known to be XY and destined to develop as males.

Four mature females with XX sex chromosomes spawned the eggs studied. These females were bred first with four XX males and subsequently with four YY males. In an initial control experiment, 40 progeny of each mating were raised to sexual maturity, with the result that all of the XX fish were of the female phenotype and all of the XY progeny were of the male phenotype.

Thereafter, embryos of known XX or of known XY genotype were collected at embryonic stage 26-1, at which time the germ cells are migrating to the gonadal primordium from the extraembryonic endoderm; at embryonic stage 30, at which time the primordial germ cells have just completed migration to the gonadal primordium; and at hatching, at which time gonial proliferation is beginning (*5, 6*). At each of these three stages, 16 XX and 16 XY specimens were collected and prepared by Gamo's method (*6*). This technique requires fixation in Bouin's solution, dechorionation of the embryos, embedding in paraffin with a melting point of 53° to 55°C, and serial sectioning at 6 μm.

The origin of the germ line and differentiation of the gonads has been described in *O. latipes* (*6, 7*), but no use was made of embryos of known genotypic sex. In our work, the first in which germ cells were studied in embryos whose sex was known with certainty, there was no sex difference in the number of primordial germ cells either during migration to the gonadal primordium or after arrival in the primordium (Table 1). At hatching, there was only a slight sex difference in the number of germ cells with the onset of their proliferation. This proliferation culminates within 2 days after hatching in significantly more germ cells in presumptive ovaries than in presumptive testes, as has been reported for the d-rR strain of *O. latipes* (*7, 8*), a time when sex differentiation of germ cells has taken place (*8*). This we confirmed in progeny of known sex genotypes produced by other breeders in the d-rr strain (Table 2).

Earlier and more mitoses in the entire embryonic gonadal rudiment of the heterogametic than of the homogametic sex is currently claimed (*9*) to cause differentiation of the mammalian testis (and of the avian ovary) and to be a function of the "odd" sex chromosome which is the Y in mammals. If this interesting concept applies to a teleost such as *O. latipes* (in which the "odd chromosome" is the Y and is associated with differentiation of a testis), it is not applicable to the number of germ cells proliferated by hatching and shortly thereafter, because the number was not larger in the testis than in the ovary.

In conclusion, the number of primordial germ cells, as originally segregated in *O. latipes*, is the same in homogametic embryos (XX females) and heterogametic embryos (XY males). At a later stage of development, mitosis of these cells within the gonads results in more germ cells in females than in males.

Table 2. Number of germ cells at 2 days after hatching in the d-rr strain of *O. latipes*: Animals of known genotypic sex produced by ten pairs of parents.

Animals	Germ cells (mean ± S.E.)	Range	
10XX	157.4 ± 20	81–354	$P < .001$
10XY	76.0 ± 20	39–136	

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Isotachysterol$_3$ and 25-Hydroxyisotachysterol$_3$: Analogs of 1,25-Dihydroxyvitamin D$_3$

Abstract. *Isotachysterol$_3$, 25-hydroxyisotachysterol$_3$, and isovitamin D$_3$ have been synthesized and tested for biological activity. Like 1,25-dihydroxyvitamin D$_3$, isotachysterol$_3$ stimulates intestinal calcium transport and bone calcium mobilization in anephric rats, whereas 25-hydroxyvitamin D$_3$ does not. Although isovitamin D$_3$ is biologically active in normal rats it is inactive in anephric rats.*

Vitamin D must first be hydroxylated on carbon-25 (C-25) in the liver (*1, 2*) and then on carbon-1 (C-1) in the kidney (*3*) before it can carry out its physiologic functions. Unlike vitamin D or its 25-hydroxy derivative, 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$D$_3$) is capable of stimulating both intestinal calcium transport and bone calcium mobilization in anephric rats (*4*). These results make clear the potential use of 1,25-(OH)$_2$D$_3$ in the treatment of patients with hypocalcemia, impaired intestinal calcium transport, secondary hyperparathyroidism, and osteodystrophy due to renal dysfunction. However, be-

Table 1. Intestinal calcium transport and bone calcium mobilization response to isotachysterol$_3$, isovitamin D$_3$, 5,6-*trans*-vitamin D$_3$, 25-hydroxyisotachysterol$_3$, and 25-OH-D$_3$ in normal and anephric rats.

