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The role of nitrate, nitrite and N-nitrosamines in carcinogenesis of colon tumours following ureterosigmoidostomy

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Summary. Urinary diversion in both a rat model for ureterosigmoidostomy and in ureterosigmoidostomy patients result in an increased incidence of colon tumours. Bacterial and chemical investigations on feces-urine mixtures from both the rat model and ureterosigmoidostomy patients showed the presence of a complex nitrate-reducing bacterial flora in both rats and humans. This bacterial flora actively reduced urinary nitrate to nitrite in humans and increased the endogenous formation of N-nitroso compounds. No evidence of urinary nitrate reduction and increased nitrosamine formation in the rectosigmoid of rats was found. The results support the N-nitrosamine theory of carcinogenesis of the colon following ureterosigmoidostomy in humans, but not in rats. As the rat model induces colon carcinomas, factors other than the increased endogenous formation of N-nitroso compounds in the rectosigmoid may contribute to the initiation of colon carcinomas following ureterosigmoidostomy.

Key words: Ureterosigmoidostomy – Colon carcinoma – Carcinogenesis – Nitrosamines

Ureterosigmoidostomy as a means of urinary diversion has been established worldwide for more than 70 years. This type of diversion is especially favoured for children with bladder exstrophy due to the psychological and social advantages of continence and missing stoma. A recognized complication of ureterosigmoidostomy is the development of adenocarcinomas of the colon at the site of ureterocolic anastomosis as first reported by Hammer in 1929 [18]. In a recent literature review by Stewart [38], 94 cases of such tumours have been published; 54 being adenocarcinomas and 26 adenomas of the colon, 4 urothelial tumours, 1 lymphoma and 8 colonic tumours of unclear classification. At present, at least 100 cases of colon tumours at the ureterocolonic anastomosis are known [21, 38]. The incidence of developing colon carcinomas following ureterosigmoidostomy ranges between 3.5 to 13.3%, resulting in an 80–550 times increased risk as compared to the general population [13, 29, 35, 37, 39, 45]. As a result of this increased risk, several researchers have argued against the use of ureterosigmoidostomy in children [35, 37–39].

Several theories have been postulated to account for the carcinogenesis of colon tumours following ureterosigmoidostomy. These theories include irritative changes of the colonic epithelium caused by urine [16, 27, 35, 45], chronic urothelial irritation by feces, epithelial instability at the borderline between urothelium and colonic epithelium [14] and the influence of fresh colonic sutures exposed to the feces-urine mixture [14]. The theory favoured by most research is the increased formation of N-nitroso compounds in the colon as a result of bacterial reduction of urinary nitrate by the mixed bacterial flora of the intestine and bacterially catalyzed nitrosation of endogenous amines present in both feces and urine [4, 6, 37, 38, 40].

The following study was designed to investigate both the N-nitrosamine theory in ureterosigmoidostomy patients [36] and in a recognized animal model for ureterosigmoidostomy [5, 14]. Furthermore, the potential prophylaxis of colon carcinomas after ureterosigmoidostomy was investigated using the uroprotector sodium-2-mercaptoethanesulphonate (mesna) and the urothelial surfactant sodiumpentosanpolysulphate.

Materials and methods

Patients and human studies

Samples of rectal feces-urine mixtures were obtained from 24 ureterosigmoidostomy patients with a mean observation period of 13 years (2–46 years) attending the Urology Department of the University of Heidelberg for routine follow-up observation. Rectal feces-urine samples were obtained for chemical analysis of nitrate, nitrite, volatile and nonvolatile N-nitroso compunds. Aerobic bacterial assays were obtained from the colon contents of 18 of the 24 patients. A control group of 20 healthy volunteers without urinary diversions was obtained from the scientific staff of the German Cancer Research Center.

