turate toxicity, body weight, and cardiac lipofuscin concentration (3). Aging appears to be associated with diminished control of reactivity, or increasing randomization.

The decreased dye concentrations in the old animals could be due to tissue alterations at several levels; there might be decreased capillary permeability *per se,* a decreased polysaccharide/collagen ratio might result in less tissue hydration and less of a fluid phase in which diffusion could occur, or more densely cross-linked collagen might provide a barrier to diffusion. Decreased passage of dye might also be the consequence of diminished capillary and tissue mobility because of increased collagen cross-linking.

If such age-related decreases in permeability are found to occur throughout the body, it would be possible to explain how most of the homeostatic mechanisms and physiologic processes decline in efficiency with increasing age.

Summary. Passage of an intravenous dye into the skin of mature and old rats was measured following the intradermal injection of histamine. Less dye accumulated in the skin of old rats. This alteration in permeability may represent the mechanism by which increased cross-linking of collagen results in diminished physiologic reactions.

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Inhibitory Effect of L-Ascorbate on Tumor Formation in Urinary Bladders Implanted with 3-Hydroxyanthranilic Acid* (33916)

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Previous reports (1–3) have shown the following: (a) 3-hydroxyanthranilic acid (3-HOA) is unstable under certain simulated physiologic conditions and oxidatively decomposes, (b) 3-HOA is frequently unstable in urine, especially in urine of some tumor patients, and (c) 3-HOA in urine can be stabilized *in vitro* and perhaps *in vivo* by the presence of high levels of L-ascorbate, an anti oxidant.

Since 3-HOA or perhaps an oxidative product(s) produces uroepithelial tumors when implanted into mice bladders (4, 5) and also is a suspected bladder carcinogen in man (6), this experiment was done to investigate the effect of elevated urinary levels of ascorbate in mouse urine on the carcinogenicity of 3-HOA implanted into mouse bladders.

Methods. A 2 × 2 factorial experiment was designed to test the interaction of ascorbate and 3-HOA on: (a) survival of mice, (b) number of malignant tumors induced, and (c) the total number of tumors induced.

Cholesterol pellets containing 3-HOA were prepared as described by Bryan *et al.* (7).

Swiss albino female mice (60–120 days old) were anesthesized with pentobarbital and ether. Pellets were then inserted into bladders of four groups of mice (A, B, C, D) by the technique of Jull (8) as modified by Allen *et al.* (4). Two groups (A and B) received pellets of cholesterol alone, and two groups (C and D) received cholesterol pellets containing 3-HOA. The total number of mice in each group treated originally and the number that survived 40 weeks after surgery

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TABLE I. Data Summary of the Effects of Ascorbic Acid and 3-Hydroxyanthranilic Acid and Their Interactions on the Development of Bladder Tumors in Mice.

Group no.	Chemical implanted in cholesterol pellets into mouse bladders	Ascorbate fed in drinking water (mg/100 ml)	No. of mice treated originally	No. of mice which survived 40 weeks	Tumors observed in 40 weeks		
					Pre-malignant	Malig-nant	Total
A	Cholesterol alone	None	58	49	3	2	5
B	Cholesterol alone	250	33	23	1	2	3
C	3-HOA[a]	None	70	46	1	8	9
D	3-HOA	250	51	37	1	2	3

[a] 3-Hydroxyanthranilic acid.

is shown in Table I.

After surgery the mice were returned to their cages and fed Purina mice pellets *ad libitum*. Mice in groups B and D only were fed L-ascorbate (250 mg/100 ml) in their drinking water *ad libitum*. Any further increase in concentration of ascorbate in the drinking water offered to groups B and D would have caused a decreased daily intake of water in groups B and D compared to that in groups A and C.

To evaluate the effects of the feeding of L-ascorbate in drinking water upon the urinary ascorbate level, two groups of mice were studied purely for the purpose of evaluating urinary ascorbate levels. One group was fed L-ascorbate (250 mg/100 ml) in their drinking water *ad libitum* and the other group was kept on the same food and was fed drinking water *ad libitum* but with no addition of L-ascorbate. Urinalysis (9) from these two groups of mice indicated that ascorbate concentration in the urine of the group receiving ascorbate in their drinking water was approximately 400 mg/100 ml as compared to 16 mg/100 ml in the group not receiving ascorbate. On the basis of this experiment groups of mice (B and D) that received ascorbic acid in the drinking water should have had a significantly higher ascorbate level in the urine than the groups (A and C) that did not receive ascorbate.

