Vitamin D and the Supravalvar Aortic Stenosis Syndrome

The Transplacental Effects of Vitamin D on the Aorta of the Rabbit

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Recent reports have indicated that idiopathic infantile hypercalcemia, a disease related to deranged vitamin D metabolism,¹⁻⁴ may be a feature of the nonfamilial, congenital supravalvar aortic stenosis syndrome.⁵⁻⁸ Other manifestations of this syndrome include mental retardation, a peculiar "elfin" facies, narrowing of peripheral pulmonary and systemic arteries, strabismus, inguinal herniae, and dental abnormalities.⁹ Congenital supravalvar aortic stenosis and peripheral pulmonary arterial stenosis also occur in a familial and a sporadic form unassociated with the other features of the syndrome.¹⁰⁻¹³ It is not known if the multipletsystem involvement in the supravalvar aortic stenosis syndrome is genetically determined or whether some or all of the features are related to a derangement of maternal or fetal vitamin D metabolism, or a combination of the two.

A paucity of information exists regarding the effects on the fetus of induced maternal hypervitaminosis D, and there appears to be no direct evidence for transplacental passage of vitamin D.¹⁴⁻¹⁶ Studies concerning hypervitaminosis D in the nonpregnant animal emphasize the vascular toxicity of the vitamin.¹⁷⁻²³ Administration of excessive vitamin D to animals has been shown to produce dose-related vascular lesions, ranging from subendothelial edema to calcification, which are most pronounced in the ascending aorta just above the aortic sinuses. The pathogenesis of these vascular abnormalities is unclear.¹⁹

The present study was undertaken to determine whether vitamin D crosses the placenta, and to explore the relationship between hypervitaminosis D in the mother and the development of supravalvar aortic stenosis in the offspring. Blood levels of vitamin D from female rabbits that received excessive amounts of vitamin D and from their offspring were therefore determined, and the proximal aortae of these animals were examined anatomically.

Methods

Adult, white, New Zealand rabbits were bred so that each female was mated with a different male. Eight females fed a stock diet* were designated as controls. In addition, three females were fed the stock diet especially prepared without vitamin D supplements, and these are referred to as "vitamin D-deficient mothers." Eight females were fed the stock diet and, starting on the day after observed copulation and continuing until delivery, were given intramuscular vitamin D (activated ergosterol in cotton seed oil†) in divided doses every other day for a total dose of 1.5 million units. These animals are referred to as "mothers given vitamin D" (1.5 million units).

*Stock diet: Protein, 18.0%; fat, 2.5 to 4.0%; fiber, 13.5%; ash, 9.0%; calcium, 1.3 to 1.6%; phosphorus, 0.3 to 0.5%; iodine, 0.003%; salt, 0.75 to 1.0%; carotene, 40 gamma/g; pantothenic acid, 8.4 g/lb; niacin, 15.75 mg/lb; thiamin, 2.0 mg/lb; riboflavin, 3.2 mg/lb; biotin, 0.09 mg/lb; vitamin C, 0.70 mg/lb; vitamin D, 768 mg/lb; vitamin E, 30 mg/lb; choline, 350 mg/lb.
†Hi-Deratol injectable, Brewer and Co., Inc., Worcester, Massachusetts.
Three groups of five females each were fed the stock diet and in similar fashion were given intramuscular vitamin D for 30 days (the length of the rabbit's gestational period) beginning the day after observed copulation, for total amounts of 2.5, 3.5, and 4.5 million units, respectively.

Five control mothers and five given vitamin D (1.5 million units) were sacrificed with their progeny shortly after delivery. Prior to sacrifice blood was drawn from each mother for determination of calcium, phosphorus, alkaline phosphatase, cholesterol, total protein, albumin, lipoprotein phenotype,24 and vitamin D. Similarly, blood for calcium, lipoprotein phenotype, and vitamin D determinations was obtained from each neonate. To obtain an adequate amount of serum for the vitamin D assay, samples were pooled from as many as three newborns from the same litter. The vitamin D bioassay25 measured the response of rachitic rats to serum fed by stomach tube with the standard line test, as modified by Shue and associates.26 The rachitogenic diet and methods described in the A.O.A.C. methods of analysis were employed.27 Four or more rats were used for each assay.

Autopsy was performed on each of the five control and on each of the five mothers given vitamin D (1.5 million units) and on their offspring. The tissues were fixed in 10% Formalin, dehydrated in alcohol, embedded in paraffin, and sectioned serially at 6 μ intervals. The sections of heart and great arteries of each of the offspring were stained and examined histologically. In addition to hematoxylin and eosin, the Reinhart (for mucopolysaccharides), elastic van Gieson (for elastic tissue), Sudan black (for fat), and periodic acid-Schiff (for glycogen) stains were employed.

