Age Variation in Rat Skin Permeability* (33915)

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Alteration in permeability has been postulated to be the most important underlying defect in mammalian aging, and the one responsible for many of the diverse debilities in older animals. Permeability changes have also been invoked to relate the debilities of age to the age-related cross-linking of collagen (1).

Very few studies have dealt directly with age changes in permeability *in vivo*. It has been reported that cartilage permeability decreases with age, and that intradermally injected fluid is absorbed much more slowly from the skin of old human beings than from young (2). The present study was undertaken to ascertain if there is an age difference in skin permeability to an intravascular dye, Evans blue, following intradermal histamine injection.

Methods. Female rats of the CD*F strain, descendents of pathogen-free animals obtained from The Charles River Breeding Laboratories were used. Preliminary studies of intravascular concentrations of Evans blue, and hematocrits, determined by periodic blood sampling from the orbital sinus indicated no detectable age differences in blood volume or hematocrit. Eight mature rats, 10–13 months old, and eight old rats, 23–28 months of age were studied. They were anesthetized with ether, and injected with 2 mg of Evans blue/100 g of body weight, in 1.0 ml of 0.15 *M* NaCl, by way of the saphenous vein. Ten min later, they were injected intradermally at four abdominal sites with 100 μg of histamine in 0.05 ml of 0.15 *M* NaCl. Thirty min after histamine injection the rats were sacrificed by a blow on the head.

The shaved abdominal skins were excised and pinned down. Each of the four injection sites and two control sites in each skin were excised with a 12-mm cork borer. Each sample was weighed, and then macerated and gelatinized by heating in 1.0 ml of 0.1 *N* NaOH in a boiling-water bath 20 min. The mixture was cooled, and neutralized with 0.1 ml of 1.0 *N* HCl. Pyridine, 0.4 ml, was added, and the mixture was heated at 70° for 40 min. The mixtures were centrifuged, and the supernatant solutions were mixed with 1.5 ml of ether. After separation, absorbance of the clear lower layer was read at 600 mμ. Evans blue standards were run through the procedure along with the skin samples. Absorbance per gram of skin for control samples was substracted from values for the injection sites. From standard values the amount of Evans blue per gram of skin was calculated. An average of the four injection sites was determined for each animal.

TABLE I. Evans Blue in Skin of Rats Following Histamine Injection.

Rat age (months)	Dye conc (μg/g of skin)[a]
10	76
10	85
10	125
10	84
12	53
13	78
13	99
13	93
23	69
23	56
24	58
24	35
24	117
24	83
25	10
28	30

[a] $p = .05$ for difference between mature and old.

Results. More dye passed into the skin of mature animals than old animals. A marked variability in values for old animals was observed (Table I).

Discussion. The variability in values for the old group is consistent with other studies showing an increasing variability with age in such factors as sensitivity to radiation, barbi-

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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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