Scanning Microscopy

Volume 4 | Number 3 Article 14

6-11-1990

Intracellular Penetration and Accumulation of Radiographic Contrast Media in the Rat Kidney

A. Nordby
University of Trondheim

K. E. Tvedt
University of Trondheim

J. Halgunset
University of Trondheim

O. A. Haugen
University of Trondheim

Scanning Microscopy, Vol. 4, No. 3, 1990 (Pages 651-666)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/90$3.00+.00

INTRACELLULAR PENETRATION AND ACCUMULATION OF RADIOGRAPHIC CONTRAST MEDIA IN THE RAT KIDNEY

A. Nordby *, K. E. Tvedt #, J. Halgunset, O. A. Haugen.

Department of Pathology and # Institute of Cancer Research,
University of Trondheim, N-7006 Trondheim, Norway.

(Received for publication October 30, 1989, and in revised form June 11, 1990)

Abstract

Radiographic iodine-containing contrast media (meglumine calcium metrizoate, iohexol and meglumine sodium ioxaglate) were injected intravenously in rats. At various intervals after exposure, in situ cryofixation of kidneys was performed. Thin, freeze-dried cryosections were examined by electron microscopy and X-ray microanalysis. In endothelial cells, erythrocytes and tubular cells high dry weight concentrations of iodine were found. Twenty-four hours after iohexol was injected, no trace of iodine was found in the plasma, microvilli or the nuclei of the tubular cells. Small organelle-like compartments in the cytoplasm of the proximal tubular cells contained high concentrations of iodine, whereas no iodine was found in the surrounding cytoplasm. Since no metabolism of contrast medium has been demonstrated, the iodine signals must be emitted from contrast medium molecules. Other elements were also measured, with the concentrations being always within the ranges found in tubular cells of control animals. The detection of intracellular contrast thus does not seem to be an artifact due to cell injury, but rather represents a physiological event in healthy cells in the rat kidney. Our results are in contradiction to the prevailing opinion that contrast media do not enter healthy cells. However, previous conclusions have been based on the use of conventional preparation methods, and the highly water soluble contrast molecules may have been lost during the different steps of fixation and processing.

Key words: Radiographic contrast media, iodine, rat kidney, intracellular penetration, freeze-dried, X-ray microanalysis, cryo-ultramicrotomy, elemental composition.

*Address for correspondence:
Asbjørn Nordby, Department of Radiology,
University of Trondheim, N-7006 Trondheim,
Norway. Phone No (+47) 07-997815
Fax No (+47) 07-997821

Introduction

Radiographic contrast media are preparations which are injected intravascularly in patients undergoing X-ray examination in order to enhance the contrast of the radiograph. Due to their hydrophilic properties, urographic contrast media will be widely distributed in the extracellular body water, and are generally held not to penetrate into healthy cells with intact cytoplasmic membranes (Kormano, 1979). The contrast agent molecules are thought to be excluded by the lipid layer of the cell membranes and therefore remain extracellularly (Speck, 1988). However, a small fraction of the contrast agent enters the intracellular compartment in certain tissues, such as the liver and other glandular organs (Dean & Plewes, 1984). The mechanism by which this internalization occurs is not known (Otto, 1980; Barnhart, 1984).

About two hours after intravenous injection of contrast media a nearly complete distributional equilibrium has been established between the plasma and the interstitial fluid (Grönberg et al, 1983). Within 24 hours more than 90 % of the given dose can be recovered unmetabolized in the urine (Cattell et al, 1967).

The modern, low osmolal radiographic contrast media are considered to be of very low general toxicity (Almén & Golman, 1987). This has resulted in a tendency towards increased use of contrast media even in "risk" patients (Rapoport et al, 1982), although the use of high doses may be expected to increase the possibility of adverse reactions (Shehadi, 1985; Harnish et al, 1987).

Contrast media reactions also influencing the kidney function have been reported (Shehadi, 1975; Ansell et al, 1980; Shehadi & Toniolo, 1980). The mechanism by which contrast media can produce renal failure is still not known, but various hypotheses have been advanced. Some investigators suggest a decreased glomerular filtration rate as an important element in the pathogenesis (Mudge, 1980). However, the mechanism behind such an interference with the glomerular function remains unknown. On the other hand, Moreau et al (1980)

and Moreau (1982) have suggested that kidney tubular cells may take up contrast media by pinocytosis. The internalized contrast agent may have a toxic effect on the tubular cells, thus producing kidney damage. But their attempts with autoradiography and electron microscopy failed to demonstrate contrast media within the cells.

Many of the unwanted side effects of contrast media might be explained by intracellular passage into the cells and interference with intracellular processes (Caillé et al, 1980; Parvez et al, 1985). In a series of experiments we have shown that the hydrophilic contrast media can enter into cultured cells (Nordby et al, 1989 b), and interfere with their proliferation (Nordby et al, 1987). The amounts of contrast media which can be extracted from exposed cell monolayers are dose and time dependent (Nordby et al,1989 a).

The purpose of the present work was to extend our previous studies on cells in vitro to an in vivo model, examining whether our findings in cultured cells are also applicable to selected cell types in vivo. Due to the important role played by the kidneys in the excretion of contrast media, and the above mentioned problems with contrast media induced renal failure, we chose to investigate the fate of intravenously administered contrast media in the rat kidney.

Materials and Methods

Contrast Media Exposure

A total of 12 male Wistar rats (350-450 g) were used. A cannula was inserted into a tail vein of the experimental animal and a bolus dose of contrast medium corresponding to 500 mg I/ kg body weight (b.w.) was injected. The following contrast media (Fig. 1) were used: high osmolal ionic meglumine calcium metrizoate (Isopaque Cerebral ®, 280 mg I/ml, 1460 mOsm/kg H_20, Nycomed A/S, Oslo, Norway); low osmolal nonionic iohexol (Omnipaque®, 300 mg I/ml, 690 mOsm/kg H_20, Nycomed A/S) and ionic dimer meglumine sodium ioxaglate (Hexabrix ®, 320 mg I/ ml, 600 mOsm/kg H_20, Laboratoire Guerbet, Paris, France). One control animal recieved no injection.In addition, one animal was given an intravenous injection of 2 ml /kg b.w. mannitol of osmolality comparable to that of the contrast media preparations used (900 mOsm/ kg H_2O).

Specimen Preparation

One kidney from each animal was used, and the tissue sample was fixed by rapid cryofixation in vivo at various intervals after injection of contrast media: at 5 and 15 seconds, 1 and 10 minutes, 3 and 24 hours and one week after intravenous injection of iohexol. At 24 hours two animals were used. As for meglumine calcium metrizoate the kidney was fixed 20 minutes after injection, whereas for meglumine sodium ioxaglate fixation was performed after 15 minutes. When mannitol was used, the kidney sample was taken one minute after injection. The fixation of the kidneys was performed under general anaesthesia. The animal was given an intraperitoneal injection of a mixture of midazolam/fentanyl/ fluanizon at a dose of 2 ml/kg b.w. When the exposure was 10 minutes or less, the animal was anaesthesized before the injection of contrast media. In the other cases the anaesthetic agent was administered just before termination of the exposure period. At the appropriate time a full midline abdominal incision was made, and the gut partly displaced from the abdominal cavity. The kidney was gently dissected free from the retroperitoneal fat and lifted forward. Without prior interruption of the circulation, cryofixation was performed by quickly clamping a part of the kidney between the polished copper jaws of a pair of pliers, precooled in liquid nitrogen. Thereafter the sample was rapidly removed, placed into a liquid nitrogen filled jar, and was continuously kept at this temperature until sectioning. Sections of 0.25 µm thickness were prepared in a Reichert-Jung Ultracut/FC4 cryoultra-microtome with the specimen temperature kept at -120^0 C. After each selected section, 100 µm was trimmed off the specimen before another section was collected. The sections were collected on nickel grids glued on graphite retainers. The sections were pressed between two retainers which were seated in a transportable press (Tvedt et al, 1984). Secured inside the closed press, the sections were transferred to an external freeze-drier and lyophilized at -80^0 C for two hours. After being gradually warmed to room temperature overnight, the sections were secured by covering the retainer with an upper formvar film.

