Connective Tissue IX: Effects of Hypervitaminosis A upon Connective Tissue Chemistry.* (28989)

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Massive amounts of Vit. A have been shown by Fell and Mellanby(1) to effect the release of acid polysaccharides from cartilage *in vitro*. This effect of excess Vit. A has been explained by Lucy *et al*(2) to be a result of release of endogenous proteolytic enzymes from lysosomes. These enzymes degrade the protein-polysaccharide complex of the matrix of cartilage releasing the acid polysaccharides from the tissue(3).

To date the effect of hypervitaminosis A upon the soft connective tissue (dermis) of the whole animal has not been studied. This paper describes the effects of large amounts of this vitamin upon the chemistry of the skin *in vivo*.

Materials and methods. Five groups of 6 male Sprague-Dawley rats weighing 140-160 g each were used. One group of animals remained untreated as a control, the other groups received daily doses of 150,000 I.U. of Vit. A palmitate in corn oil (Nutritional Biochemical Co.) intraperitoneally. One group of 6 animals each was sacrificed after 1, 2, 3 and 4 days of Vit. A. The abdominal skin (about 3 g) from each sacrificed rat (including the controls) was shaven, removed and dissected free of adhering fat, fascia and muscle. Aliquots of each skin were hydrolyzed in 10 ml/g of 4 N HCl for 8½ hours at 100°C. The bulk of the tissue samples was pooled, aliquots removed for lipid determination, and the remaining tissue divided into 3 equal samples representing one group of animals from each day of sacrifice. These pools were then extracted sequentially in the cold with 0.15 M NaCl, 0.5 M NaCl and 0.5 M citrate buffer as has been described previously(4). These fractions have been shown to extract quantitatively the "ground substance," salt soluble collagens and acid soluble collagens respectively(4,5). Samples from each extract were hydrolyzed in HCl as described above, and the hydrolysates from both the whole skin and the various fractions were analyzed for their hexosamine (6), hydroxyproline(7) and nitrogen(4) contents. The unhydrolyzed extracts were analyzed for their fucose(8) and sialic acid(9) contents. Lipids were determined as described previously(10).

These results were expressed as the mean and standard deviation of triplicate analyses of triplicate extracts from each group of animals. Means differing by more than 2 standard deviations were found to be significantly different by the "t" test ($p<.05$). The standard deviation of each mean never exceeded 7% of that mean.

Results. The effects of daily Vit. A injection upon total dermal concentration of hexosamine, collagen (from hydroxyproline), cholesterol, phospholipid and triglyceride are shown in Table I. These results are expressed in mg/mmole of total dermal nitrogen to obviate variations in tissue water content. The data of this Table indicate that daily administration of massive doses of Vit. A palmitate were without effect upon total dermal concentrations of lipids and collagen. Total dermal concentration of hexosamine, however, was significantly reduced by administration of Vit. A.

The dermal concentrations of hexosamine, nitrogen, hydroxyproline, fucose and sialic acid soluble in isotonic saline of animals receiving various doses of Vit. A are expressed in Table II per mmole of total dermal nitrogen. These results indicate that concentration of hexosamine and hydroxyproline in the connective tissue was profoundly decreased with Vit. A administration. Moderate decreases of fucose and nitrogen in this fraction were also observed with repeated doses of Vit. A.

* Supported in part by a grant-in-aid from N.I.H., P.H.S., Bethesda, Md.

TABLE I. Total Dermal Lipid, Hexosamine and Collagen Contents in mg per mmole of Total Nitrogen of Skins from Rats Which Have Received Daily Doses of Vit A.

Dosage	Cholesterol	Phospholipid	Triglyceride	Hexosamine	Collagen†
0	.80	2.0	12	.48	35.4
1	.80	1.8	14	*.32	37.3
2	.81	1.8	12	*.29	34.5
3	.78	1.6	14	*.29	35.0
4	.71	1.4	11	*.27	36.0

* Significantly different from 0 ($p < .05$).
† 1 μmole of hydroxyproline = 1 mg of collagen(5).

The hexosamine, nitrogen and hydroxyproline contents of the 0.5 M NaCl and citrate extracts of the connective tissue from animals receiving various doses of Vit. A are presented in Table III per mmole of total dermal nitrogen. These results demonstrate that hypervitaminosis A resulted in a marked decrease in hexosamine content of the saline extract. This decrease was paralleled by moderate decrease in the nitrogen and hydroxyproline content of this fraction with repeated doses of Vit. A. The primary effect of hypervitaminosis A upon the chemistry of the citrate extract was to increase profoundly the hydroxyproline concentration of this fraction.

Subtraction of the sum of soluble components from the total dermal concentrations of hexosamine, nitrogen and hydroxyproline permits the calculation of the insoluble concentration of these materials remaining in the tissue. These results, expressed per mmole of total dermal nitrogen are presented in Table IV. This Table indicates that there is essentially no change in concentration of insoluble hexosamine, nitrogen and hydroxyproline with Vit. A administration.

