Hexosamine and Hydroxyproline Contents of Tissues in Scurvy.* (26600)

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Although the formation of connective tissue is defective in scurvy(1,2) the role of ascorbic acid in the process is not clear. The possible metabolic defect in ascorbic acid deficiency might be the inability of the tissues to hydroxylate proline to form hydroxyproline(3) and incorporation of an "active" hydroxyproline into collagen(4). The view, however, is contradictory to the observation of Mitoma and Smith(5) that hydroxylation of proline is not decreased by L-ascorbic acid deficiency in guinea pigs. According to them L-ascorbic acid possibly participates in the maturation of fibroblasts and thus exerts an indirect effect on collagen synthesis. A deficiency of L-ascorbic acid in guinea pigs either interferes with the synthesis of new collagen or results in its destruction as it is produced(6). There is a decrease in the activity of oxidative enzymes(7-9) in scorbutic guinea pigs. The generalised lowered cellular metabolic activities might also result in defective production of matrix. Collagen which is a basic building material of connective tissue is a protein. Insulin is involved in biosynthesis of proteins(10). Scorbutic guinea pigs suffer from hypoinsulinism(11-13). It is therefore possible that insulin deficiency associated with scurvy might interfere with formation of collagen. Hexosamine and hydroxyproline which are respectively the principal constituents of the ground substance and the collagen fibers of connective tissues, therefore, were estimated in the different tissues of normal, scorbutic and insulin-treated scorbutic guinea pigs.

Experimental. Male guinea pigs weighing from 250 to 300 g were used. Selection of the animals, feeding them a scorbutogenic diet(14), separation of the animals into groups containing one normal, one scorbutic and one insulin-treated scorbutic guinea pig and injection of insulin into the animals intended for insulin treatment were the same as described previously(15). On the fourth week of the experiment all the animals were killed by decapitation and portions of liver, kidney, bone, cartilage, aorta, lung, skeletal muscle and skin were analyzed for hexosamine and hydroxyproline. The tissues were dried, defatted and hydrolyzed by the method of Blix(16). The hydrolysate was used for estimation of hexosamine(16) and hydroxyproline(17).

Results. The results are given in Tables I and II. Hexosamine content of aorta, kidney, skeletal muscle and bone was increased significantly in scorbutic guinea pigs. Lung, liver, cartilage and skin of the animals under different treatments did not show any change in hexosamine content. Prolonged insulin treatment of the scorbutic animals did not influence the hexosamine content of the tissues studied. The hydroxyproline content of the cartilage and kidney decreased significantly in the scorbutic guinea pigs. The other tissues studied did not show change from normal. Prolonged insulin treatment, however, not only enhanced the hydroxyproline of cartilage and kidney but also those of lung and skin.

Discussion. In a number of tissues of scorbutic guinea pigs hexosamine, which is the principal constituent of the ground substance of connective tissue, was significantly increased in comparison to normal controls. This might be due to increased biogenesis or decreased catabolism of hexosamine in scurvy. Insulin treatment of scorbutic animals did not alter the hexosamine content of tissues, evidently indicating that insulin had no role in the process. According to Dorfman and Schiller(18) insulin deficiency adversely affects the synthesis of mucopolysaccharides. This concept, however, may not be applicable to connective tissue metabolism in scorbutic guinea pigs. Ascorbic acid possibly plays a direct role in the process.

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TABLE I. Hexosamine Content of Tissues of Guinea Pigs. Values are g/100 g of dry tissue.

Tissue	Condition of animal		
	Normal	Scorbutic	Insulin-treated scorbutic
Aorta	.64 ±.045	1.14 ±.067	1.31 ±.11
Lung	.68 ±.03	.70 ±.02	.69 ±.03
Liver	.47 ±.02	.47 ±.06	.47 ±.03
Skin	.50 ±.03	.48 ±.04	.49 ±.03
Cartilage	1.81 ±.14	2.71 ±.20	2.86 ±.16
Kidney	.24 ±.007	.81 ±.06	.76 ±.04
Skeletal muscle	.34 ±.02	.66 ±.047	.62 ±.06
Bone	.39 ±.026	.67 ±.019	.67 ±.038

Values are mean ± stand. error. Sixteen animals in each group.

In a few tissues of the scorbutic animals hydroxyproline, which is the principal constituent of collagen, was decreased considerably and after prolonged insulin treatment of these animals, hydroxyproline content of the tissues was increased. The interference with collagen synthesis in some tissues, therefore, might be due to a lack of insulin which results from scurvy(11-13).