X-ray Microanalysis

The sections were examined and analyzed in a JEOL 100 CX electron microscope equipped with an ASID-4 D scanning attachment and a Kevex 7000 energy-dispersive spectrometer. During X-ray microanalysis the instrument was operated at 100 kV, 1-2 nA probe current, recording the X-ray signals emanating from the specimen while the electron probe was scanning an area of the section measuring 0.2-10 µm^2. The exact probe current was monitored using a Faraday cup after the probe current had been stabilized. The quantitation of the various elements was performed by the continuum method (Hall et al, 1973) assuming proportionality between the concentration of an element and the peak-to-background ratio of the generated X-radiation. The procedure for data processing and correction for instrument contribution was made as described elsewhere (Tvedt et al, 1987).

Iodine concentrations were estimated by comparing the intensity of the X-ray signals to those emanating from PVP-solutions of various, known iodine-concentrations processed and analysed in a manner identical to the one applied to the tissue samples. Four different polyvinyl-pyrrolidone (PVP)-

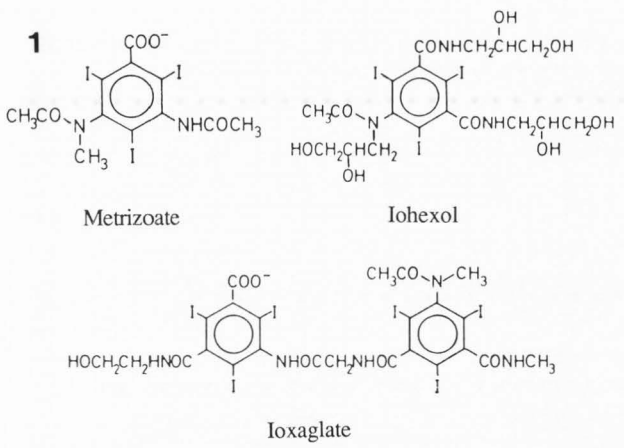

Fig. 1. Chemical structures of the radiographic contrast media used in the present work.

solutions, one containing no iodine, three containing 10.2, 29.2 and 59.1 mmol iodine per kg dry weight (d.w.) respectively, were used. Repeated analyses of these standards demonstrated a linear dependence of the iodine-specific X-ray intensity on the actual iodine concentration, the coefficient of determination being $R^2 = 0.821$.

Analyses were performed in erythrocytes and plasma within kidney capillaries, in tubular lumina, and in the nuclei, cytoplasm and identifiable cytoplasmic organelles of endothelial and tubular epithelial cells.

Statistical Evaluation

Differences between the estimated iodine concentration in different locations, including different standards were evaluated using the two-sided Student's t-test with 0.05 as the significance limit. An average estimated iodine concentration differing significantly from the estimate in the no-iodine standard will be reported as "significant amounts of iodine" (Nordby et al,1989 b).

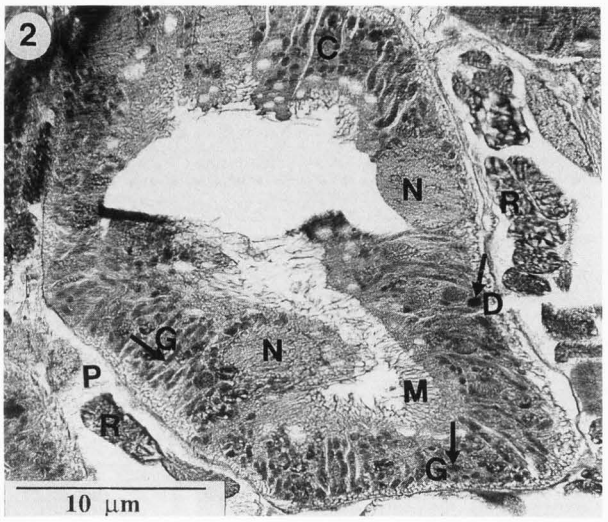

Fig. 2. Transmission electron micrograph of a freeze-dried section through a proximal tubule of a rat kidney, one minute after intravenous injection of iohexol (500 mg I/kg b.w.). N, nucleus; C, cytoplasm; D and arrow, "black organelle"; G and arrow, "grey organelle"; M, microvilli; R, erythrocyte; P, plasma.

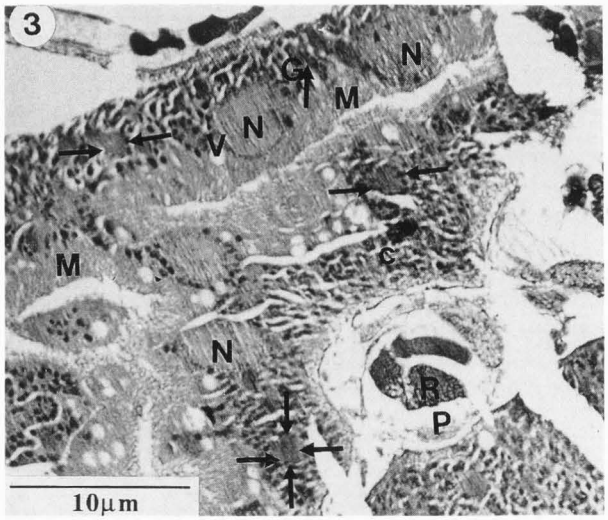

Fig. 3. Transmission electron micrograph of a freeze-dried section through a proximal tubule of a rat kidney, 3 hours after intravenous injection of iohexol (500 mg I/kg b.w.). N, nucleus; C, cytoplasm; G and arrow, "grey organelle"; M, microvilli; R, erythrocyte; P, plasma; V, vacuole; arrows, grey electron dense regions with high concentration of iodine.

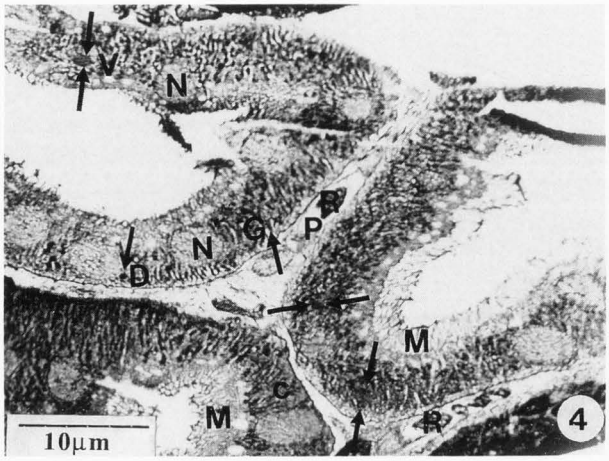

Fig. 4. Transmission electron micrograph of a freeze-dried section through proximal tubuli of a rat kidney, 24 hours after intravenous injection of iohexol (500 mg I/kg b.w.). N, nucleus; C, cytoplasm; D and arrow, "black organelle"; G and arrow, "grey organelle"; M, microvilli; R, erythrocyte; P, plasma; V, vacuole; arrows, grey electron dense regions with high concentration of iodine.

Table 1. Estimated concentration (mmol/kg d.w.) of various elements, and molar Na:K ratio at different localizations in different cells of one control rat kidney.

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro		Mean	76	323	96	149	288	44	4	4	0.243	10
cytes		SD	38	24	10	13	27	6	4	4	0.141	
Endo	Nucl	Mean	74	674	829	97	253	6	4	2	0.114	4
thelium		SD	40	57	35	14	24	3	6	5	0.071	
Plasma		Mean	816	322	309	190	590	10	23	7	3.628	10
		SD	663	155	179	64	408	12	21	20	3.512	
Prox	Nucl	Mean	63	682	690	126	314	1	5	1	0.094	10
tubulus		SD	37	49	60	13	52	3	6	4	0.056	
	Cyto	Mean	49	356	502	142	198	4	4	2	0.138	10
	plasm	SD	30	31	47	22	22	2	4	5	0.085	
"Black		Mean	28	85	102	373	183	4	1	3	0.335	10
organelles"		SD	10	17	36	67	32	2	2	2	0.121	
"Grey		Mean	49	236	343	201	189	4	2	2	0.215	10
organelles"		SD	14	46	153	73	56	4	4	4	0.063	
Dist	Nucl	Mean	12	561	778	90	110	1	5	0	0.022	10
tubulus		SD	24	131	179	11	40	3	5	3	0.045	
	Cyto	Mean	34	292	425	120	89	2	4	1	0.112	10
	plasm	SD	28	46	78	10	61	2	3	2	0.086	

The animal recieved no special treatment prior to anesthesia. n is the number of cells, in each category, or areas in plasma, analyzed. The figures given are the mean and the standard deviation of the values obtained for individual cells or areas.

Table 2. Estimated concentration (mmol/kg d.w.) of various elements, and molar Na:K ratio at different localizations in different cells of a rat kidney, one minute after the animal was given an intravenous dose of mannitol (2ml/kg b.w., 900 mOsm/kg H_2O).

