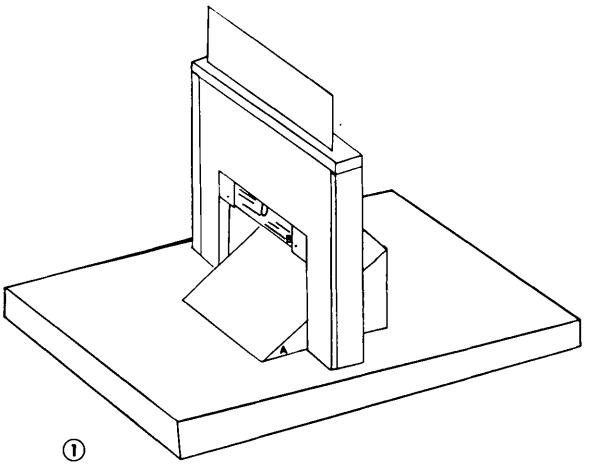

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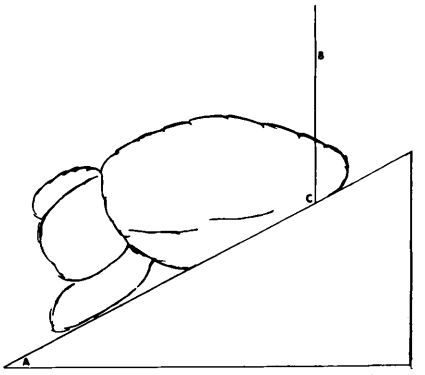

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FIG. 1. A- Wooden block cut at an angle of 28°. B- Blade perpendicular to the base of the instrument.

FIG. 2. A and B- as in Fig. 1. C- Brain being transversely cut to present an anterior section slanting posteriorly and forming an angle of 118° with its ventral surface.

by the technician, and the rapid adjustment will increase his efficiency. If a great deal of work is involved the utility of this device is evident.

The drawing skill of Robert Marier and the technical assistance of Paul Ricard are gratefully acknowledged.

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Stabilization of Urinary 3-Hydroxyanthranilic Acid by Oral Administration of L-Ascorbic Acid.* (32547)

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Certain metabolites of dietary L-tryptophan produce uro-epithelial tumors when implanted into urinary bladders of mice(1). One such metabolite is 3-hydroxyanthranilic acid (3-HOA). This metabolite or a derivative thereof, if present in urine in sufficient concentration, could possibly produce tumors of the urinary bladder in man.

Therefore, data concerning true quantities of 3-HOA excreted in urine of tumor patients may be important in studying the etiology of tumors of the urinary bladder; yet, obtaining such data is complicated or prevented perhaps because of instability of 3-HOA in some urines. Data obtained in our laboratory(2,3) show that 3-HOA is unstable under certain simulated physiologic conditions, and is oxidatively decomposed in some samples of urine during incubation at 37°C for six hours. Since urine ordinarily remains in the bladder for at least six hours (overnight), and since voided samples of it are not always promptly analyzed for 3-HOA, these data suggest that amounts of 3-HOA found in some samples of voided urine are not necessarily a reliable index of true quantities formed, but rather may be the amounts remaining after decomposition of this metabo-

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lite in the interim between formation and analysis.

Consequently, some method for stabilization of urinary 3-HOA in such interim may be important to allow adequate estimation of its presence. Since we have observed, *in vitro,* that 3-HOA is stable in aqueous solutions containing L-ascorbate, we have investigated the possibility of use of this substance as an anti-oxidant for stabilization of urinary 3-HOA.

Methods. A consecutive series of 4 overnight (midnight to 7:00 A.M.) specimens of urine was collected from each of 5 tumor patients. After collection of the second specimen, each patient was given, orally, L-ascorbic acid, 500 mg, 3 times each day for 2 days to cause spillage into the urine of ascorbate during collection of the last 2 specimens. Concentration of ascorbate in each urine specimen was determined by the method of Hughes(4).

Since urine containing 3-HOA may remain in the bladder for periods of at least 6 hours (overnight), percentage recovery of 3-HOA added to urine samples was determined after this time period. A known amount (300/μg) of 3-HOA was added to 20 ml of urine in duplicate (Samples A & B) and allowed to incubate together with duplicate control samples (no 3-HOA added in samples C & D) for 6 hours at 37°C in air. After the incubation period, all samples of urine were analyzed for amounts of 3-HOA by the method of Michael *et al*(5). The mean amount of 3-HOA found in control samples C & D was subtracted from that found in samples A & B to correct for endogenous 3-HOA before calculation of percentage recovery of added 3-HOA. Results are expressed as percentages of 3-HOA remaining after 6 hours.