Compound	Amount (μg)	Animals (No.)	Ca in serum (mg/100 ml) (mean ± S.E.)	^{45}Ca serosal/^{45}Ca mucosal (mean ± S.E.)
		Normal		
None	0	6	4.3 ± 0.1	1.8 ± 0.2
Isovitamin D$_3$	5	6	6.5 ± .1	3.5 ± .3
Isotachysterol$_3$	5	6	6.3 ± .1	3.2 ± .3
25-Hydroxyisotachysterol$_3$	5	6	6.4 ± .1	3.0 ± .2
25-OH-D$_3$	0.25	6	6.4 ± .1	3.2 ± .2
5,6-*trans*-vitamin D$_3$	5	6	6.0 ± .1	3.5 ± .1
		Anephric		
None	0	6	3.7 ± 0.1	1.2 ± 0.2
Isovitamin D$_3$	5	6	3.8 ± .1	1.2 ± .1
Isotachysterol$_3$	5	6	5.0 ± .1	3.2 ± .2
5,6-*trans*-vitamin D$_3$	5	6	4.9 ± .1	3.5 ± .2
25-OH-D$_3$	0.25	6	3.9 ± .1	1.4 ± .2

cause of the limited amount of 1,25-$(OH)_2D_3$ available to the clinician, only a few patients with advanced renal diseases have been treated with the metabolite. Brickman *et al.* have reported that 1,25-$(OH)_2D_3$ is effective in stimulating intestinal calcium transport and raising serum calcium and phosphorus concentrations in three uremic patients (*5*).

Our 21-step chemical synthesis of 1α,25-$(OH)_2D_3$ provides the possibility that large quantities of this hormone may become available (*6*). However, the length and expense of this procedure prompted an examination of analogs of 1,25-$(OH)_2D_3$ which might be less expensive and less difficult to prepare. One approach taken has been the 180° rotation of the A ring of vitamin D_3 by isomerization, thus placing the 3β-hydroxyl in the position occupied by the 1α-hydroxyl of 1,25-$(OH)_2D_3$ (*7, 8*). Thus 5,6-*trans*-vitamin D_3 (Fig. 1) is capable of stimulating both intestinal calcium transport and bone calcium mobilization in anephric rats, while its 25-hydroxy derivative appears only to stimulate intestinal calcium transport (*7, 8*).

Because of the possible usefulness of 1,25-$(OH)_2D_3$ analogs in medicine, we tested the biological activity of two other isomers of vitamin D_3, which also have the 3β-hydroxyl function in the geometric position occupied by the 1α-hydroxyl of 1,25-$(OH)_2D_3$ (Fig. 1). Isotachysterol$_3$, 25-hydroxyisotachysterol$_3$, and isovitamin D_3 were synthesized and shown to be capable of inducing intestinal calcium transport and bone calcium mobilization in normal animals. It was surprising, however, to find that isotachysterol$_3$ but not isovitamin D_3 is biologically active in anephric rats.

Isotachysterol$_3$ and 25-hydroxyisotachysterol$_3$ were prepared from vitamin D_3 and 25-hydroxyvitamin D_3 (25-OH-D_3), respectively, according to the procedure of Inhoffen *et al.* (*9*). Fifty milligrams of vitamin D_3 or 25-OH-D_3 were dissolved in diethyl ether and treated with boron trifloride etherate for 24 hours. The reaction mixture was extracted with water and diethyl ether, and the product in the ether layer was chromatographed as described for 5,6-*trans*-vitamin D_3 and its 25-hydroxy derivative (*7*). Both isotachysterol$_3$ and 25-hydroxyisotachysterol$_3$ exhibited a characteristic ultraviolet absorption spectrum, with maxima at 302, 290, and 280 nm (*9*), and each appeared as one component on gas-liquid chromatography and on thin-layer chromatography. The mass spectrum of isotachysterol$_3$ showed a molecular ion *m/e* at 384, and fragments at 369, 271, 259, 253, and 230 (Fig. 2). Isovitamin D_3 was prepared as reported (*10*), and the product was chromatographed as described above for isotachysterol$_3$. This product had an ultraviolet absorption spectrum with maxima at 300, 288, and 278 nm (*9, 10*) and chromatographed as a single homogeneous peak on gas-liquid chromatography and thin-layer chromatography. The mass spectrum displayed a molecular ion *m/e* at 384 and fragments at 369, 271, and 253 (Fig. 2). Because the ultraviolet absorption spectra for isotachysterol$_3$ and isovitamin D_3 are so similar it is very difficult to tell them apart. However, it is possible to distinguish one isomer from the other by mass spectrometry because the fragmentation pattern for each of these isomers is quite distinct.

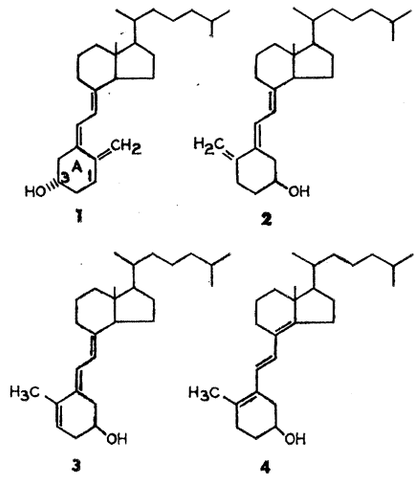

Fig. 1. Structures of (**1**) vitamin D_3, (**2**) 5,6-*trans*-D_3, (**3**) isovitamin D_3, and (**4**) isotachysterol$_3$.