Table 1. Nitrate, nitrite, N-nitrosamines in urine-feces mixture from 24 ureterosigmoidostomy patients

	NO_2 µmol/l	NO_3 mmol/l	NDMA nmol/l	NPIP nmol/l	NPYR nmol/l	NMOR nmol/l	NSAR nmol/l	NMPA nmol/l	NAzCA nmol/l	NPRO nmol/l	NTCA nmol/l	NMTCA nmol/l	Total nmol/l
Positive:	20	23	24	7	11	8	20	7	3	24	24	24	24
Mean:	31.43	0.25	5.31	0.90	2.08	1.13	8.37	1.12	0.61	22.37	41.62	14.40	97.90
S.D.:	40.82	0.24	4.79	1.54	2.79	1.94	7.88	2.20	1.83	18.72	28.15	18.08	66.54
Range:													
Lower:	ø	ø	1.35	ø	ø	ø	ø	ø	ø	2.78	8.02	0.57	20.51
Upper:	147.83	0.92	20.30	4.39	10.0	7.33	25.76	8.22	7.69	85.42	114.81	69.32	290.97

Animals and experimental studies

One hundred and thirty outbred Wistar rats (Hanover, FRG), 200 g body weight at the start of the experiment were kept under conventional conditions (two animals per Makrolon III cage; temperature 23 ± 2°C; relative humidity 55 ± 10%; illumination period 12 h/day). All animals received a low, nitrosamine diet (1,320 N Altromin, Lage, FRG). Animals ($n = 120$) were operated using a procedure similar to that described by Crissey and Gittes [5, 14]. The bladder neck was ligated, the bladder dome excised and anastomosis of the remaining patch and intact bladder trigonum to an opening cut in the anterior wall of the rectosigmoid. According to the recommendation of Gittes [14], rats received continuous antibiotic coverage (carbenicilline-indanyl, 127 mg/l in drinking water) to avoid ascending pyelonephritis and perianal ulceration. The calculated dose based on an average consumption of 30 ml drinking water per day was 19.1 mg/Kg/day. Two days after operation the rats were prospectively randomized into 3 groups of 40 animals. Group I acted as a control and received only continued antibiotic coverage. Group II animals received the uroprotector sodium-2-mercaptoethanesulphonate (Mesna) at a dose of 350 mg/kg/day, group III received the urothelial surfactant sodium pentosanpolysulphate (30 mg/kg/day). Group IV consisted of the remaining 10 animals which acted as an unoperated control group.

Feces-urine mixtures were randomly selected and aerobically cultured 4–247 days postoperatively from 14 animals in groups I and III and from 18 animals in group II. Feces samples were aerobically cultured from all 10 controls animals in group IV.

Collection and storage of urine and feces samples

Human samples. Mid-morning rectal feces-urine samples were collected in polyethylene containers containing 4 ml 2.5 M sodium hydroxide and 1 mM 2-(ethylmercurymercapto)benzoic acid (Janssen Chimica, Brüggen, FRG) to prevent artefact nitrosamine formation and bacterial growth. The samples were centrifuged at 5,000 r.p.m. for 30 mins at 10°C to separate solid feces and the clear supernatant removed for analysis.

Rat samples. Feces-urine samples were collected over 24-hours using metabolic cages to which 10 ml of 2.5 M sodium hydroxide containing 1 mM 2-(ethylmercurymercapto)benzoic acid and 1 mM morpholine (as a control for artefact formation) was added to the sample collection tubes prior to sample collection. After 24-hours, the metal cages were washed down with a further 5 ml of water. The feces-urine mixture was made up to 50 ml in volume with distilled water, thoroughly mixed and centrifuged as above to obtain a clear supernatant for analysis.

Urinary analysis of nitrate, nitrite and N-nitroso compounds

The analysis of nitrate, nitrite and volatile N-nitroso compounds was made using previously reported methods [44]. Nonvolatile N-nitrosamino acids were analyzed using N-nitrosoisonipecotic acid (NiNIP) as an internal standard instead of N-nitrosoazetadine-4-carboxylic acid (NAzCA) as previously reported [44]. Additional gas chromatographic methods [43] were used to confirm the presence of nonvolatile N-nitrosamino acids in rectal urine samples.

Statistical analysis

Comparison of nitrate, nitrite and nitrosamine concentration in human urine was made using the two-tailed rank sum test test according to Wilcoxon. Results obtained from animal experiments were evaluated using the exact test of Fisher.