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro		Mean	124	400	133	140	331	50	3	4	0.303	10
cytes		SD	79	51	34	32	70	4	3	4	0.191	
Plasma		Mean	611	296	617	154	434	17	19	1	2.352	3
		SD	136	90	440	30	82	15	8	2	1.413	
Prox	Nucl	Mean	94	806	897	136	258	2	4	2	0.134	11
tubulus		SD	55	138	198	39	49	4	6	4	0.128	
	Cyto	Mean	102	473	684	191	219	4	6	4	0.210	10
	plasm	SD	69	138	186	52	56	2	6	2	0.111	
"Black		Mean	36	137	134	383	202	5	3	2	0.284	9
organelles"		SD	14	48	78	94	41	2	2	2	0.132	
"Grey		Mean	39	235	318	165	193	1	2	4	0.164	10
organelles"		SD	12	25	96	43	54	5	5	4	0.047	
Microvilli		Mean	484	435	610	136	453	5	17	1	1.119	8
		SD	116	34	57	19	98	4	6	2	0.282	

n is the number of cells, in each category, or areas in plasma, analyzed. The figures given are the mean and the standard deviation of the values obtained for individual cells or areas.

Results

Morphology

Electron microscopy of freeze-dried cryosections revealed a fairly well preserved morphology (Figs. 2-4). Glomeruli and tubules were easily identified, as were capillary endothelial cells and intravascular erythrocytes. The different parts of the tubular apparatus showed characteristic features permitting the distinction to be made between proximal and distal tubules, the former being characterized by their conspicuous brush border of closely packed, slender microvilli.Sections through the thin limb of Henle´s loop were also recognized without difficulty. Despite

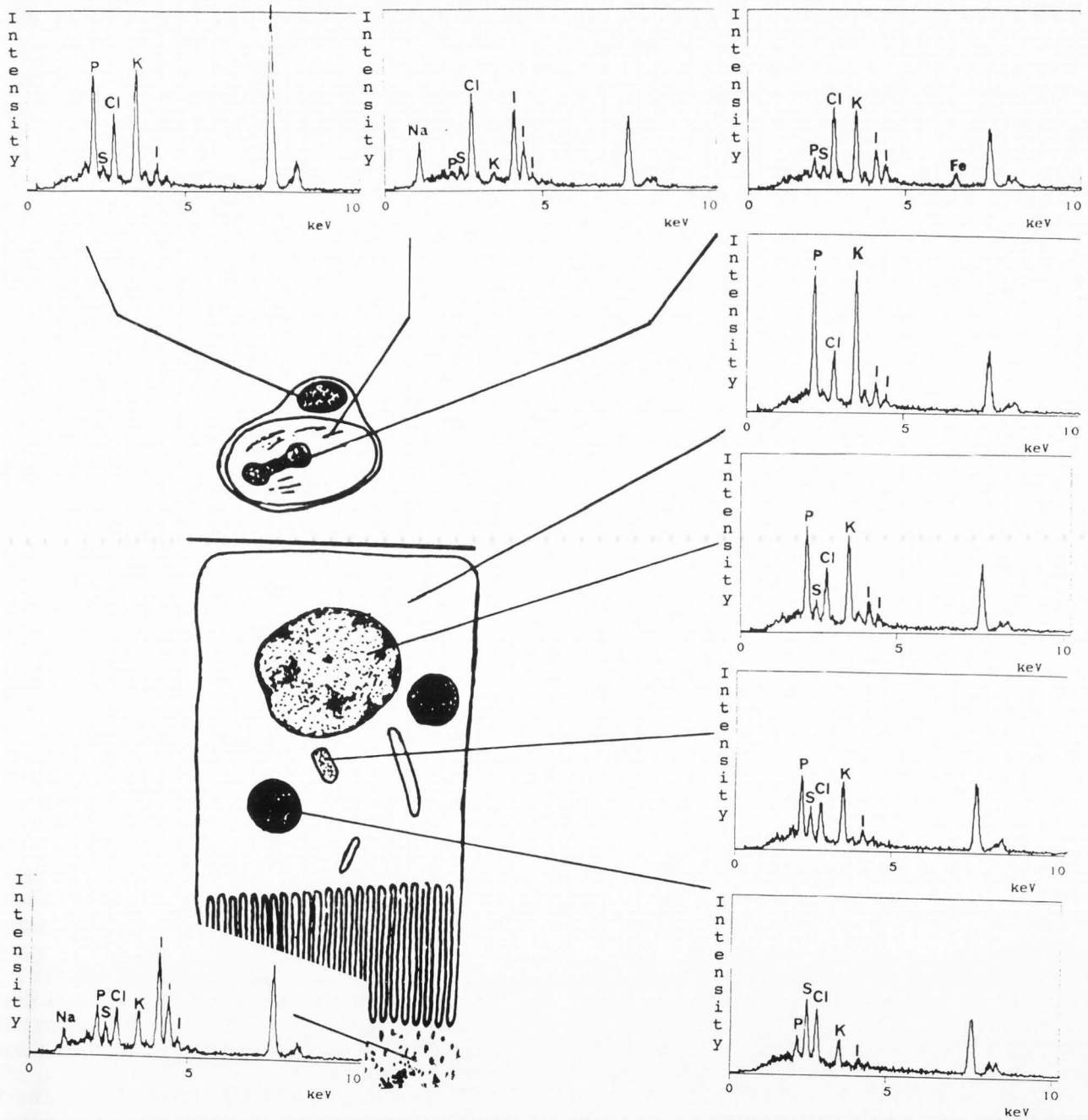

Fig. 5. Schematic illustration of a proximal tubular cell and a capillary, one minute after intravenous injection of iohexol with typical X-ray spectra obtained by single microanalysis of different parts of the cell. The peaks corresponding to some elements are marked by their chemical symbol. The three major iodine peaks are readily observed.