To evaluate anatomic changes in growing offspring given small daily quantities of vitamin D, three control mothers, three vitamin D-deficient mothers, and three mothers given vitamin D (1.5 million units) were not bled or sacrificed after delivering their offspring. After parturition the three vitamin D-deficient mothers were fed the stock diet and permitted to nurse their offspring. To avoid the consumption of large amounts of vitamin D from maternal milk, the offspring of the mothers given vitamin D (1.5 million units) were nursed by control mothers. The latter offspring as well as those of the controls were thereafter fed 250 units of vitamin D per day.9 Whenever death occurred spontaneously in one of the offspring of the mothers given vitamin D (1.5 million units), a rabbit of the same age born to a control mother and to a vitamin D-deficient mother was sacrificed. All of the remaining offspring were sacrificed at 3 months of age. Gross and microscopic examinations of the hearts and great vessels were carried out in each rabbit.

**Results**

The results of the analyses of the serum of the mothers and offspring are presented in table 1. The levels of vitamin D were strikingly different in the various groups (table 1). The levels in the mothers given vitamin D (1.5 million units) and in their offspring were 7 and 9 times greater than the controls, respectively. Other than the levels of vitamin D there were no statistically significant differences in any of the other measurements between the groups of mothers (table 1). The calcium levels were somewhat lower and the phosphorus values somewhat higher in the mothers given vitamin D (1.5 million units) when compared to the controls. Significantly higher serum calcium levels were observed in the offspring of the mothers given vitamin D (1.5 million units) when compared to the controls. The high serum calcium levels noted in this study are in the same general range as those reported for the normal rabbit by others.28 Cholesterol values were not significantly higher in the mothers given vitamin D (1.5 million units), and there were no qualitative differences in the electrophoretic lipoprotein phenotypes. The control mothers and the mothers given vitamin D (1.5 million units) had chylomicron, beta (β-), and alpha (α-) lipoprotein bands of equal staining intensity. All of the offspring showed less intense β- and α-lipoprotein bands than their parents, but there were no differences in staining intensity between the two groups of offspring.

**Anatomic Observations**

**Adult Females**

There were no gross or microscopic abnormalities in the hearts or aortae of the control mothers. All of the females given 2.5, 3.5, and 4.5 million units of vitamin D died spontaneously within 65 days after their first injection of the vitamin, and all that con-
The entire aorta of each of these adult rabbits showed advanced changes, including irregular depressions in the intimal wall, focal calcium deposits, and foci of degeneration and necrosis of the aortic media. The aortae of the mothers given vitamin D (1.5 million units) showed similar but less striking changes. The alterations were most pronounced in the proximal portion of the aorta.

**Offspring**

*Control Newborns Sacrificed after Delivery*

The aortic roots of the 23 control neonates were histologically normal. The normal invagination or plica at the upper margin of the sinuses of Valsalva was observed in some sections of each of these aortae, indicating that the plica does not extend around the entire inner circumference. The medial thickening that accounted for this invagination appeared moderately exaggerated (fig. 1 right) in five of these 23 aortae, but in none was it believed to be of sufficient proportion to narrow the aortic lumen significantly.

**Older Control Offspring**

With the exception of a ventricular septal defect in one, no other abnormalities of the heart or aorta were found in these 12 rabbits. The normal supravalvar aortic plica did not appear unusually prominent in any of these animals.

**Offspring of Vitamin D-Deficient Mothers**

No abnormalities were observed in these nine rabbits. The supravalvar aortic plica was present in each, but in none did it appear to be exaggerated.

**Newborns of Mothers Given Vitamin D (1.5 Million Units)**

The aortae of 14 of the 18 newborns in this group were normal, and 11 of these 14 aortae showed an exaggeration of the supravalvar plica similar to that observed in the five controls mentioned above (fig. 1 right). The higher incidence of this finding in this vitamin D group when compared to the controls is significant ($P < 0.001$). Four newborn rabbits,
each from a different litter, had abnormal aortae. In each animal there was a prominent annular protrusion at the superior margin of the sinuses of Valsalva resulting in significant narrowing of the luminal circumference at this level (fig. 2). The protrusion was caused entirely by proliferation of the media; the intima was normal. There was widening of the spaces between the elastic fibrils in the inner media. Stains for glycogen, fat, calcium, and mucopolysaccharides did not reveal abnormal deposits of these substances in the medial lesions.

