conclude that the "physiological" permeability barrier resides within the alpha (probably including the mesos) layer, and while it was thought that the beta layer could play some role in reducing integument permeability, its function was interpreted as primarily mechanical. Their conclusions are compatible with our findings, since the mesos layer was disturbed during the cellophane stripping (*12*).

Since lipids are known to determine epidermal permeability in numerous terrestrial organisms, including certain amphibians (*13*), it is surprising that lipids were not investigated previously in relation to limiting water exchange in reptiles. It has been known since the 1950's that extracting lipids with organic solvents increased the permeability of mammalian skin (*14*), but this information has been overlooked by comparative physiologists (*15*). In mammals, the function of the epidermal permeability barrier apparently depends on intercellular lipids derived from epidermal lamellar bodies (Odland bodies, membrane-coating granules) (*16*). Similar bodies were found in the reptilian mesos layer, but their function remains to be clarified (*17*).

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6. Both epidermis and nylon net (2 by 3 mm mesh) were secured to the top of the container (volume, 2.4 ml) by a paraffin seal at the edges and were trimmed to conform to the container's circumference (4.24 cm). The water-saturated wick was separated from the epidermis by a small air space. Containers were weighed every 4 hours, and rates of water loss were computed from data for intervals during which rates of loss were linear, indicating the establishment of steady-state kinetics.
7. The NaOH reduces sulfide bonds; the urea splits hydrogen bonds. See H. D. Onken and C. A. Moyer, *Arch. Dermatol.* **87**, 584 (1963); A. G. Matoltsy, A. M. Downes, T. M. Sweeney, *J. Invest. Dermatol.* **50**, 19 (1968).
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Rat Model for Carcinogenesis in Ureterosigmoidostomy

Abstract. *A rat model is used to study the carcinogenesis that occurs when urine is surgically diverted into the fecal stream, as in ureterosigmoidostomy. Adenocarcinoma of the colon occurs adjacent to the urine inlet. It is completely prevented by proximal diversion of the feces, implying that fecal carcinogens are activated locally by the urine or the urothelium.*

Adenocarcinoma of the colon mucosa is a recognized complication of ureterosigmoidostomy, an operation used for many years to make up for the loss of the bladder in cancer surgery, extrophy of the bladder, bladder incontinence, and other conditions. The tumor, which develops adjacent to the junction of the ureter with the bowel, occurs 500 times as often as in the population at large and, in children so operated, 7000 times as often as in all persons under age 25. The latency period is 5 to 50 years (*1*).

In the past two decades, many thousands of children have had an isolated segment of sigmoid colon (a colon "conduit") interposed between their ureters and a cutaneous stoma, thus dispensing with the need for their abnormal urinary bladder. It is not known whether adenocarcinoma will appear in this large group, because the etiological mechanisms behind carcinogenesis in ureterosigmoidostomized patients have not been investigated. We used a rat model to test our own hypothesis of the etiological factors involved in this continuing problem.

Adenocarcinoma of the bladder is the most common kind of tumor developed in patients with extrophy of the bladder, a rare congenital malformation in which microscopic islands of colonic epithelium are present on the urothelial surface of the urine-bathed bladder (*2*). We hypothesized that colonic epithelium is especially susceptible to urine-borne carcinogens, and therefore planned long-term experiments in which rats were treated with carcinogens active in either the urinary tract or the gastrointestinal tract. The variables were considered to be urine, feces, and the two different epithelia. We joined the urinary stream to the colon as in a ureterosigmoidostomy or excluded the feces from the urine-bathed distal colon segment by a proximal colostomy (Fig. 1). Our results clearly indicate that a rat model is adequate for the study of carcinogenesis in ureterosigmoidostomy (Table 1). Adenocarcinoma of the colon appeared adjacent to the junction of the epithelia where the urine joined the fecal stream. And, contrary to our hypothesis, the colonic mucosa did not prove to be particularly susceptible to urine-borne carcinogens. No adenocarcinoma appeared at the bladder-colon junction unless feces were flowing by.

To simulate ureterosigmoidostomy in rats, we divided the urethra and both vasa to free the bladder base with its attached ureters and lateral vascular pedicles. We resected most of the dome of the bladder and used a running 7-0 suture of polyglycolic acid to join the remaining patch to an opening cut in the anterior surface of the rectal wall. To exclude the feces and simulate a colon conduit, the

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Table 1. Incidence of colonic tumors in rats surviving surgery or treatment with carcinogens.

Carcinogen	Operation and tumor site				
	None	Ureterosigmoidostomy		Colostomy and "rectal bladder"	
		Bladder-bowel junction	Distant bowel	Bladder-bowel junction	Distant bowel
None	0/38	4/6	0/6	0/11	0/11
DMH	10/40	3/5	0/5	0/10	4/10
FANFT*	0/38	1/1	0/1	0/6	0/6
DMH and FANFT*	25/35	4/5	1/5	0/4	0/4
Total	35/151	12/17	1/17	0/31	4/31

*These carcinogens caused transitional cell tumors of the bladder in 16 of 89 rats.

same dissection was done but the descending colon was transected, the proximal segment joined to the abdominal skin, and the ureterovesical patch sewn onto the rectal stump with the same suture. The urine then drained through this rectal bladder. Control surgical groups received transabdominal bilateral vasectomy only.