Abbreviations of N-nitroso compounds:

NDMA	N-Nitrosodimethylamine
NDEA	N-Nitrosodiethylamine
NPIP	N-Nitrosopiperidine
NPYR	N-Nitrosopyrrolidine
NMOR	N-Nitrosomorpholine
NAzCA	N-Nitrosoazetidine-4-carboxylic acid
NiNIP	N-Nitrosoisonipecotic acid
NSAR	N-Nitrososarcosine
NMPA	3-(N-Nitroso-N-methylamino)propionic acid
NPRO	N-Nitrrosoproline
NTCA	N-Nitrosothiazolidine-4-carboxylic acid
NMTCA	N-Nitroso-2-methylthiazolidine-4-carboxylic acid
NHPRO	N-Nitrosohydroxyproline

Results

Human studies

The concentrations of nitrate, nitrite and N-nitrosamines in the rectal feces-urine mixtures of 24 ureterosigmoidostomy patients are presented in Table 1 and the concentrations in urine samples from 20 control volunteers in Table 2. Urinary nitrite was not detected in 20 control urine samples whereas in rectal feces-urine samples from ureterosigmoidostomy patients a mean nitrite concentration of 31.43 ± 40.82 µmol/l (range 0–147.83 µmol/l) was found. The mean nitrate concentration of 0.25 ± 0.24 mmol/l (range 0–0.92 mmol/l) in ureterosigmoidostomy patients was significantly lower ($p < 0.001$) than the mean concentration of 0.93 ± 0.39 mmol/l (range 0.33–1.82 mmol/l) found in control urine samples.

N-Nitrosodimethylamine (NDMA) was the only volatile nitrosamine detected, and was present in two urine samples in the control group at a concentration of 2.68 nmol/l in both urine samples, this level was signifi-

Table 2. Nitrate, nitrite, N-nitrosamines in urine from 20 control volunteers

	NO_2 mmol/l	NO_3 mmol/l	NDMA nmol/l	NDEA nmol/l	NPIP nmol/l	NPYR nmol/l	NSAR nmol/l	NPRO nmol/l	NTCA nmol/l	NMTCA nmol/l	Total nmol/l
Positive:	∅	20	2	∅	∅	∅	13	20	20	20	20
Mean:	∅	0.93	0.22	∅	∅	∅	2.29	9.69	24.95	20.19	57.33
S.D.:	∅	0.39	0.70	∅	∅	∅	2.34	3.12	16.34	18.55	33.87
Range:											
Lower:	∅	0.33	∅	∅	∅	∅	∅	5.45	6.14	7.62	25.16
Upper:	∅	1.82	2.68	∅	∅	∅	9.61	17.90	63.63	87.28	178.42

Table 3. Bacterial status of 18 ureterosigmoidostomy patients

	Proteus	Entero-cocci	Klebs.	E. coli	Citrob	Morgan morg.	Strepto-cocci	Aerobes spp.	Pseudo-monas	Staph. coagul
Positive:	3/18 16.7%	11/18 61.1%	6/18 33.3%	16/18 88.9%	3/18 16.7%	1/18 5.6%	2/18 11.1%	4/18 22.2%	1/18 5.6%	2/18 11.1%
CFU	10^6/g	10^5/g	10^4/g							
Positive:	13/18 72.2%	4/18 22.2%	1/18 5.6%							

CFU = Colony forming units

cantly increased to 5.31 ± 4.79 nmol/l (range 1.35–20.32 nmol/l) in ureterosigmoidostomy patients. Rectal urine samples also contained N-nitrosopiperidine (NPIP) at a mean concentration of 0.90 ± 1.54 nmol/l in 7/24 samples (range 0–4.39 nmol/l), N-nitrosopyrrolidine (NPYR) at a mean concentration of 2.08 ± 2.79 nmol/l in 11/24 samples (range 0–10.0 nmol/l) and N-nitrosomorpholine (NMOR) at a mean concentration of 1.13 ± 1.94 nmol/l in 8/24 samples (range 0–7.33 nmol/l).

A similar significant increase was found for the excretion of nonvolatile N-nitrosamino acids in ureterosigmoidostomy patients. N-Nitrosoarcosine (NSAR) was increased from 2.29 ± 2.34 nmol/l to 8.37 ± 7.88 nmol/l ($p < 0.009$), N-nitrosoproline (NPRO) from 9.69 ± 3.12 nmol/l to 22.37 ± 18.72 nmol/l ($p < 0.0001$) and N-nitrosothiazolidine-4-carboxyline acid (NTCA) from 24.95 ± 16.34 nmol/l to 41.62 ± 28.15 nmol/l ($p < 0.017$). a significant decrease in the excretion of N-nitroso-2-methylthiazolidine-4-carboxylic acid (NMTCA) from 20.19 ± 18.55 nmol/l in the control group to 14.40 ± 18.08 nmol/l in ureterosigmoidostomy patients ($p < 0.017$) was found. In addition to these nonvolatile N-nitroso compounds, 1.12 ± 2.20 nmol/l 3-(N-nitroso-N-methylamino)propionic acid (NMPA) and 0.61 ± 1.83 nmol/l N-nitrosoazetadine-4-carboxylic acid (NAzCA) were found in 3 and 7 rectal urine samples, respectively. The total concentration of both volatile and nonvolatile N-nitroso compounds was significantly increased from 57.33 ± 33.87 nmol/l (range 25.16–178.42 nmol/l) in the control group to 97.90 ± 66.54 nmol/l (range 20.51–290.97 nmol/l) in ureterosigmoidostomy patients ($p < 0.0052$).