Older Offspring of Mothers Given Vitamin D (1.5 Million Units)

Ten rabbits died spontaneously at 2 to 20 days of age. The cause of their deaths was not apparent at autopsy. There were no cardiac or aortic abnormalities in the four that lived less than 10 days and in two that died at 16 and 20 days of age, respectively. Supravalvar aortic abnormalities were noted in the four remaining rabbits, representing different litters. The aorta of a 7-day-old rabbit appeared normal on gross examination, but on microscopic examination an area of medial thickening and early degeneration just above the aortic sinuses was apparent. A 10-day-old rabbit had a nonobstructing fibrous band stretched across the lumen of the ascending aorta at the superior margin of the sinuses of Valsalva and a particularly prominent plica of the wall at this level. The aortae of the other two rabbits, a 20-day-old (fig. 3) and a 14-day-old (fig. 4), were severely narrowed in the region immediately above the sinuses of Valsalva by triangular-shaped protrusions or hillocks, and the aortic lumina distal to these sites were widened. These protrusions into the lumen resulted from localized thickening of the aortic media. In addition to an apparent increase in the elastic fibers and smooth muscle cells in the media, small foci of degeneration and round-
cell infiltration also were present. The arrangement of the elastic fibers and smooth muscle cells in the hillock produced an irregular pattern (fig. 3). A focal aneurysmal protrusion of the ascending aorta was observed in one animal (fig. 3). In the other animal, calcium deposits also were present in the media (fig. 4). The intima in each rabbit appeared normal, but the adventitia in one was focally thickened and invaginated into the media in a wedgelike fashion (fig. 3).

The remaining six rabbits in this group were sacrificed at 3 months of age. The changes in their aortae resembled quite closely the findings in the mothers given vitamin D (1.5 million units). The proximal aorta in these six animals showed generalized irregularities of the wall with degeneration and calcification of the media. The supravalvar area was not narrowed or disproportionately thickened, however, when compared to the rest of the ascending aorta.

Discussion

Three specific anatomic types of supravalvar aortic stenosis (SAS) have been described in the human.29, 31 The hour-glass type is the most common and is characterized by extreme thickening of the aortic media producing a constricting annular ridge. The membranous type is produced by a semicircular diaphragm with a small central opening stretched across the lumen of the aorta. The hypoplastic type is characterized by uniform hypoplasia of the ascending aorta. A fourth anomaly, of no functional significance, consists of a nonobstructing band or cord stretched across the lumen of the aorta at the level of aortic leaflets.

According to Perou,29 the most common basic lesion is an angulation and exaggerated infolding of the wall with a thickened and focally disorganized medial layer capped by a zone of intimal thickening. The infolding may be shaped like a hillock or triangle

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**Figure 2**

The aortic roots of two newborns (left and center) whose mothers were given vitamin D (1.5 million units) show prominent annular protrusions impinging on the lumen at the superior margin of the sinuses of Valsalva. A higher magnification of the lesion (brackets) in a third newborn is shown on the right. The narrowing of the lumina results entirely from proliferation of the media. S. V., sinus of Valsalva; Ao., ascending aorta; A. V., aortic valve; A. L., anterior leaflet of mitral valve. Hematoxylin and eosin; × 39 (left); × 63 (center); × 97 (right).
Figure 3

Heart and aorta in a 20-day-old rabbit whose mother had received vitamin D. (Upper left)

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Figure 4

The aortic root in a 14-day-old rabbit whose mother was given vitamin D. (Left) The wall of the aorta just above the sinuses of Valsalva is markedly thickened (brackets) and bulges into the lumen. A close-up of this area is shown in the photomicrograph on the right. Areas of necrosis and focal deposits of calcium are apparent. A. V., aortic valve. Hematoxylin and eosin; × 25 (left); × 160 (right).

whose base is formed by the abnormal media and whose apex points toward the lumen. Foci of necrosis, round-cell infiltration, and calcification also have been noted in the hillock. Similar to the vascular changes in the animal given excessive vitamin D, the intrin-

Figure 3 (continued)

The anterolateral wall of the left ventricle (L. V.) and left atrium has been removed, and the aorta (Ao.) opened. The lumen immediately above the aortic valve (A. V.) is narrowed and the aortic wall is thickened. The ostium of the right coronary artery (C. A.) is dilated. A focal protrusion or aneurysm (arrow) also is present in the ascending aorta. (Lower left) Section of wall of ascending aorta, aortic valve cusp (A. V.), and ventricular septum (V. S.). The prominent supravalvar aortic lesion (brackets) is triangular-shaped, with the apex pointing toward the lumen. The aortic valvar cusp is folded back toward the heart. Hematoxylin and eosin; × 7. (Upper right) Close-up view of the irregular pattern of the smooth muscle cells in the supravalvar area of thickening. Hematoxylin and eosin; × 107. (Lower right) View of another region of the supravalvar lesion. At this site the adventitia has invaginated in a wedgelike fashion (arrow) into the thickened media. Elastic van Gieson; × 25.
sic pathology involves the inner half of the media. In SAS the elastic fibers are broken and disorganized. There are focal increases in interstitial fibrous tissue and an irregular increase in smooth muscle. The adventitia is usually normal, but in one of Perou's patients it invaginated into the media in a wedgelike fashion.