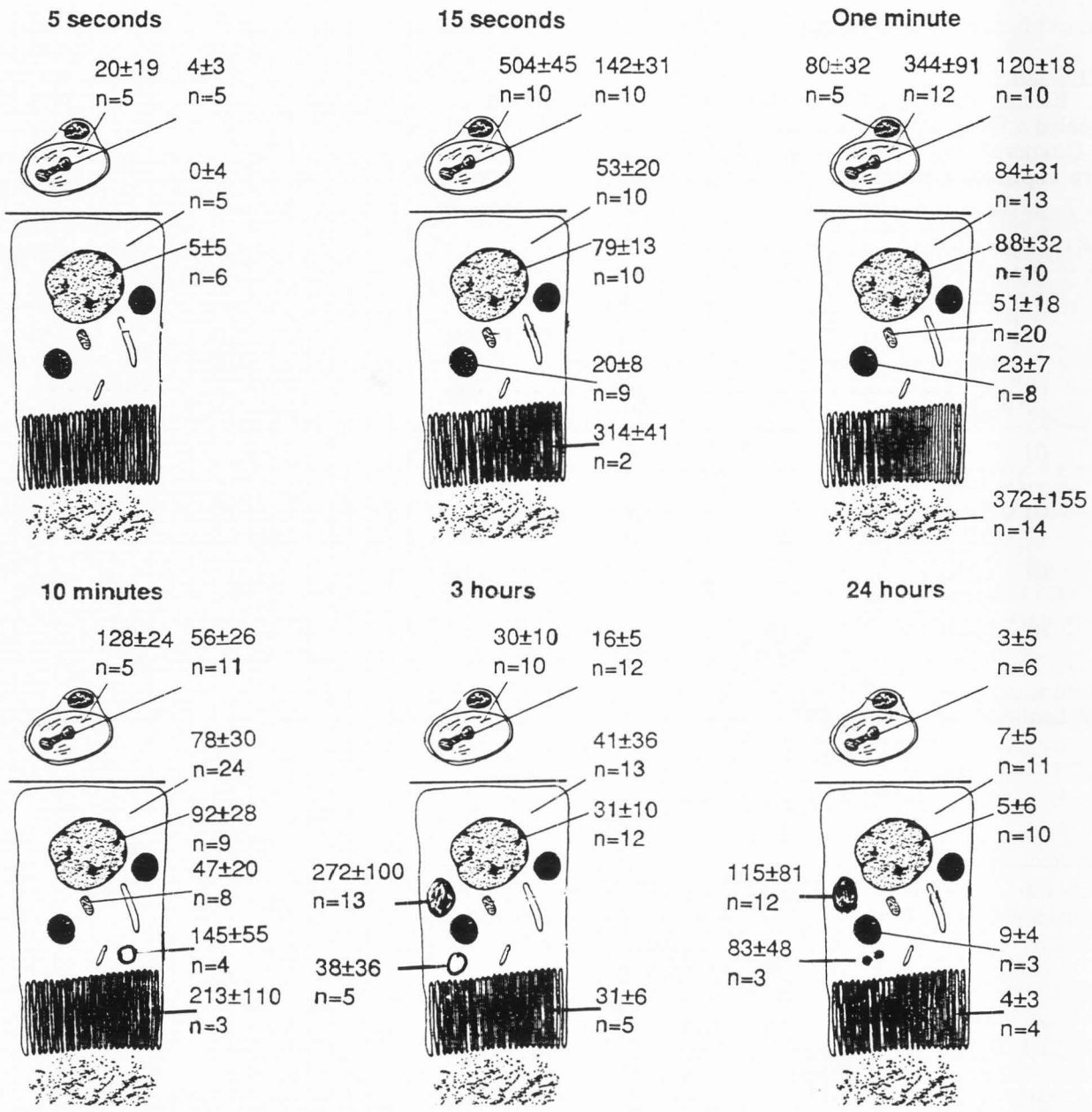

Fig. 6. Schematic illustration of a proximal tubular cell and a capillary after exposure to intravenously administered iohexol at different times. The values correspond to the results given in the tables. Mean iodine concentration and standard deviation are shown. "n" is the number of cells or areas in plasma or tubular lumen, analyzed.

ice-crystal artifacts, the size of which increased with the distance from the renal capsule, and which imparted a lattice-like overall structure to the tissue, some intracellular details were resolved. The nuclei were easily seen. In the cytoplasm of the tubular cells, distinct structures, descriptively called "black organelles" or "grey organelles" and elongated, electron-dense structures, reminiscent of those seen in conventionally prepared specimens, and which are due to rodlike mitochondria oriented parallel to the cell axis, were distinguished. However, the ice-crystal-induced small-scale distortion of the structure precluded an unequivocal identification of these cytoplasmic organelles.

In the kidneys which were freeze-fixed between 10 minutes and 24 hours after intravenous injection of contrast medium, homogeneous regions of intermediate electron density, called "grey regions", were noticed. Such grey regions were not observed in the control kidneys, and they were not found in the

Tables 3 a through g. Estimated concentration of various elements, and molar Na:K ratio at different localizations in different cells of rat kidneys at different time exposure to a single intravenous injection of iohexol (500 mg I/ kg b.w.). n is the number of cells or areas in the plasma or tubular lumen, analyzed. The figures given are the mean and the standard deviation of the values obtained for individual cells or areas. The analyzed regions are described in the tables. "Black organelles" are homogeneous regions which were electron dense and which could be distinguished as discrete organelles. "Grey organelles" are less electron dense, homogeneous regions which could be distinguished as discrete organelles. "Grey regions" are parts of the cells which were less electron dense and of different sizes. "Black dense organelles" are very small electron dense regions which also contained iron.

Table 3a. Elemental concentrations (mmol/kg d.w.) in rat kidney 5 seconds after intravenous injection of iohexol.

	Locali-zation		Element Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	32	368	115	179	259	50	2	4	0.088	5
		SD	8	41	35	12	25	5	3	3	0.022	
Plasma		Mean	1160	120	149	228	588	8	23	20	14.828	5
		SD	545	105	175	63	192	6	18	19	10.942	
Prox tubulus	Nucl	Mean	59	659	660	201	137	3	2	5	0.088	6
		SD	29	45	35	16	23	2	3	5	0.041	
	Cyto plasm	Mean	64	349	526	199	107	4	3	0	0.189	5
		SD	17	47	53	57	11	4	1	4	0.064	

Table 3b. Elemental concentrations (mmol/kg d.w.) in rat kidney 15 seconds after intravenous injection of iohexol.

	Locali-zation		Element Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	46	289	92	120	238	39	-7	142	0.164	10
		SD	30	30	10	17	30	5	4	31	0.129	
Plasma		Mean	503	38	42	83	168	2	-15	504	18.3	10
		SD	57	17	11	11	22	2	6	45	12.6	
Prox tubulus	Nucl	Mean	34	768	802	136	245	1	1	79	0.044	10
		SD	28	110	198	25	46	4	5	13	0.040	
	Cyto plasm	Mean	55	354	524	171	159	6	5	53	0.154	10
		SD	27	84	81	49	42	2	6	20	0.079	
"Black organelles"		Mean	13	56	98	374	202	4	5	20	0.290	9
		SD	11	24	49	54	22	2	9	8	0.285	
Microvilli		Mean	276	211	294	84	169	2	-7	314	1.481	2
		SD	51	87	64	18	18	6	8	41	0.853	

animal examined one week after exposure.

X-ray Microanalysis of Control Kidneys

The X-ray microanalytical data from the two control animals, one that received no injection and one that recieved an intravenous dose of hypertonic mannitol, are shown in Table 1 and 2, respectively. The molar ratio of intracellular sodium to potassium (Na:K-ratio) was estimated to be in the order of 0.02 - 0.3 in all cell types examined. The estimated iodine concentrations were very low, 0 - 7, (median 2), mmol/kg dry weight (d.w.), and not significantly above zero when compared to the standard containing no iodine.

X-ray Microanalysis of Kidneys Exposed to Iohexol

Elemental concentrations at various intervals after intravenous administration of a bolus dose of iohexol, are shown in Tables 3, a through g, and Figures 5 through 7. In no instance did we detect any severe deviation from the control values of the intracellular Na:K-ratios. Thus, there was no evidence of cell damage with disturbance of the normal permeability characteristics of the cytoplasmic membranes. No intracellular iodine was seen in the kidney fixed 5 seconds after injection, and at this time point the iodine signals detected in the blood plasma were only slightly above the control level. However,

Table 3c. Elemental concentrations (mmol/kg d.w.) in rat kidney one minute after intravenous injection of iohexol.

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	112	307	111	119	313	39	-3	120	0.369	10
		SD	35	39	33	23	31	6	3	18	0.136	
Endo thelium	Nucl	Mean	92	653	847	125	292	7	-2	80	0.150	5
		SD	42	86	121	46	25	12	5	32	0.095	
Plasma		Mean	742	130	148	118	469	6	-7	344	10.03	12
		SD	230	115	88	32	154	3	8	91	7.41	
Prox tubulus	Nucl	Mean	73	618	690	120	255	2	0	88	0.121	10
		SD	28	80	154	39	57	3	5	32	0.047	
	Cyto plasm	Mean	106	364	490	123	205	5	2	84	0.311	13
		SD	56	61	77	24	44	3	7	31	0.201	
"Black organelles"		Mean	23	96	142	335	200	3	4	23	0.203	8
		SD	25	46	65	74	25	3	5	7	0.257	
"Grey organelles"		Mean	50	221	326	214	175	5	0	51	0.236	20
		SD	30	112	164	86	29	3	4	18	0.110	
	Lumen	Mean	384	194	265	94	239	2	-11	372	4.01	14
		SD	156	108	151	34	106	3	8	156	5.80	
Dist tubulus	Nucl	Mean	43	632	483	86	243	2	2	62	0.086	9
		SD	25	146	170	34	43	2	3	10	0.050	
	Cyto plasm	Mean	132	400	542	114	227	4	0	126	0.365	12
		SD	81	82	139	24	34	4	6	81	0.283	

Table 3d. Elemental concentrations (mmol/kg d.w.) in rat kidney 10 minutes after intravenous injection of iohexol.