Results. A total of 20 urine samples from 5 tumor patients were analyzed for percentage recovery of added 3-HOA. Fig. 1 shows the mean percentage recovery of added 3-HOA from these urine samples together with mean urinary ascorbate concentrations in specimens 1-4. In specimens 1 and 2, collected before ascorbate administration, mean percentage recovery of added 3-HOA was about 50%, and the mean urinary ascorbate concentration relatively low, about 0.1 mg%. In specimens 3 and 4, collected during ascorbate administration, percentage recovery of added 3-HOA was about 100% and urinary ascorbate levels were elevated as expected.

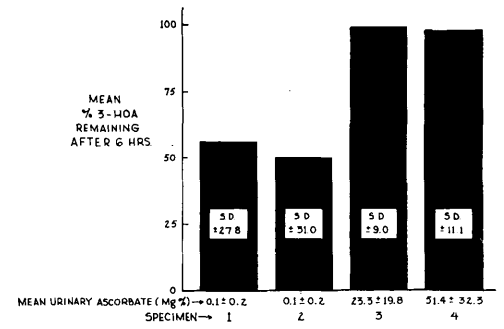

FIG. 1. Effect of elevated levels of urinary ascorbate on stability of 3-hydroxyanthranilic acid in urine from patients with tumors of the urinary bladder.

An analysis of variance was performed using a random complete block design where the blocks were the individual patients(6). The analysis indicated that percentage recovery of added 3-HOA in urine specimens 1 and 2 differed significantly from those in urine specimens 3 and 4 at the 0.01 level. There was no significant difference in recovery between specimens 1 and 2 or between specimens 3 and 4.

Discussion. The data from these experiments show that significant amounts of 3-HOA can decompose in urine samples of some tumor patients if such samples are allowed to incubate at 37°C for 6 hours, and that the presence of high levels of L-ascorbate in such samples inhibits this decomposition. The results suggest that significant amounts of urinary 3-HOA may decompose during its retention in the urinary bladder, and that such decomposition can be prevented by the oral administration of L-ascorbic acid.

Some investigators(7,8) have found high urinary levels of 3-HOA in patients with tumors of the bladder; yet others(9) observed lower urinary levels of 3-HOA in some of their patients than in normal subjects. Stabilization of urinary 3-HOA by oral administration of L-ascorbic acid before collection of urine specimens perhaps would give more reliable data concerning the urinary excre-

tion of 3-HOA by patients with tumors of the bladder.

Summary. A method is presented for the stabilization of urinary 3-hydroxyanthranilic acid (3-HOA) to enable the acquisition of more reliable data concerning the urinary excretion of 3-HOA by patients with tumors of the urinary bladder. The data show that significant amounts of 3-HOA can decompose in urine samples of some tumor patients if such samples are allowed to incubate at 37°C for 6 hours, and that the presence of high levels of L-ascorbate in such samples inhibits this decomposition.

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Differences in Colonic Staining with Technical Variations in Fluorescent Antibody Conjugation.* (32548)

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Immunologic mechanisms have been implicated in the course of ulcerative colitis(1-3). However, there have been conflicting reports regarding the demonstration of circulating antibodies to colonic mucosa in patients with this disease(4,5). Application of immunohistochemical techniques has led to emphasis on the cytoplasm of the colonic mucosal epithelial cell as the site of an antigen to which serum globulins from certain ulcerative colitis patients bind(6-9). On the other hand, some investigators have been unable to demonstrate a specific colonic mucosal epithelial antigen, using sera from ulcerative colitis patients (2,4,5). To understand better the possible reasons for these discrepancies, the present studies were designed to evaluate several variables involved in the direct fluorescent antibody technique and to demonstrate factors which may result in nonimmunospecific staining of the colonic mucosa. Careful attention was directed to the uniformity of labeling, fluorescein:protein ratios and final protein concentrations of globulin conjugates.

Materials and methods. The fluorescent antibody conjugation and staining methods used were essentially those of Goldstein *et al* (10,11), as previously utilized in this laboratory(5,12). The experimental design is shown in Fig. 1. Globulin fractions of whole serum were obtained by precipitation with cold saturated ammonium sulfate and gel filtration with a Sephadex G-50 column. Pressure dialysis was employed to adjust globulin fractions to a concentration of 1.25% before the conjugation procedure, and two 8 ml (100 mg) globulin preparations of a given serum separately were conjugated with fluorescein. One 100 mg globulin preparation was con-

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