Aerobic rectal feces-urine mixture cultures from ureterosigmoidostomy patients showed mixed bacterial flora in 94.4% (17/18) of the patients. In 94.4% (17/18) of patients, at least one strain of nitrate-reducing bacteria (Proteus mirabilis or vulgaris, Klebsiella pneumoniae or oxytoca and E. coli) was detected. The bacterial count was 10^6 colony forming units (CFU)/g in 72.2% (13/18), 10^5 CFU/g in 22% (4/18) and 10^4 CFU/g in 5.6% (1/18) of the rectal feces-urine samples (Table 3).

Animal studies

The concentrations of nitrate, nitrite and N-nitrosamines in the feces-urine mixture of operated animals (Groups I–III) and urine of unoperated rats (group IV) are shown in Table 4. The mean nitrate concentration of 918 µg/24 h (range 395–1,345 µg/24 h) and the mean nitrite concentration of 57 µg/24 h (range 0–396 µg/24 h) in the control urine samples (group IV) were significantly higher ($p < 10^{-6}$–10^{-8}) than in the feces-urine mixtures of the operated animals in groups I-III. Nitrate and nitrite were not detected in the feces of control animals in group IV. No significant differences in the nitrate and nitrite concentrations in feces-urine mixtures of operated animals in groups I–III were observed 5 days, 3 and 8 months following ureterosigmoidostomy.

There was no significant differences between the NDMA concentrations found in feces-urine mixtures in groups I–III and the separately collected feces and urine samples from animals in group IV. The mean levels of NDMA found were between 5.2 and 10.5 ng/24 h (range

Table 4. Nitrate, nitrite, N-nitrosamines in urine-feces from rats

	5 days			3 months			8 months		
	Nitrate (μg/day)	Nitrite (μg/day)	NDMA (ng/day)	Nitrate (μg/day)	Nitrite (μg/day)	MDMA (ng/day)	Nitrate (μg/day)	Nitrite (μg/day)	MDMA (ng/day)
Group 1									
Mean	8.04	3.21	9.80	28.5	12.0	8.75	1.0	ø	6.13
Positive	18%	14%	100%	50%	50%	100%	5%	ø	100%
Range	0–121	0–48	5–23.5	0–125	0–60	5–22.5	0–20	ø	2.5–12.5
Group 2									
Mean	19.7	6.7	8.82	6.42	0.83	7.08	ø	ø	5.25
Positive	21%	18%	97%	25%	8%	100%	ø	ø	95%
Range	0–175	0–67	0–21	0–36	0–10	5–10	ø	ø	10.0
Group 3									
Mean	13.69	1.88	9.05	11.27	10.91	8.64	8	ø	6.5
Positive	16%	16%	97%	36%	36%	100%	20%	ø	100%
Range	0–190	0–20	0–18	0–47	0–40	5–17.5	0–40	ø	5–7.5
Group 4 (urine)									
Mean	–	–	–	918	57	10.5	–	–	–
Positive	–	–	–	100%	95%	100%	–	–	–
Range	–	–	–	395–1345	0–396	7.5–15	–	–	–
Group 4 (feces)									
Mean	–	–	–	ø	ø	9.2	–	–	–
Positive	–	–	–	ø	ø	100%	–	–	–
Range	–	–	–	ø	ø	5–12.5	–	–	–

Group 1 = operated control (untreated); Group 2 = Mesna; Group 3 = Natriumpentosanpolysulphate; Group 4 = unoperated control