It is probable that the hydroxylation of proline for transformation of precollagen to mature collagen is dependent on insulin. In the relative insufficiency of the hormone in the scorbutic condition the mature collagen fibers are not properly formed, leading to disorganization of the connective tissue. The ground substance freed from the binding force of the proliferating strands of collagen fibers depolymerizes and the mucopolysaccharides eventually find their way into the circulation. This causes an increase in the serum level of mucoproteins in scorbutic guinea pigs as reported earlier(19).

TABLE II. Hydroxyproline Content of Tissues of Guinea Pigs. Values are g/100 g dry tissue.

Tissue	Condition of animal		
	Normal	Scorbutic	Insulin-treated scorbutic
Aorta	3.67 ±.18	3.79 ±.22	4.15 ±.13
Lung	1.35 ±.05	1.38 ±.04	2.29 ±.23
Liver	.36 ±.02	.38 ±.03	.42 ±.03
Skin	4.86 ±.19	4.83 ±.20	7.52 ±.23
Cartilage	2.89 ±.19	1.14 ±.13	2.90 ±.13
Kidney	.74 ±.03	.36 ±.03	.69 ±.04
Skeletal muscle	1.23 ±.18	1.29 ±.16	1.61 ±.24
Bone	2.28 ±.12	2.30 ±.13	2.29 ±.14

Values are mean ± stand. error. Sixteen animals in each group.

Summary. The hexosamine and hydroxyproline contents of aorta, lung, skin, kidney, skeletal muscle, bone and cartilage were determined in normal, scorbutic and insulin-treated scorbutic guinea pigs. In the scorbutic guinea pigs hexosamine content significantly increased in aorta, kidney, skeletal muscle and bone and no change was observed in lung, liver, cartilage and skin. Insulin treatment had no effect on these contents. Hydroxyproline content decreased significantly only in kidney and cartilage of scorbutic guinea pigs. Prolonged insulin treatment of the scorbutic animals produced a significant increase in the hydroxyproline contents of cartilage, kidney, lung and skin.

The possible implications of the results have been discussed.

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Incorporation of S^{35} from L-Methionine in Tumor-bearing and Protein-depleted Rats.* (26601)

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Data have been presented to demonstrate an increase in the specific activity of certain plasma and tissue proteins in protein-depleted or tumor-bearing animals given S^{35} methionine. For example, Garrow found an increased uptake of S^{35} from labelled methionine in plasma proteins of depleted dogs(1) and malnourished children(2). Waterlow (3) reported a greater incorporation of S^{35} in the viscera of protein-depleted rats. Allison *et al.*(4) demonstrated an increased specific activity of serum proteins following an injection of S^{35} methionine into tumor-bearing or protein-depleted dogs. These and other data suggest a shift in the dynamic state of protein metabolism in depleted or tumor-bearing animals that could involve a number of cellular structures. The following experiments were performed to study the incorporation of S^{35} from labelled methionine into cellular proteins of liver and tumor tissues and into serum protein fractions of control, protein-depleted and tumor-bearing rats.

Methods. Male Wistar rats, 100-150 g, were divided into 3 groups of 30 animals each and placed on a diet containing 18% of casein as previously described by Allison *et al.* (5). On the first day of experiment a suspension of a Walker 256 carcinosarcoma was transplanted into one group, while on the seventh day another group was started on a protein-free diet. All animals were sacrificed after 17 days of experimentation. On day 7 of the experiment 10 rats from each group were placed in metal metabolism cages and daily food, urine and fecal collections were made for the next 10 days. The nitrogen concentration of the sample was determined by the micro-kjeldahl method.

At ½, 1, 2, 4, 6 and 12 hours before autopsy, 5 rats in each group were injected intraperitoneally with 0.2 mg of S^{35} L-methionine per kg of body weight. All rats were anesthetized with sodium pentobarbital (50 mg/kg) and sacrificed by exsanguination. The labelled tissue proteins were extracted and purified by the method of Zamecnik *et al.* (6). Liver and tumor tissues were fractionated into various cellular fractions by differential centrifugation. The nuclei were prepared in non-aqueous medium by the method of Carruthers *et al.*(7), and the mitochondria, microsomes, and cell sap were separated by a modification of the method of Hogeboom *et al.*(8). Serum was separated electrophoretically into various fractions by paper electrophoresis and dyed with alcoholic bromphenol blue, while total serum protein was determined by the copper sulfate falling drop method. All radioactive samples were counted in a windowless proportional flow counter and corrected for background, self-absorption, radioactive decay, back-scattering, geometry and body weight of the animals.

Results. The data in Table I demonstrate that the tumor-bearing rats were in positive nitrogen balance. If it is assumed that tumor tissue was essentially a "nitrogen trap", (9) then subtracting the tumor nitrogen from the total balance leaves the carcass (body minus tumor) near nitrogen equilibrium. During the same time period, the protein-depleted animals were in marked negative balance. These data are in agreement with these of Allison *et al.*(4) who reported a cor-

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