Male Wistar-Furth rats (N = 320) were assigned to the three surgery groups and to three carcinogen groups: N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), a urinary tract carcinogen; dimethylhydrazine (DMH), a bowel carcinogen; or both carcinogens. Control groups received no carcinogen. The FANFT was added to powdered food (0.2 percent) and fed for 16 weeks (*3*). The DMH was injected once weekly (20 mg, subcutaneously) for 16 weeks (*4*). There were heavy losses from the surgical groups during the first few months—not directly from the surgery but from urinary obstruction, pyelonephritis, and perianal ulcerations. Rats surviving carcinogen treatments (16 weeks) were autopsied if they then died, and all the remaining survivors were killed and autopsied 33 weeks after completion of the treatments. The gastrointestinal tract and bladder were examined in formalin-fixed specimens with a dissecting microscope, and all gross tumors and suspicious areas of mucosa were examined histologically.

As Table 1 shows, few rats survived for autopsy in most of the surgical groups. But there were large exophytic tumors of the bowel mucosa at the junction of the bladder patch and the colon in 12 of the rats with the simulated ureterosigmoidostomy. These tumors resembled the tumors found at the junction of the two streams in ureterosigmoidostomized humans, and were distinctly different from the bowel polyps that occurred randomly in parts of the lumen of the large bowel in rats treated with DMH. Indeed, histologic examination was necessary to show that the epithelium involved in the tumor formation at the junction of the two epithelia was intestinal. The junctional tumors were found 8 to 11 months after surgery.

In contrast, no tumors of the junctional colonic epithelium developed when feces were excluded from the junction. When these "rectal bladder" rats were treated with DMH, 4 of 10 developed polypoid tumors of the colonic mucosa, presumably because of the excretion of DMH in the urine. These tumors were distant from the junction and similar to those seen in the intact animals. An incidental finding was an unexpectedly high incidence of bowel tumors in intact animals with both FANFT and DMH.

We hope that other investigators will use this model as they work toward a solution of the vexing clinical problem of colonic cancer in ureterosigmoidostomized patients. Some cases have been reported to occur years after the urine was rerouted away from the colon because of complicating ascending infections. The ureteral stump was left attached to the colonic mucosa, from which tumors arose. This suggested to some that chronic irritation from the old suture line might be a factor (*5*).

Since a fecal stream is necessary for the local carcinogenesis, it is possible that a nonuriniferous junction of transitional epithelium and colonic epithelium exposed to the fecal stream might be similarly carcinogenic. This alternative has not yet been investigated but is technically feasible. Our current hypothesis is that hydrolytic enzymes in the urine activate conjugated carcinogens in the stool and are active at the junction of the two streams, where they are both in the greatest concentration. The possibility of a local activator produced by the urothelium or of a mechanical (irritation) effect alone seems less likely but deserves further investigation. Clearly, the model suggests that the thousands of children with colon conduits do not have an increased risk of developing adenocarcinoma of their colonic mucosa.

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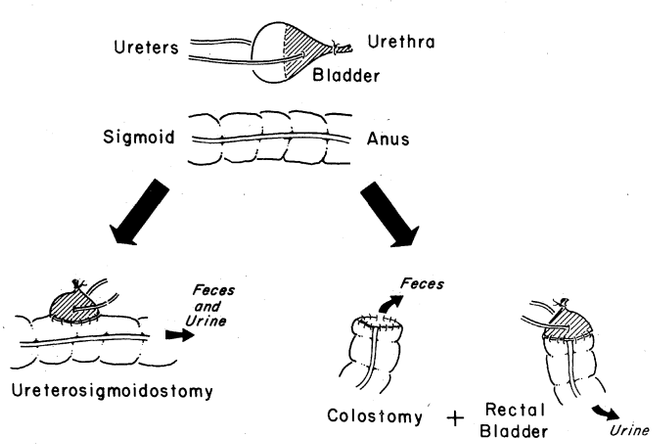

Fig. 1. Schematic representation of the diversion of urine to the large bowel, with or without proximal diversion of the feces. The base of the bladder is kept in continuity with the ureters, and its blood supply from the lateral pedicles is maintained after the urethra is divided and the dome of the bladder resected. The bladder patch is rotated 90° and attached to the rectosigmoid passing behind it. No special preparation of the colon of the animal is needed. The proximal colostomy converts the diversion into an exact analog of that used for "rectal bladder" urinary diversion, but is also a reasonable analog for the colon conduit used in children. These procedures are best done with the aid of low magnification, and require careful technique to avoid fistula and ureteral obstruction.

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