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	87	320	143	170	288	52	1	56	0.275	11
		SD	61	35	127	26	35	29	7	26	0.193	
Plasma		Mean	452	182	220	129	465	9	14	128	7.68	5
		SD	168	168	184	40	62	11	13	24	10.60	
Prox tubulus	Nucl	Mean	69	507	565	95	284	2	0	92	0.145	9
		SD	36	106	205	29	48	4	4	29	0.081	
	Cyto plasm	Mean	87	306	436	101	201	5	1	78	0.334	24
		SD	76	107	129	30	66	6	4	30	0.364	
"Grey organelles"		Mean	29	263	361	191	231	5	3	47	0.156	8
		SD	38	225	289	119	70	5	2	20	0.215	
Vacuoles		Mean	156	165	203	134	205	1	1	145	1.001	4
		SD	49	48	71	39	60	4	4	55	0.410	
Microvilli		Mean	330	162	428	82	311	3	-6	213	2.20	3
		SD	188	80	63	29	200	4	2	110	0.84	

in the animal sacrificed 15 seconds after injection, the plasma iodine dry weight concentration attained the highest value seen in these experiments. In kidney samples taken at successively longer intervals after administration of contrast medium, the estimated iodine concentrations were successively lower, being barely detectable at 3 hours.The estimated plasma iodine concentrations were closely paralleled by the concentrations found in the tubular fluid, the latter being actually measured by scanning small areas of the microvillous brush border of proximal tubules. Small, but significant amounts of iodine appeared in the tubular cells at 15 seconds, diffusely distributed in the cytoplasm as well as in the nuclei. The more

Table 3e. Elemental concentrations (mmol/kg d.w.) in rat kidney 3 hours after intravenous injection of iohexol.

	Localization		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythrocytes		Mean	45	362	92	117	509	51	3	16	0.129	12
		SD	47	31	12	16	80	7	3	5	0.141	
Plasma		Mean	1705	191	191	198	1168	10	29	30	13.36	10
		SD	1034	100	113	67	578	9	19	10	10.86	
Prox tubulus	Nucl	Mean	71	738	778	102	482	3	0	31	0.097	12
		SD	22	57	157	20	96	2	4	10	0.034	
	Cytoplasm	Mean	83	367	542	111	331	6	2	41	0.224	13
		SD	49	89	85	11	73	3	6	36	0.100	
"Grey regions"		Mean	45	162	236	190	184	7	-12	272	0.336	13
		SD	27	136	189	13	89	4	9	100	0.180	
Vacuoles		Mean	319	433	536	133	543	9	19	38	0.824	5
		SD	175	105	136	29	123	4	17	36	0.615	
Microvilli		Mean	206	425	592	94	442	8	7	31	0.489	5
		SD	55	29	31	20	15	6	7	6	0.149	

Table 3f. Elemental concentrations (mmol/kg d.w.) in one rat kidney 24 hours after intravenous injection of iohexol.

	Localization		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythrocytes		Mean	98	344	191	166	299	40	4	3	0.291	6
		SD	39	91	83	67	77	14	6	5	0.090	
Prox tubulus	Nucl	Mean	95	802	985	170	211	4	4	5	0.134	10
		SD	84	157	192	48	27	3	4	6	0.111	
	Cytoplasm	Mean	117	375	602	186	170	8	5	7	0.299	11
		SD	79	58	65	55	73	4	7	5	0.158	
"Black organelles"		Mean	58	135	217	368	205	5	1	9	0.412	3
		SD	42	54	137	83	35	2	2	4	0.181	
"Grey regions"		Mean	49	173	262	261	180	9	-6	115	0.300	12
		SD	25	76	97	48	69	3	6	81	0.152	
"Black dense organelles"		Mean	58	166	266	330	132	64	0	83	0.337	3
		SD	26	36	48	82	17	49	6	48	0.102	
Microvilli		Mean	434	404	526	165	405	4	14	4	1.089	4
		SD	180	72	74	15	85	4	4	3	0.454	

Table 3g. Elemental concentrations (mmol/kg d.w.) in rat kidney one week after intravenous injection of iohexol.

	Localization		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythrocytes		Mean	48	266	65	154	217	38	3	2	0.186	10
		SD	43	55	16	14	51	6	4	3	0.141	
Prox tubulus	Nucl	Mean	92	631	678	162	202	2	9	2	0.172	10
		SD	71	139	103	22	51	3	5	6	0.165	
	Cytoplasm	Mean	68	306	475	164	146	6	4	1	0.229	11
		SD	33	75	96	51	20	2	4	4	0.121	
"Black organelles"		Mean	35	105	167	448	172	4	2	0	0.343	2
		SD	7	14	9	38	9	1	3	3	0.115	
"Black dense organelles"		Mean	43	231	332	226	161	36	2	3	0.190	2
		SD	1	48	67	80	52	38	2	2	0.042	

Table 4. Estimated concentrations (mmol/kg d.w.) of various elements, and molar Na:K ratio at different localizations in different cells of a rat kidney, 20 minutes after exposure to meglumine calcium metrizoate (500 mg I / kg b.w.).

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	75	247	85	180	231	45	4	24	0.279	8
		SD	60	63	22	28	59	5	5	8	0.178	
Plasma		Mean	2007	176	325	296	1231	5	25	145	22.14	7
		SD	1325	146	354	86	788	9	13	78	19.04	
Prox tubulus	Nucl	Mean	95	695	593	183	213	4	-6	163	0.137	11
		SD	48	57	83	27	69	3	6	22	0.074	
	Cyto plasm	Mean	76	409	495	147	138	5	0	103	0.182	11
		SD	42	48	67	24	29	3	6	15	0.082	
"Black organelles"		Mean	14	30	57	369	176	4	1	13	0.516	10
		SD	6	12	31	76	47	3	2	6	0.214	
Microvilli		Mean	469	439	534	154	426	4	7	77	1.240	7
		SD	254	105	115	19	156	5	6	14	1.052	

n is the number of cells or areas in the plasma, analyzed.The figures given are the mean and the standard deviation of the values obtained for individual cells or areas.

Table 5. Estimated concentrations (mmol/kg d.w.) of various elements, and molar Na:K ratio at different localizations in different cells of a rat kidney, 15 minutes after exposure to meglumine sodium ioxaglate (500 mg I/ kg b.w.).

	Locali-zation		Element									
			Na	K	P	S	Cl	Fe	Ca	I	Na:K	n
Erythro cytes		Mean	55	398	121	182	335	58	10	16	0.134	6
		SD	39	43	59	41	57	7	15	5	0.092	
Plasma		Mean	2315	256	178	401	1511	16	33	243	9.41	5
		SD	575	97	79	150	461	9	23	95	1.90	
Prox tubulus	Nucl	Mean	141	751	824	213	273	3	11	48	0.181	11
		SD	102	107	186	31	39	5	25	29	0.138	
	Cyto plasm	Mean	79	407	581	184	183	5	4	27	0.192	10
		SD	50	56	70	28	43	4	2	22	0.111	
"Black organelles"		Mean	24	40	63	427	180	5	3	4	0.543	3
		SD	24	10	20	49	18	5	7	4	0.611	
Vacuoles		Mean	1343	254	289	313	1008	4	68	454	3.37	3
		SD	2133	171	191	238	1512	4	45	221	4.38	
Microvilli		Mean	664	339	440	204	491	4	5	139	1.96	2
		SD	184	2	27	29	54	6	6	5	0.56	

n is the number of cells or areas in the plasma, analyzed. The figures given are the mean and the standard deviation of the values obtained for individual cells or areas.

electron-dense, organelle-like structures did not at this stage contain more iodine than the surrounding cytoplasm. However, in kidney samples from animals sacrificed at successive time points, an apparent, progressive reduction of the concentration of iodine-containing substances took place. At 3 hours the concentration of diffusely distributed cytoplasmic iodine was much lower than that found at 10 minutes. On the other hand, there was a corresponding apparent increase of the amount of iodine found in the morphologically distinct structures of intermediate electron density called "grey regions".

At 10 minutes after exposure the iodine concentration in the cytoplasm of the tubular cells was higher near the apical cell membrane than in the basal part of the cells.

In the two animals exposed to iohexol for 24 hours, no iodine-containing substance could be detected in the capillaries nor in the tubular lumina. In the proximal tubular cells no iodine was found in the

nuclei nor in the cytoplasm, except for the small intracytoplasmic compartments of moderate electron density called "grey regions", and in the very dense black structures, which contained high local concentrations of iodine.

In the animal sacrificed one week after exposure to iohexol, no iodine was detected in any location.

X-ray Microanalysis of Kidneys Exposed to Other Contrast Media

After injection of meglumine calcium metrizoate or meglumine sodium ioxaglate, highly significant amounts of iodine were found both in the nuclei and the cytoplasm of the proximal tubular cells, the tissue samples being taken 15 and 20 minutes after injection, respectively (Tables 4 and 5). In the kidneys exposed to meglumine sodium ioxaglate, some vacuolar structures contained very high concentration of iodine.

Discussion

In the present work we have demonstrated high intracellular dry weight concentration of iodine in the erythrocytes and capillary endothelium as well as in the kidney tubular cells of rats exposed to iodine-containing contrast media. The iodine appears in the cells within a few seconds after injection, and traces of iodine remain detectable for at least 24 hours.