0–23.5 ng/24 h), the limit of detection was 2.5 ng/24 h. Analysis of randomly selected urine samples from control animals (group IV) and feces-urine mixtures from animals in groups I–III did not show the presence of nonvolatile N-nitroso compounds (e.g. NPRO and NSAR), the limit of detection was 0.1 μg/24 h. Aerobic cultures of feces-urine mixtures from animals in groups I–III and of feces from the control animals in group IV showed complex mixed bacterial flora containing *E. coli, Proteus, Enterococcus, Klebsiella, Aerococcus spp., Aerobe spp., Lactobacillus spp., Staph. spp., Staph. aureus* and non-haemolysing *Streptococcus* (Table 5). The total bacterial counts in the four groups ranged from 10^5 to 10^7 CFU/g feces-urine mixture. There was no significant differences between the incidence of occurrence of *E. coli* in the operated animals (groups I–III) and the control group ($p > 0.2$). The occurrence of *Proteus spp.* in 64.3% (9/14) of the animals treated with sodiumpentosanpolysulphate in group III was significantly ($p = 0.05$) smaller than the 100% occurrence found in feces only cultures from control animals in group IV. No significant difference was found between the occurrence of *Proteus spp.* in control group IV and groups I and II ($p > 0.2$). *Klebsiella* was found in all operated animal groups, but was absent from the feces of control animals. In 100% (46/46) of the operated animals, at least one strain of nitrate reducing bacteria (*E. coli, Proteus* or *Klebsiella*) was found.

Discussion

The organotropic carcinogenic effects of different N-nitroso compounds in experimental animals and their metabolism are well understood [30]. In the rat, almost quantitative absorbtion of ingested doses of nonvolatile N-nitrosamines occurs in the upper gastrointestinal tract and 96–98% of an administered dose of NPRO, NSAR and N-nitrosohydroxyproline (NHPRO) is excreted unchanged in urine or feces [7, 28]. Following gastrointestinal absorbtion, volatile nitrosamines such as NDMA and NPYR are metabolically detoxified in the liver where they are also potent hepatocarcinogens [30], excretion of volatile nitrosamines does not usually occur in feces and/or urine [1, 12, 23].

The concentrations of nonvolatile N-nitrosamino acids found in urine samples from control volunteers (Table 2) are within the normally reported range [42, 44, 46]. The presence of trace levels of NDMA in two human urine samples cannot be explained. It may be speculated that the very low concentrations of NDMA (ca. 10 ng/24 h) found in urine samples from control animals (Table 4) may have resulted from the dropping of rat diet containing traces of NDMA into the urine collection containers during the collection period. This observation is supported by the fact that the rat diet was routinely analysed at monthly intervals during the experimental period and found to contain a mean concentration of 1.4 μg/kg NDMA (range 1.1–1.9 μg/kg). On three occa-