All surviving mice were killed at the end of 40 weeks after surgery. They were examined postmortem in a manner previously described by Jull (8). The bladders were distended by the injection of Bouin's solution and fixed for 24 hr before they were cut open and examined with a hand lens. Selected material from each mouse bladder was then removed for sectioning and microscopic examination. Tumors found were classified as malignant or premalignant, the malignant tumors showing frank invasion into submucosa and muscle.

Results. A summary of the data of the experiment is presented in Table I. These data were adjusted for initial distribution and treated statistically by a chi-square analysis. In some instances a Z test was made (10).

Prior to testing the interaction of 3-HOA and ascorbate and their effect on the number of malignant tumors induced and the total number of tumors induced, it was necessary to determine whether or not the probability of survival was the same for all four experimental groups. The results of an analysis of information (11) concerning survival is reported in Table II. The analysis showed that the number of mice surviving the surgical procedure was independent of the presence or absence of ascorbic acid or 3-HOA, and that

TABLE II. Analysis of Information on Survival of Mice Implanted with 3-Hydroxyanthranilic Acid and Fed Ascorbate.

Source	*df*	*I*[b]	*p*
Vitamin C	1	0.20	ns[d]
3-HOA[a]	1	2.98	ns
Vitamin C + 3-HOA[c]	1	0.742	ns

[a] 3-Hydroxyanthranilic acid.
[b] Information.
[c] Adjusted for initial distribution.
[d] Nonsignificant.

there was no interaction of these two factors in altering the probability of survival of the mice.

A chi-square test (after adjustment for initial distribution) of the interaction between 3-HOA and ascorbate in affecting the number of malignant tumors induced was significant ($0.05<p<0.10$). It was decided to test the equality of the proportion of mice with malignant tumors that received 3-HOA and the proportion of mice with malignant tumors that received 3-HOA and ascorbic acid using a Z test (10). The test was significant at the 0.05 level. One may conclude that urinary ascorbate inhibited the expression of the anticipated carcinogenic effect of 3-HOA. A similar analysis (chi-square) was done on the total number of tumors induced. There was no significant interaction between 3-HOA and ascorbate. However, in view of the results of the preceding analysis, it was decided to test the equality of the proportion of mice with total tumors that received 3-HOA and the proportion of mice with total tumors that received 3-HOA and ascorbic acid with a Z test. The results of this test indicates that elevated urinary levels of ascorbate significantly ($p<0.07$) inhibited the expression of the anticipated carcinogenic effect of 3-HOA.

Discussion. From these data one may conclude that the probability of survival was the same for all four experimental groups and that ascorbic acid inhibited the carcinogenic effect of 3-HOA reported by Allen *et al.* (4) and Bryan *et al.* (5).

By what mechanism(s) ascorbate inhibits the carcinogenic effect of 3-HOA on mice bladders is of interest. Since 3-HOA is easily oxidized in urine but is stabilized by high levels of urinary ascorbate in such urine, one hypothesis to explain such mechanism is that urinary ascorbate simply acts as an antioxidant on urinary 3-HOA *in vivo* and prevents its oxidative decomposition as postulated by Schlegel *et al.* (12). If this hypothesis is true, urinary 3-HOA is not the primary bladder carcinogen, but rather some oxidative product(s) of 3-HOA.

Experiments are underway in this laboratory to test the inhibitory effect of ascorbate on the carcinogenicity to mice bladders of some of the other carcinogenic tryptophan metabolites.

Summary. A 2×2 factorial experiment was done to test the interaction of ascorbic acid and 3-hydroxyanthranilic acid on the development of malignant and total tumors in the urinary bladders of mice. An analysis of the data showed that the probability of survival was the same in all groups of mice and that ascorbic acid inhibited the anticipated carcinogenic effect of 3-hydroxyanthranilic acid.

Since the evaluation of the two groups of mice receiving 3-hydroxyanthranilic acid pellet implantation with and without oral administration of vitamin C was of utmost importance in our final evaluation of our results, we asked for an authoritative and unbiased opinion, and Dr. Charles E. Dunlap, M.D., Professor and Chairman, Department of Pathology, Tulane University School of Medicine, most graciously consented to review all of the slides. We gratefully acknowledge Dr. Dunlap's cooperation. The authors, also, gratefully acknowledge the assistance of M. Clinton Miller, Ph.D., Department of Biostatistics, Tulane University School of Medicine in the statistical analysis of data.

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