It is important to recognize that X-ray microanalysis only detects the elements present in the specimen without providing any information about the chemical compound into which the various elements engage. Thus, this technique cannot positively identify the contrast agent molecule as such. However, the intravascular contrast media are biologically very inert substances which seem not to be metabolized to any appreciable degree. In an earlier paper we reported on the analysis of extracts from monolayer cell cultures which had been exposed to contrast media for varying periods of time (Nordby et al, 1989 a). When subjected to high performance liquid chromatography, the extracts showed peaks that co-chromatographed with pure contrast medium. Such peaks were not detected in extracts from unexposed cells, and no additional, unidentified peak appeared after exposure. Thus, although different contrast agents seem to be readily internalized by cultured cells (Nordby et al, 1989 b), we were unable to demonstrate any metabolization of these compounds in vitro (Nordby et al, 1989 a). Golman and Almén (1984) claim that contrast media are not metabolized to any significant degree in vivo, and Olsson et al, (1983) found no metabolites of iohexol in serum or urine of healthy volunteers after intravenous injection. Taken together these findings represent strong evidence that contrast agents are not chemically modified in the body, and that any detected iodine therefore can be taken as a marker of the contrast agent molecule.

Davidson (1966) injected very high doses of the contrast medium directly into the renal artery of dogs during surgery. He reported that tritiated sodium diatrizoate was internalized in the proximal tubular cells, evaluated by microautoradiographic studies. But he found no activity related to the red blood cell. Other attempts at microscopic localization of contrast media have failed (Moreau et al, 1980), probably due to difficulties of fixation (Mützel, 1981). Contrast agents, being polar, water-soluble molecules, will readily be washed out of the tissues during fixation using chemical methods. The results shown in this paper could not have been revealed without the techniques of rapid freeze-fixation and cryo-ultramicrotomy combined with the high resolution of electron microscopy and X-ray microanalysis.

The cellular uptake of contrast media might be a sign of cellular damage resulting in a breakdown of the cytoplasmic membrane barrier (Abraham et al, 1980). However, the animals were exposed to concentrations and doses of contrast media equivalent to those used during angiography in humans, and any

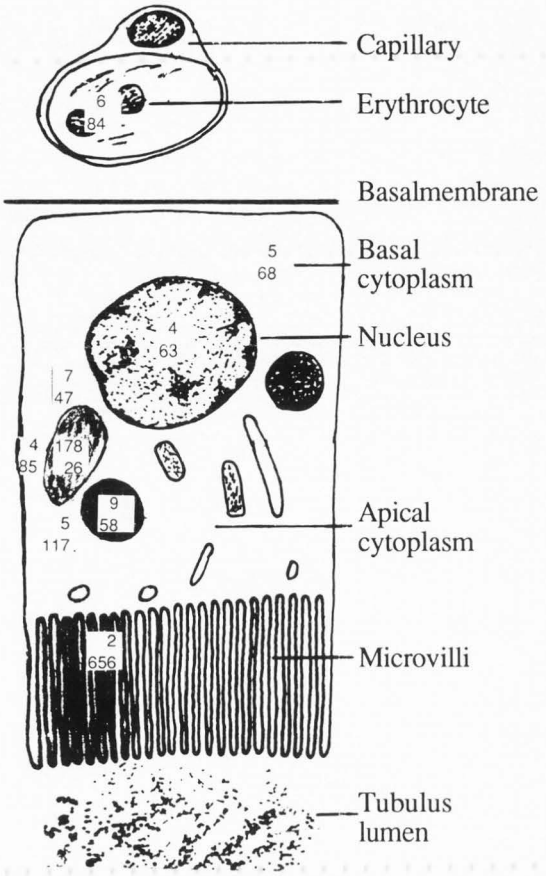

Fig. 7. Schematic illustration of a proximal tubular cell and a capillary, 24 hours after intravenously administered iohexol (500 mg I/ kg b.w.). The iodine (on the top) and sodium concentration calculated after single analysis at different places in the cell are marked.

disruption of the membrane integrity with a resulting abnormally high permeability would be expected to produce a leakage of monovalent cations down their respective concentration gradients. The molar ratio of intracellular sodium to potassium (Na:K-ratio) represents a sensitive indicator of cation leakage, and will rapidly increase in the case of membrane damage. During the experiments reported in the present paper, no such disturbance of the Na:K- ratio was observed. In the control animals and those exposed to contrast media the ratio was always found to be in the range of 0.03 to 0.3, which corresponds very well to normal values reported elsewhere (Hall & Gupta, 1984), as do the concentrations of other elements measured. Thus, the cellular internalization of contrast media does not seem to be an artifact due to cell injury, but rather a physiological event in healthy rat erythrocytes, endothelial cells and kidney tubular cells.

The present work confirms and extends the results reported in our previous papers where we showed that cultured cells of various types internalize contrast media upon exposure (Nordby et al, 1989 b), and that such agents may cause perturbations of the cell physiology by other mechanisms than pure osmotic stress (Nordby et al, 1987). Although the conditions of exposure were not directly comparable to the clinical situation in terms of dose and duration, it appears that at least some of the results obtained in vitro may apply to the intact organism.

Despite the fact that both cultured cells and rat kidney cells internalize contrast agents, there are some noteworthy differences in the reaction patterns. In cultured cell lines the internalized contrast agent remained diffusely distributed throughout the cell (Nordby et al, 1989 b), whereas in the kidney tubules this was not the case. Within 15 seconds after intravenous injection of iohexol, iodine was detected with a diffuse distribution in the nuclei as well as in the cytoplasm of the tubular cells. After the first few minutes, a redistribution apparently started, with progressive concentration of iodine in certain areas of the cytoplasm, while the rest of the cell was gradually cleared. The regions which accumulated iodine during this second phase showed characteristic appearances, and as a rule were easily identified on morphological grounds (Figs. 3&4). These were seen as more or less well circumscribed, nearly round structures with a homogeneous interior of medium or high electron density. The most electron dense organelles also contained high levels of iron, and therefore were tentatively identified as secondary lysosomes (Heptinstall et al, 1974).

The mechanism behind the movements of contrast media into and within the kidney tubular cells is not known. However, it may be speculated that the contrast agent molecules enter the tubular cell from the tubular lumen, especially since the iodine concentration was higher in the apical than in the basal part of the cell after 10 minutes` exposure. The luminal membrane of the tubular cell contains various passive and active transport systems, which could be responsible for the rapid entry. The contrast media may pass through leak channels, or active endocytotic processes like pinocytosis may be involved. The intracellular redistribution, with a concentration of the internalized contrast media in limited regions and the buildup of steep concentration gradients in the cytoplasm, may imply an energy-dependent system of recognition and active transport. After 24 hours exposure the iodine concentration varied from nearly zero to more than 100 mmol/ kg d.w. over a distance of 0.2 µm in both animals. Such transport systems are well developed in tubular cells, constituting the biochemical basis for the tubular secretion. Alternatively, the concentration process may be due to binding of contrast agents to some immobile macromolecular structure in these locations.

Although we have demonstrated the internalization of contrast media in kidney tubular cells of the male rat, we found no morphological or X-ray microanalytical evidence of cell injury. It may be that the tubular cells are especially adapted to handle various potentially harmful, small-molecular substances, and that the rapid sequestration of contrast media into restricted areas of the cell, should be interpreted in this perspective. It would therefore be of great interest to examine in what way contrast agents are handled by other cell types. Cryofixation and X-ray microanalysis of freeze-dried cryosections would seem to be particularly well suited for this kind of investigation. Furthermore, there could be some variation between different species. It has been reported that a "small degree of tubular secretion of diatrizoate" takes place in rats (Mudge et al,1971). Thus, there seems to be the need for a reassessment of the fate of contrast media in the human body, using sensitive methods like the one described in the present paper. Such investigations are being carried out in our laboratory.

Aknowledgment

The authors thank Mrs. Marit Westad and Mrs. Heidi Hjelmeland for skillful technical assistance. Dr. A. Nordby was a research fellow of the county of Sør-Trøndelag during this work. K.E. Tvedt is a scientist of the Norwegian Cancer Society.