Discussion. Hypervitaminosis A has been demonstrated to be without effect upon the lipid composition of the skin. Since all results were expressed per mmole of total dermal nitrogen, these results could not be affected by variations in the water and fat content of the skin. It is obvious from these results that the primary action of hypervitaminosis A upon the chemistry of the connective tissue was to produce a marked decrease in the amounts of hexosamine containing materials within this tissue. Since the major sources of hexosamine in the connective tissue are glycoproteins and acid mucopolysaccharides, it is these two substances then which are most profoundly affected by hypervitaminosis A. Since one dose of Vit. A was sufficient to effect the loss of over 60% of the hexosamine in the "ground substance," while no significant decrease in nitrogen content of this fraction was observed, it is suggested that this represents the loss of acid mucopolysaccharides rather than glycoproteins from the tissue. With further doses of Vit. A, decreases in the fucose and nitrogen contents of the "ground substance" were also observed. Presumably this represents the loss of fuco-glycoprotein from the tissue with

TABLE II. Amount of Dermal Hexosamine (Hex), Hydroxyproline (Hypro), Fucose and Sialic Acid (in μMoles/mMole Total Nitrogen) and Nitrogen (as % of Total) Soluble in 0.15 M NaCl from the Skin of Rats Receiving Various Amounts of Vit. A.

Dosage	Hex	Nitrogen (%)	Hypro	Fucose	Sialate
0	1.84	13	1.93	.60	.05
1	* .77	11	*1.10	*.48	.04
2	* .62	12	* .74	*.34	.04
3	* .50	* 9.0	* .74	*.36	.05
4	* .39	* 8.1	* .27	*.27	.06

* Significantly different from 0 ($p < .05$).

TABLE III. Amount of Dermal Hexosamine (Hex) and Hydroxyproline (Hypro) in μMoles/mMole Total Nitrogen and Nitrogen Soluble (as % of Total) in 0.5 M NaCl and Citrate Buffer (pH 3.0) from the Skin of Rats Receiving Various Doses of Vit. A.

	Saline			Citrate		
Dosage	Hex	N	Hypro	Hex	N	Hypro
0	.73	12	3.45	0	.22	2.60
1	*.18	11	3.05	0	.22	*9.25
2	*.14	* 9.0	*2.72	0	*.16	*6.75
3	*.15	* 9.0	*2.30	0	*.10	*5.30
4	*.13	* 8.0	*1.62	0	*.16	*7.45

* Significantly different from 0 ($p < .05$).

TABLE IV. Insoluble Dermal Hexosamine (Hex) and Hydroxyproline (Hypro) in μMoles/mMole Total Nitrogen and Nitrogen as % of Total in the Skin of Rats Receiving Various Doses of Vit. A.

Dosage	Hex	Nitrogen	Hypro
0	.80	53	27
1	.79	57	24
2	.74	63	24
3	.76	62	24
4	.76	59	26

massive doses of Vit. A. Since the "ground substance" of rat skin contains only glucosamine and *no* galactosamine(11) the mucopolysaccharide disappearing from the "ground substance" must be hyaluronic acid and perhaps heparin. Similarly, decreases in concentration of hexosamine in the 0.5 M NaCl extract occurred initially without parallel losses of nitrogen and therefore presumably represent a loss of the mucopolysaccharide content of this fraction with Vit. A administration. Since this fraction has been shown to contain only galactosamine and no glucosamine(11), the mucopolysaccharides lost from this fraction presumably was chondroitin sulfate.

Associated with hypervitaminosis A was a marked increase in dermal concentration of citrate soluble hydroxyproline, or collagen. Despite this change in the concentration of soluble collagen, hypervitaminosis A was without significant effect upon the chemistry of the insoluble portion of the connective tissue.

Summary. In general, the initial primary effect of hypervitaminosis A upon the chemistry of the connective tissue appeared to be a marked loss of hyaluronic acid and chondroitin sulfate from the saline soluble components of the tissue. With further doses of Vit. A there appeared to be a significant decrease in the glycoprotein content of these fractions. To this degree these chemical studies essentially confirm the effects of Vit. A described by others for cartilage *in vitro,* upon the soft connective tissues *in vivo.*

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Received October 18, 1963. P.S.E.B.M., 1964, v115.

Breakdown Products of Urea and Uremic Syndrome.* (28990)

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During the past 150 years the accumulation of urea, ammonium cyanate(1), amino acids, guanidine, uric acid, phenols and other substances, both organic and inorganic, in the blood of uremic animals and patients has lead to their being implicated in the uremic syndrome(2). No definitive proof has been presented that any of these substances are directly responsible for the symptoms of uremia.

Urea, since it is the major metabolic end product found in uremic blood, has been the substance most often considered to be responsible for the symptoms of uremia. A proof to the contrary was offered by Merrill(3) who observed no difference in the extent of clinical improvement in the uremic syndrome following extracorporal dialysis even if the

* These studies were supported by U. S. Public Health Service grant.