

The Influence of Dihydrostreptomycin upon Avillous Hyperplasia and Duodenal Ulceration in the Pantothenate-Deficient Mouse (34296)

JOSEPH SERONDE, JR.
(Introduced by Lois M. Zucker)

Harriet G. Bird Memorial Laboratory, Stow, Massachusetts 01775

Focal avillous hyperplasia of the duodenal mucosa occurs in Princeton mice fed a stock diet composed of natural foodstuffs. On this regimen, the lesions are small and infrequent, but their incidence and average size increases if the animals are fed a complete, chemically defined diet; and a more marked increase occurs if pantothenic acid is withheld (1). Lesions are composed of closely packed, overgrown Crypts of Lieberkühn, and appear grossly as flat, thickened plaques, located principally on the dorsal and ventral surface of the mucosa, just distal to the pyloric valve. Chronic duodenal ulcers also occur in pantothenic acid deficient Princeton mice (2), and seem invariably to be located upon the preexisting lesions of avillous hyperplasia.

The incidence and severity of avillous hyperplasia is greater in mice housed in pans filled with pine shavings than in those kept in suspended mesh-bottomed cages. This difference is especially clear-cut if the animals are fed a natural foodstuffs diet ($p < 0.001$). When they are fed a complete chemically defined diet, the significance diminishes ($p = \text{slightly} < 0.025$). Control of coprophagy is the usual reason for caging rodents on wire mesh, and is important in assuring the proper expression of many experimental vitamin deficiencies. However, in the present instance, our findings with mesh-bottomed cages suggest an improvement in general hygiene, perhaps through a change in quantity and character of bacteria in the animals' environment. Preliminary experiments to test this supposition showed oxytetracycline and penicillin to have no effect upon avillous hyperplasia; but dihydrostreptomycin gave rise to the following results.

Materials and Methods. Princeton mice (2) were used exclusively. The complete, chemically defined diet (designated 2520 in Table I) was composed, in grams percent, of vitamin "free" casein, 18.0; glucose, 71.0; modified (3) Wesson salt mixture, 3.7; cotton seed oil (containing 1500 IU of vitamin A as carotene; 100 U of vitamin D₃; and 5 mg of alpha tocopheryl acetate), 5.0; 1-cystine, 0.20; cysteine-HCl, 0.10 as antioxidant; and cellulose flour, 2.0 as a vehicle for the vitamin supplement whose composition in milligrams per 100 g of diet follows: thiamine-HCl, 1.0; pyridoxine-HCl, 1.0; riboflavin, 2.0; niacin, 4.0; calcium pantothenate, 4.0; pteroylglutamic acid, 0.2; biotin, 0.02; B₁₂, 0.0012; inositol, 20; para-aminobenzoic acid, 10.0; choline chloride, 100.0. The pantothenic acid-deficient diet (designated 2517 in Table I) was identical to the above described diet, excepting for the omission of that vitamin.

All animals were fed *ad libitum*. They had access to the stock diet prior to complete weaning at 21 days, and were thereafter placed on experiment when males had attained a body weight of at least 14 g, and females 12 g, at about 4 weeks of age. At this time they were transferred from enameled pans containing pine shavings to wire-mesh-bottomed hanging cages. For the first week on experiment, the chemically defined diets were modified by mixing them with 25% of powdered stock diet (natural foodstuffs), to improve their acceptance, and to reduce the number of spontaneous casualties during the first few days. Such deaths could generally be attributed to starvation. Thereafter, the experimental diets were fed undiluted. Both sexes were used, and were caged separately, 5 or 6 to a cage (18 × 20 × 20 cm).

TABLE I. The Effect of Dihydrostreptomycin (DHSM) upon Incidence and Area of Focal Avillous Hyperplasia and Chronic Duodenal Ulcer.^a

		Focal avillous hyperplasia							Chronic duodenal ulcer incidence
		Incidence		Area					
Diet		(%)	(No.)	Mean	SE	D/F	<i>t</i>	<i>p</i>	
2520	—	80.0	(36/45)	3.20	0.479	58	0.204	>0.5	0
2520	DHSM	77.5	(25/31)	3.04	0.636				0
2517	—	100.0	(51/51)	11.82	0.184	108	8.961	<<0.001	51% (26/51)
2517	DHSM	97.0	(59/61)	5.02	0.691				$\chi^2 = 20.844$ 11% (7/61)

^a No. 2520 is the complete diet; 2517 is pantothenate-deficient.

In the first experiment, 31 mice were fed the complete, chemically defined diet, to which 0.01% of dihydrostreptomycin was added. Forty-five controls received the unmodified diet. In the second experiment, 61 mice were fed the pantothenate-deficient diet, containing the same concentration of dihydrostreptomycin, and 51 controls received the unmodified deficient diet.

After eating the undiluted diets for 4 weeks, animals were killed with ether, and the upper gastro-intestinal tracts were filled with 10% formalin at once. After approximately 10 min, stomachs and duodenum were removed, opened with an incision following the line of the greater curvature, and pinned out under additional formalin, in order to harden delicate mucosal structures sufficiently to withstand washing with running water. Lesions were then examined and measured under a dissecting microscope containing a micrometer eyepiece.

Results. Findings are presented in Table I, which shows that dihydrostreptomycin did not affect the incidence of focal avillous hyperplasia in either diet group. In animals fed the complete diet (2520), the antibiotic had likewise no effect upon the average area of lesions. However, in pantothenic acid deficiency (2517), dihydrostreptomycin reduced the average area of lesions by about one half, a finding calculated to be highly significant. The total absence of duodenal ulcers in ani-

mals receiving a complete diet is in accord with many previous observations of the phenomenon. Dihydrostreptomycin markedly reduced the incidence of duodenal ulcers in pantothenic acid-deficient mice, the control incidence being 51%, and the experimental incidence being 11%. The area of avillous hyperplasia lesions in pantothenate deficient mice receiving dihydrostreptomycin (5.02 mm²) was not significantly greater than that of mice fed the complete diet without antibiotic (3.20 mm²).

Conclusions and Summary. Dihydrostreptomycin lowered the incidence of chronic duodenal ulcers in pantothenate-deficient mice. It also reduced the average size of a lesion, focal avillous hyperplasia, thought to be precursive. These findings raise the possibility that an organism may participate in the pathogenesis of both lesions, since lowering of resistance to infection is a well-documented effect of pantothenic acid deficiency (4), and in rats may be a major cause of death (5).

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