References

Abraham JL, Higgins CB, Newell JD. (1980). Uptake of iodinated contrast material in ischemic myocardium as an indicator of loss of cellular membrane integrity. Am J Pathol 101 : 319-327

Almén T, Golman K. (1987). Contrast media pharmacokinetics in the assessment of renal failure. In: Parvez Z, Moncada R, Sovak M (eds.). Contrast

media: Biologic effects and clinical application. Volume I, Boca Raton: CRC Press, Inc., pp 77-95

Ansell G, Tweedie MCK, West CR, Evans P, Couch L. (1980). The current status of reactions to intravenous contrast media. Invest Radiol 15: S32-S39

Barnhart JL. (1984). Hepatic disposition and elimination of biliary contrast media. In: Sovak M (ed.). Radiocontrast agents. New York: Springer-Verlag, pp 367-418

Caillé JM, Guibert-Tranier F, Howa JM, Billerey J, Calabet A, Piton J. (1980). Cerebral penetration following metrizamide myelography. J Neuradiology 7: 3-12

Cattell WR, Fry IK, Spencer AG, Purkiss P. (1967). Excretion urography. I. Factors determining the excretion of Hypaque. Br J Radiol 40: 561-571

Davidson AJ. (1966). Micro-autoradiographic study of the distribution of tritiated sodium diatrizoate in the canine kidney. Invest Radiol 1: 271-280

Dean PB, Plewes DB. (1984). Contrast media in computed tomography. In: Sovak M (ed.). Radiocontrast agents. New York: Springer-Verlag, pp 479-523

Golman K, Almén T. (1984). Urographic contrast media and methods of investigative uroradiology. In: Sovak M (ed.). Radiocontrast agents. New York: Springer-Verlag, pp 127-191

Grönberg T, Sjöberg S, Almén T, Golman K, Mattsson S. (1983). Noninvasive estimation of kidney function by X-ray fluorescence analysis. Elimination rate and clearance of contrast media injected for urography in man. Invest Radiol 18: 445-452

Hall TA, Anderson HC, Appleton T. (1973). The use of thin specimen for X-ray microanalysis in biology. J Microsc 99: 177-182

Hall TA, Gupta BL. (1984). The application of EDXS to biological sciences. J Microsc 136: 193-208

Harnish PP, Morris TW, Fischer HW. (1987). Drug action of intravascular contrast media. In: Parvez Z, Moncada R, Sovak M (eds.). Contrast media: Biologic effects and clinical application. Volume II, Boca Raton: CRC Press, Inc., pp 27-47

Heptinstall RH, Kissane JM, McCluskey RT, Porter KA (eds.). (1974). Sundry condition affecting the renal tubulus. In: Pathology of the kidney. Boston: Little, Brown and Company, pp 1043-109

Kormano M. (1979). Volume of distribution of contrast media in blood. Acta Radiol Diagn 20: 33-38

Moreau J-F.(1982). Nephrotoxicity of urographic contrast media. In: Amiel M (ed.). Contrast media in radiology. New York: Springer-Verlag, pp 195-200

Moreau J-F, Droz D, Noel L-H, Leibowitch PJ, Michel J-R. (1980). Tubular nephrotoxicity of water-soluble iodinated contrast media. Invest Radiol 15: S54-S60

Mudge GH. (1980). Nephrotoxicity of urographic radiocontrast drugs. Kidney Int 18: 540-542

Mudge GH, Berndt WO, Saunders A, Beattie B. (1971). Renal transport of diatrizoate in the rabbit, dog and rat. Nephron 8: 156-172

Mützel W. (1981). Properties of conventional contrast media. Discussion. In : Felix R, Kazner E, Wegener OH (eds.). Contrast media in computed tomography. International workshop Berlin 1981. Amsterdam, Excerpta Medica, pp 27-30

Nordby A, Halgunset J, Haugen OA. (1987). Cytostatic effects of radiographic contrast media in synchronized cell cultures. Invest Radiol 22: 678-684.

Nordby A, Thorstensen K, Halgunset J, Haugen OA, Solberg S. (1989 a). Effects on the ATP content of cultured cells after radiographic contrast media exposure. Evidence for accumulation of contrast media in cultured cells. Acta Radiol 1989; 30: 541-547.

Nordby A, Tvedt KE, Halgunset J, Kopstad G, Haugen OA. (1989 b). Incorporation of contrast media in cultured cells. Invest Radiol 1989; 24: 703-710.

Olsson B, Aulie Å, Sveen K, Andrew E. (1983). Human pharmaco-kinetics of iohexol. A new nonionic contrast medium. Invest Radiol 18: 177-182

Otto H. (1980). Die Rolle der Leberzelle bei der Elimination hepatotroper Röntgenkontrastmittel. (Significance of the liver cell in elimination of cholegraphic agents). Radiologe 20: 16-23.

Parvez Z, Talat K, Moncada R. (1985). Ultrastructural changes in rat aortic endothelium during contrast media infusion. Invest Radiol 20: 407-412

Rapoport S, Bookstein JJ, Higgins CB, Carey PH, Sovak M, Lasser EC. (1982). Experience with metrizamide in patients with previous anaphylactoid reactions to ionic contrast agents. Radiology 143: 321-325

Shehadi WH. (1975). Adverse reactions to intravascularly administrated contrast media. A comprehensive study based on a prospective survey. AJR 124 : 145-154

Shehadi WH. (1985). Death following intravascular administration of contrast media. Acta Radiol Diagn 26: 457-461

Shehadi WH, Toniolo G. (1980). Adverse reaction to contrast media.A report from the committee on safety of contrast media of the international society of radiology. Radiology 137: 299-302

Speck U. (1988). Basic pharmacology of iodinated contrast media. In: Carr DH (ed.). Contrast media. New York: Churchill Livingstone, pp 1-23

Tvedt KE, Kopstad G, Haugen OA. (1984). A section press and low elemental support for enhanced preparation of freeze-dried cryosections. J Microsc 133: 285-290

Tvedt KE, Kopstad G, Haugen OA, Halgunset

J. (1987). Subcellular concentration of calcium, zinc, and magnesium in benign nodular hyperplasia of the human prostate. X-ray micro-analysis of freeze-dried cryosections. Cancer Res 47: 323- 328

Discussion with Reviewers.

A. J. Morgan: Would the authors kindly provide more information on their standards prepared in a PVP matrix? For example, what iodine compounds were actually used for this purpose; how were these compounds solubilized without risking the sublimation of iodine; how were the standards frozen prior to cryosectioning?
Authors : Various amounts of potassium iodide, which is readily solubilized in aqueous liquids, were dissolved in a 30 per cent PVP (50,000 mean m.w.) solution. Four different standards were prepared, containing 0, 10.2, 29.2, and 59.1 mmol I/ kg d.w. The standards were stabilized by freezing: A drop of the PVP-standard was transferred to a large formvar film and frozen by clamping with a pair of pliers, precooled in liquid nitrogen. The discs of frozen PVP standard were stored in liquid nitrogen until sectioning.

A. J. Morgan : Halides are generally considered to be very unstable under electron irradiation. Did you observe any instability of the iodine signal during the course of your experiments; if so, what steps did you take to minimize iodine loss?
Authors : Some mass loss inevitably occurs during analysis and this might affect the iodine more than other elements.We were not able to detect any selective loss of iodine in our preparations, neither in the standards nor in the kidney samples.

G. M. Roomans : Why was 100 µm trimmed off between sections? Was extensive ice crystal damage not a problem with sections from deeper within the tissue?
Authors : In order to use ordinary statistical methods the observations must be mutually independant. Therefore, we had to make sure that no cell could be analyzed more than once. In order to achieve this, we trimmed off the specimen between sections a distance well above the diameter of any cell. The applied freezing method (see Materials and Methods) produces a flattened specimen with two well frozen surfaces. This provides uniform sectioning and permits the specimen to be oriented in such a way that the plane of sectioning is parallel to the direction of freezing. This eliminates the problem of increasing ice crystal size with increasing the number of sections.

G. M. Roomans : Why did you carry out more extensive experiments with one particular contrast substance (iohexol)?
Authors : The high osmolal ionic contrast media are rapidly being replaced by the new generation of low osmolal contrast media in radiological practice. Iohexol belongs to the latter group and has already become the most widely used preparation in Scandinavia. Therefore, we felt that this substance deserved a more thorough examination than the others.