Table 5. Bacterial status of rats

		E. coli	*Proteus*	*Entero cocci*	*Klebs.*	*Aeorococcus spp.*	*Lactobacillus*	*Staph. spp.*	*Streptococci*	*Aerobes spp.*
Group 1, 14 Animals										
Positive:	*n*	13	11	14	4	4	11	7	4	ø
	%	92.9	78.6	100	28.6	28.6	78.6	50	28.6	ø
CFU:	*Median*	2×10^{7}	5.3×10^{5}	1.8×10^{8}	1.3×10^{6}	5.7×10^{5}	1.3×10^{3}	6.3×10^{6}	1.6×10^{9}	ø
Range:	*Lower*	1.3×10^{3}	4.3×10^{2}	3.7×10^{6}	5.3×10^{4}	9.1×10^{4}	1.6×10^{7}	3.7×10^{4}	3.3×10^{7}	ø
	Upper	1.1×10^{10}	1.3×10^{10}	9.3×10^{9}	3.5×10^{9}	1.1×10^{6}	3.3×10^{10}	5.3×10^{7}	9.3×10^{9}	ø
Group 2, 18 Animals										
Positive:	*n*	18	18	18	9	8	11	16	8	2
	%	100	100	100	50	44.4	61.1	88.9	44.4	11.1
CFU:	*Median*	2×10^{7}	2.9×10^{6}	1.7×10^{8}	5.3×10^{5}	9.3×10^{5}	1.8×10^{8}	1.4×10^{6}	7.5×10^{7}	8.7×10^{3}
Range:	*Lower*	4.5×10^{2}	3.4×10^{3}	4.2×10^{6}	1.7×10^{4}	7.2×10^{4}	1.4×10^{6}	7.2×10^{4}	1.4×10^{7}	2×10^{2}
	Upper	1.9×10^{10}	1.2×10^{10}	2×10^{10}	3.7×10^{8}	2×10^{8}	1.4×10^{10}	2.3×10^{8}	2×10^{10}	1.7×10^{4}
Group 3, 14 Animals										
Positive:	*n*	14	9	14	12	2	6	7	2	3
	%	100	64.3	100	85.7	14.3	42.9	50	14.3	21.4
CFU:	*Median*	9.3×10^{7}	4.6×10^{6}	$6{,}3\times10^{8}$	3×10^{6}	$5{,}8\times10^{6}$	$5{,}6\times10^{8}$	1.8×10^{6}	7.5×10^{7}	6.9×10^{7}
Range:	*Lower*	2.8×10^{4}	2.8×10^{4}	5.3×10^{6}	5.3×10^{4}	$1{,}7\times10^{6}$	8.5×10^{7}	2.8×10^{5}	5.3×10^{6}	8×10^{6}
	Upper	1.7×10^{10}	1.5×10^{9}	2.4×10^{9}	1.8×10^{10}	8.8×10^{6}	9.8×10^{10}	8.3×10^{6}	1.5×10^{8}	$1{,}3\times10^{8}$
Group 4 (feces), 10 Animals										
Positive:	*n*	10	10	10	ø	1	7	8	ø	4
	%	100	100	100	ø	10	70	80	ø	40
CFU:	*Median*	1.6×10^{6}	$2{,}6\times10^{5}$	10×10^{5}	ø	6.3×10^{4}	3.2×10^{10}	2.8×10^{5}	ø	2.8×10^{4}
Range:	*Lower*	3.2×10^{4}	1.6×10^{4}	8.5×10^{4}	ø	-	1.9×10^{10}	8.5×10^{3}	ø	1.6×10^{3}
	Upper	5.1×10^{7}	2×10^{6}	3.2×10^{6}	ø	-	7.7×10^{10}	2×10^{6}	ø	5.6×10^{4}

CFU = Colony forming units
Group 1 = operated control (untreated); Group 2 = Mesna; Group 3 = Natriumpentosanpolysulphate; Group 4 = unoperated control

sions, NPYR was also found in the rat diet at concentrations between 0.1–0.4 µg/kg and traces of NPYR were also determined in urine samples collected from animals receiving diets containing NPYR.

The urinary excretion of nonvolatile N-nitroso compounds depends mainly on the dietary nitrate burden [24, 28, 42]. However experimental studies have shown that humans maintained on nitrate-free diets continues to excrete significant concentrations (26 ± 10 nmol/l) of endogenously formed N-nitroso compounds [46]. This suggests that endogenous sources of nitrate and nitrite produced by intestinal microorganisms [15, 17], peritoneal macrophages [11, 19, 20, 26, 41] and the oxidation of ammonia in the liver [10, 33] may also be involved in the endogenous formation of N-nitroso compounds. Approximately 60–70% of the ingested nitrate burden is excreted in the urine and only about 1% in feces [2]. In our investigations, we found mean urinary nitrate concentrations of 0.93 mmol/l in unoperated humans (Table 2) and 918 µg/24 h in unoperated rats. In the feces of unoperated control rats, no nitrate could be detected (Table 4).

Stewart [36, 38] first described a 10 fold increase in the excretion of 570 nmol/l total N-nitroso compounds in the urine-feces mixture of ureterosigmoidostomy patients compared to a total excretion of 56.8 nmol/l N-nitroso compounds in normal urines from healthy volunteers. On the basis of the results, and the fact that the concentrations of total N-nitroso compounds in rectal urine samples positively correlated with the total bacterial counts for nitrate-reducing organisms, Stewart postulated that the formation of N-nitroso compounds from bacterially reduced urinary nitrate and endogenous amines in feces could be a possible explanation for colon carcinoma induction following ureterosigmoidostomy.

The increased endogenous formation of N-nitroso compounds in ureterosigmoidostomy patients and the increased risk of colon cancer is in many ways analogous to the situation in patients with chronic bacterial infections of the urinary tract [8, 31] or bilharziosis [44] in which an increased risk for bladder cancer occurs. Stewart's theory [36, 38] is supported by in vivo and in vitro experiments which show the formation of N-nitroso compounds from amines and nitrite by intestinal microorganisms [3, 22, 24, 48]. Using the rat model of Crissey and Gittes for ureterosigmoidostomy [5, 14], Cohen et al. [4] reported elevated concentrations of up to 0.275 µg/ml NDMA in the feces-urine mixture of operated rats as well as the formation of 0.15 µg/ml NDMA during in vitro incubation of rat urine and feces.