The aortic wall of many normal human newborns exhibits an incomplete transverse infolding or plica at the upper margin of the sinuses of Valsalva that is conspicuous in some normal aortae but practically absent in others. SAS is thought to be a developmental exaggeration of this normal structure. The present study demonstrates that this mild angulation and infolding of the aortic wall occurs in normal rabbits with varying degrees of prominence. Exaggerated prominence of the normal plica occurred without apparent narrowing of the lumen in a significantly higher percentage of rabbits born to mothers given vitamin D during pregnancy when compared to the controls. The aortae of the offspring whose mothers were fed a diet lacking vitamin D, however, also showed a supravalvar plica, albeit of lesser prominence, suggesting that the presence of the invagination does not depend solely on some action of vitamin D.

In the present study, eight offspring exposed in utero to high levels of antirachitic substance, presumably vitamin D, and to the maternal biochemical products of excessive administration of the vitamin revealed pathological abnormalities confined to the supravalvar aortic wall. In six instances the aortic lesions seriously impinged on the lumen and demonstrated essentially all of the histological features of SAS as seen in man. The aneurysmal protrusion of the aorta of one rabbit has been observed in a similar location in SAS in man. A nonobstructing delicate fibrous band crossing the proximal aorta was present in one rabbit. In another rabbit a medial lesion was microscopically evident although no gross abnormality was detected. The six offspring which were sacrificed at age 3 months showed the pathological changes associated with vasculotoxicity in the adult animal, although they received a cumulative oral dose of only 22,000 units of vitamin D from birth, leading one to question if they were predisposed to an increased tissue sensitivity to the toxic effects of the vitamin.

From this study it may be concluded that antirachitic substance crossed the hemochorial placenta of the rabbit and that the vascular toxicity of vitamin D can be transmitted across the placenta. There are definite similarities between SAS in the human and the lesions of the aortae in the offspring whose mothers received vitamin D. From these findings it seems reasonable to suggest that an in utero derangement in vitamin D metabolism on the part of mother or fetus or of both may be responsible for SAS, especially when the latter is associated with infantile hypercalcemia. It is necessary, however, to be cautious in drawing an analogy between the experimental animal model and the clinical disease. Because of the poorly understood and highly complex interaction between environmental and genetic factors that are involved in the genesis of human malformations, it must be stressed that our study does not supply a simple explanation for the syndrome of SAS and infantile hypercalcemia, but rather raises many questions. No emphasis has been placed on the prophylactic and therapeutic implications of the experimental findings. Further investigations will necessarily be stimulated by such considerations and hopefully will clarify the basic genetic, metabolic, and pathological mechanisms involved.

Summary

Pregnant rabbits were given high doses of vitamin D to determine whether the vitamin crossed the placenta and to explore the relationship between maternal hypervitaminosis D and congenital supravalvar aortic stenosis. The blood levels of antirachitic substance in the mothers given vitamin D and their offspring were 7 and 9 times greater than in the control mothers and offspring, respectively, indicating that transplacental passage oc-
curred. Serum calcium levels in the offspring whose mothers received vitamin D were significantly higher when compared to control values. A total of 14 abnormalities of the aorta were noted in the 34 offspring whose mothers received vitamin D. Aortic lesions that appeared similar anatomically to supravalvar aortic stenosis in man were noted in six rabbits. Also, one rabbit showed a supravalvar fibrous band and another had an abnormality of the proximal portion of the aorta observed only on microscopic examination. Six additional offspring at age 3 months showed generalized vitamin D vasculotoxicity, without supravalvar narrowing of the aorta, of an advanced type commonly seen in the adult animal given massive doses of the vitamin. Thirty-five control offspring and nine rabbits born to mothers on vitamin D-deficient diets showed no abnormalities of the aorta. The results suggest that an in utero derangement in vitamin D metabolism on the part of mother or fetus, or of both may be responsible for supravalvar aortic stenosis, especially when the latter is associated with infantile hypercalcemia. The questions raised by the experimental findings are emphasized.

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Gains from Serendipity

Readers who remember Bible stories will recall that Saul, the son of Kish, was sent forth to find his father's asses, which were lost. In the discouragement of his failures to find them he consulted one, Samuel, a seer. And Samuel told him not to set his mind on them for they had been found, but to know that he was chosen to rule over all the tribes of Israel. So it was announced, and the people shouted their approval. Thus modest Saul, who went out to seek lost asses, was rewarded by a kingdom. That is the earliest record of serendipity I am aware of.—WALTER BRADFORD CANNON: The Way of an Investigator. New York, W. W. Norton & Co., 1945, p. 88; also in Serendipity and the Three Princes, Theodore G. Remer (Ed.). Norman, Oklahoma, University of Oklahoma Press, 1965, p. 176.
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