A. J. Spencer : As it stands the paper is only semiquantitative, since results of statistical tests are not shown. The authors state that "in no instance did we detect any severe deviation from the control values of the intracellular Na:K-ratios". It is not possible to assesss what "severe deviation" actually means if the tests are not shown. Statistical tests are also required for the other elements, making it clear what comparisons are being made.
Authors : As stated in the Materials and Methods section, Student´s t-test was used for comparison of group means, with a significance limit of 0.05. Statistical tests were used to evaluate the recorded iodine counts in cells exposed to contrast media as compared to the controls. Expressions like "significant amounts of iodine" etc. refer to the outcome of such testing. The use of the t-test is justified for this purpose because of our experiments were specifically designed and performed in order to detect an eventual entry of contrast media into tubular cells. However, if one were to apply similar testing procedures to all the other elements, one would run into severe problems of multiple comparisons. Since we had no a priori hypothesis about changes in the other elements, it would not be correct to perform a statistical test of any difference that might turn out to seem "interesting". An analysis of variance might have been performed, but with so many variables and so few cases, this would not have been very helpful.

We included in our study data on other elements than iodine in order to provide support to our view that the proximal tubular cells are not severely injured by contrast agents, and that the entry of iodine is not a sign of cell damage. Our argument rests principally upon the demonstration of an undisturbed Na:K-ratio. The statement "in no instance did we detect any severe deviation from the control values of the intracellular Na:K-ratios" is not quantitative, but a formal statement of "no statistically significant difference" would be of no additionally value without an assessment of the test´s power. We agree that the expression "severe deviation" is unfortunate, not due to a lack of testing, but because its exact defination is not clearly stated. In fact, we defined any Na:K-ratios below 0.6 as "not severely deviant" from the control value of 0.138±0.085. None of the cells exposed to contrast media exceeded this value. Thus, the main point of our report remains the unequivocal demonstration of iodine entry into cells which otherwise seem normal.

A. J. Spencer : You have shown that radiographic contrast media can enter kidney proximal tubule cells and suggest that media is sequestered into specific regions of the cell. However, it is obvious that some regions of the cell (mitochondria, lysosomes etc) were not analysed. Only by analysing all organelles within a cell, either by multiple static probes or by X-ray imaging techniques, can a picture of the distribution of an element be built up. Please comment.
Authors: More extensive analyses are definitely needed in order to elucidate the intracellular distribution of contrast media. In the present work it was not our intention to characterize the pharmacokinetic of contrast media in detail, but rather to provide evidence for the intracellular penetration of contrast media in vivo, a question which has been a matter of dispute. We have indeed analyzed a lot of mitochondria and other organelles. However, at present we feel that we are not always able to identify the organelles with such confidence that we can present data of this kind. Longitudinally sectioned mitochondria are easily recognized in kidney tubular cells, but on transversal sectioning the profiles are hard to distingush from those of other organelles. Thus, many interesting questions remain to be answered. Our results indicate that X-ray microanalysis of freeze-dried cryosections is the best method presently available for investigating the fate of these highly hydrophilic substances on the cellular and subcellular level.

A. J. Spencer : You suggest various pathways for contrast entry into proximal tubule cells. How do you think the media enter erythrocytes?
Authors : We can only speculate. The cytoplasmic membrane of the erythrocyte is about 100.000 times more permeable to chlorine-anions than the membrane of most other cells. Whether this is of significance to the penetration of radiographic contrast media is still an open question.

A. J. Spencer : You state in the results that after 10 minutes of exposure to iohexol that the concentration of I was higher in the apical part of the cells. Was this trend observed at other time points? Since only a single value is given for cytoplasm in tables 1-5, where were these analyses performed - all apical, all basal, cell core or a combination?
Authors : We did not find this trend at other time points. The analyses were mostly performed in the basal parts of the cells.

A. J. Spencer : The data for erythrocytes and plasma are highly variable, often showing high Na/K ratios in erythrocytes and low Na/K and Na/P ratios in plasma. This suggests contribution of the X-ray signal from plasma to the spectrum for erythrocytes, and vice versa. Please comment.
A. J. Spencer : Please comment on your use of the Na/K ratio as a measure of cell integrity, when the Na/K ratio differs by an order of magnitude in some of your control cells.
J. Wroblewski: The variability between the elemental data is very large. Is this caused by low number of cells analysed in each group or the quantitation procedure used? Please comment.
Authors : Low concentrations of sodium and calcium (in the presence of high concentrations of potassium) are notoriously difficult to measure precisely. In addition, very thin sections have been used in order to obtain high spatial resolution. Thus, the mass of the supporting formvar film represents a large fraction of the total mass excited by the electron probe, and a small error in the estimation of the background contribution from the film will affect the estimated level of all elements. Further, with thin, freeze-dried cryosections, fluids with high water content, e.g. blood plasma and tubular fluid, cause additional problems. With a water content more than 95% water, such compartments are often not successfully sectioned. Another problem is that after freeze-drying the mass is very low. Even though this to some extent has been compensated for by extending the analysing periods, there still are quantification problems due to the low X-ray intensity. In general, there appears to be an inverse relationship between the spatial precision and the precision in terms of concentration. For the question addressed in the present investigation, localization was of prime importance.

A. J. Spencer : Earlier papers have shown that radiocontrast media may affect erythrocyte function and morphology (Schiantarelli et al, 1973) and affect glomerular filtration (Larson et al, 1983). You have suggested that secondary lysosomes may be a site for sequestration of contrast medium due to the presence of high I and Fe. Does the association of I and Fe not suggest that glomerular filtration has been affected, allowing erythrocytes (or their break-down products) to enter tubule lumen and subsequently be phagocytosed by proximal tubule cells? The "black organelles" are also likely to be lysosomes since they contain high S, as previously described in kidney and other tissues (LeFurgey et al, 1989, James-Kracke et al,1979). Please speculate why this population of lysosomes does not sequester I.
Authors : Studies on the biological effects of radiocontrast media have yielded divergent results. Thus, Vaamonde et al (1989) detected no morphological changes in the erythrocytes and found only very slight effects on the kidney function in rats exposed to meglumine diatrizoate sodium in doses three times higher than the ones used in our experiments. However, in order for intact erythrocytes to enter the kidney tubules through the glomerular filtration barrier, severe damage to the latter would be required. Moreover, if either erythrocytes or their iron-containing breakdown products were reaching

the tubular lumen, we would expect to detect iron in the tubular fluid by X-ray microanalysis. We never observed such peaks in our spectra. Thus, there is nothing in our data which supports the mechanism suggested by the referee. More work will have to be done in order to fully elucidate the pathways followed by contrast media in the kidney tubular cells.

J. Wroblewski : Did you attempt to carry on X-ray microanalysis on conventionally prepared samples in order to check if also in your experimental set up iodine is lost during various preparation steps?
Authors : We have performed X-ray microanalysis on conventionally fixed cultured cells and rat-tissue after exposure to contrast media, without recording iodine signals. As the radiographic contrast media are highly water soluble molecules, it is reasonable to believe that the dehydration procedure removes such components. But we have not analyzed the loss due to the various preparation steps.

J. Wroblewski : You have described differences in the distribution of internalized contrast media between kidney cells in vivo and in vitro. Do you know if this difference is due to the fact that in vivo cells are polarised, while in conventional monolayer cultures they are not?
Authors : The epithelial cells of the rat kidney seem to internalize more contrast media than other types of cells (myocytes, endothelium, hepatocytes, white blood cells, - unpublished observations). The cells we used for in vitro experiments, were established cell lines from cervical and prostatic carcinoma, showing poor cellular differentiation. Thus the cells analyzed in the two series of experiments differ with respect to several parameters. We do not know which is most important for determining the intracellular handling of contrast media.

Additional References

James- Kracke MR, Sloane BF, Shuman H, Somlyo AP. (1979). Lysosomal composition in cultured vascular smooth muscle cells: electron probe analysis. Proc. Natl. Acad. Sci. USA 76, 6461-6465

Larson TS, Hudson K, Mertz JI, Romero JC, Knox FG. (1983). Renal vasoconstructive response to contrast media. The role of sodium balance and the renin-angiotension system. J. Lab. Clin. Med. 101, 385-391

LeFurgey A, Mandel LJ, Hawkey LA, Ingram P. (1989). Quantitative microchemical imaging of proximal tubules: Lysosomal compartmentation of sulfur and calcium during anoxia. (Abstract) Kidney Int. 35, 411

Schiantarelli P, Peroni F,Tirone P, Rosati G. (1973). Effects of iodinated contrast media on erythrocytes. I. Effects of canine erythrocytes on morphology. Invest Radiol 8: 199- 204

Vaamonde CA, Bier RT, Papendick R, Alpert H, Gouvea W, Owens B, Pardo V. (1989). Acute and chronic effects of radiocontrast in diabetic rats. Role of anesthesia and risk factors. Invest Radiol 24: 206-218