Our investigations on ureterosigmoidostomy patients show significant increases in the concentrations of nitrite and N-nitroso compounds, a significant decrease in nitrate and the presence of a mixed bacterial flora of nitrate-reducing bacteria in feces-urine mixtures compared to normal urine samples from control volunteers providing additional support for Stewart's nitrosamine

theory for carcinogenesis following ureterosigmoidostomy [36, 38]. Our results for investigations using experimental animals do not agree with the previously published results of Cohen et al. [4, 40]. The analysis of N-nitroso compounds in feces and biological samples has been a contentious area of research prone to problems arising from artefact nitrosamine formation and sample contamination [1]. In early investigations on human feces, Wang et al. [47] reported both NDMA and N-nitrosodiethylamine (NDEA) to be present in normal human feces, a finding which could not be validated by several other working groups [1, 12, 23] who suggested that the analytical method used resulted in artefactual nitrosamine formation. We have used established analytical methods to stabilize biological samples (the addition of sodium hydroxide as well as 2-(ethylmercurymercapto)benzoic acid as an antibacterial agent) and prevent artefact nitrosamine formation in addition to using a marker amine (morpholine) to check for artefact formation during sample collection and analysis. Analysis was also performed using a N-nitroso compound-specific detector (Thermal Energy Analyser). In the previously reported results by Cohen et al. [4] and Stribling et al. [40], feces-urine mixtures were not collected using suitable methods to prevent artefactual nitrosamine formation and a non-specific detection method was also used for the analysis of N-nitroso compounds. These differences in analytical methodology may well explain the contradictory results obtained in the two studies.

The absence of an increased excretion of nitrite and N-nitroso compounds in feces-urine mixtures from rats with ureterosigmoidostomy in contrast to humans with ureterosigmoidostomy may be due to the fact that nitrite as well as N-nitroso compounds (if formed endogenously) in the rat are almost totally absorbed in the rectosigmoid. In addition to which, nitrate and nitrite are metabolized by intestinal bacteria present in the rat [32, 34, 49–52]. In ureterosigmoidostomy patients, differences in the diet and availability of endogenous amines, as well as differences in the absorbtion characteristics of the rectosigmoid, may be responsible for the increased excretion of nitrate, nitrite and N-nitroso compounds in rectal urine samples as compared to the situation found in experimental animals.

An alternative explanation is that endogenous nitrosation does not occur in the rectosigmoid of rats with ureterosigmoidostomy. This theory is supported by both Lee's [23] in vitro and Mallet's [24, 25] in vivo experiments showing that nitrosation of amines by nitrite is not supported by an anaerobic intestinal bacterial flora. Nevertheless, adenocarcinomas of the colon at the urointestinal anastomosis in the rat model of Crissey et al. [5] and Gittes et al. [14] occur in both our experiments (unpublished results) and in the experiments of other authors [4–6, 14]. Failure to detect an increased level of endogenous nitrosamine formation in the rat rectosigmoid indicates that other factors may influence colon carcinogenesis following ureterosigmoidostomy such as the presence of fresh colonic sutures or instability of the borderline between colon epithelium and urothelium [14].

In conclusion, factors other than the endogenous formation of N-nitrosamines in the human rectosigmoid seem to contribute to the induction of colon carcinomas following urosigmoidostomy. If this were not the case, the development of carcinomas only at the ureterocolonic anastomosis [6, 13, 14, 18, 21, 29, 35–40], and the protective effects of either an initial colostomy or the inter-position of ileum between urothelium and colon epithelium [14] could not be explained. Whilst the N-nitrosamine theory [36, 38] cannot be rejected and experimental evidence in human studies clearly show the increased endogenous formation of N-nitroso compounds, none of the detected nitrosamines found in feces-urine mixtures from both humans and rats has been shown to induce colon carcinomas in experimental animals [9, 30].

With regard to the excellent urological long-term results and the psychosocial advantages of ureterosigmoidostomy, especially in children and in Third World countries, further investigations concerning the carcinogenesis and prophylaxis of carcinomas following ureterosigmoidostomy in comparison to other methods for urinary diversion are necessary.

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