For intestinal calcium transport and bone calcium mobilization measurements, weanling, male albino rats (Holtzman) were fed for 2 weeks a diet adequate in calcium and phosphorus but deficient in vitamin D, and then fed for an additional week on a diet low in calcum (0.02 percent) and deficient in vitamin D (*7*). At the end of the third week, groups of six rats, which were either bilaterally nephrectomized or sham-operated, received either isotachysterol$_3$, 25-hydroxyisotachysterol$_3$, isovitamin D_3, 5,6-*trans*-vitamin D_3, or 25-OH-D_3 intrajugularly in 0.05 ml of 95 percent ethanol. The animals were decapitated 20 hours later, and blood was collected. Small intestines were used immediately for measurements of intestinal calcium transport by the everted gut sac technique (*7*). The blood was centrifuged, and 0.1 ml of serum was mixed with 1.9 ml of a 0.1 percent lanthanum chloride solution. Serum calcium was determined with a Perkin-Elmer atomic absorption spectrophotometer (model 403).

Isotachysterol$_3$, isovitamin D_3, and 25-hydroxyisotachysterol$_3$ stimulate intestinal calcium transport and elicit a rise in serum calcium presumably at the expense of bone calcium (Table 1). Like 1,25-$(OH)_2D_3$ and 5,6-*trans*-vitamin D_3, isotachysterol$_3$ is able to stimulate both intestinal calcium transport and bone calcium mobilization in anephric rats (*4, 7*). Unlike isotachysterol$_3$, isovitamin D_3 and 25-OH-D_3 are incapable of inducing either intestinal calcium transport or bone calcium mobilization in anephric rats. Surprisingly, the 25-hydroxy derivative of isotachysterol$_3$ is

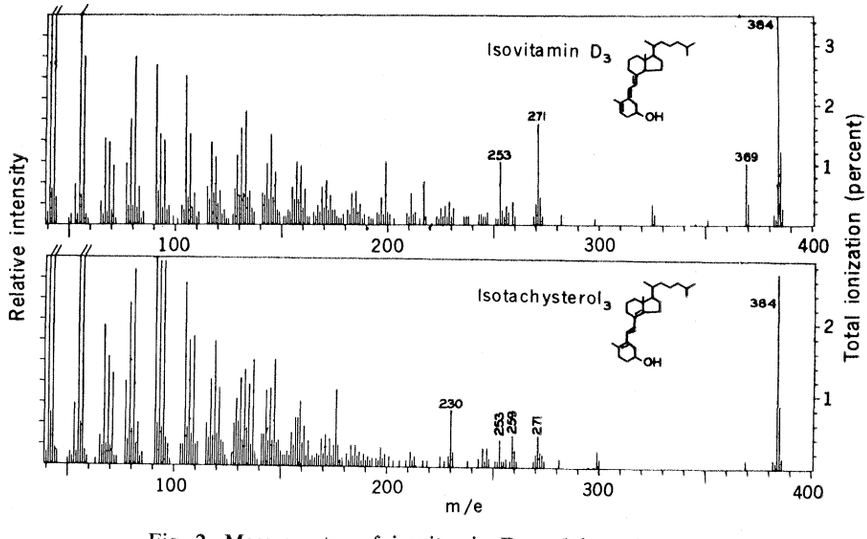

Fig. 2. Mass spectra of isovitamin D_3 and isotachysterol$_3$.

capable of inducing both intestinal calcium transport and bone calcium mobilization. The 25-hydroxy derivative of 5, 6-*trans*-vitamin D_3 is unable to stimulate bone calcium mobilization while stimulating intestinal calcium transport (*7*).

It is also surprising that isovitamin D_3 is biologically active in normal rats but not in anephric rats. Although there are many possible explanations, it seems likely that a small amount of vitamin D_3 may have remained in the preparation. This small amount, which could go undetected by either thin-layer chromatography or gas-liquid chromatography, would be metabolized only in normal animals to 1,25-$(OH)_2D_3$ and elicit a response in both the bone and intestine. Another possible explanation is that in isovitamin D_3 the A ring may not be rotated 180°, and therefore the kidney is required for a hydroxylation on C-1 to provide an analog of 1,25-$(OH)_2D_3$ which is biologically active in the intestine and bone. This problem remains to be solved.

In any case, our results demonstrate that, in addition to the 5,6-*trans* isomers, another isomer of vitamin D_3—isotachysterol$_3$—can function in bone and intestine of anephric animals. The conversion of vitamin D_3 to the isotachysterol$_3$ is as simple to carry out as the 5,6-*trans* isomerization. However, it has the added advantage that the procedure results in virtually quantitative conversion of vitamin D_3 to the isotachysterol. Thus its preparation would be less expensive than that of 5,6-*trans*-vitamin D_3, and its purification before use would be considerably simpler. Its possible utility in cases of renal osteodystrophy, therefore, seems evident.

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Transfer of Experimental Autoimmune Renal Cortical Tubular and Interstitial Disease in Guinea Pigs by Serum

Abstract. *Guinea pigs injected with rabbit tubular basement membranes and Freund's adjuvant develop progressive renal cortical tubulointerstitial disease and deposit autoantibodies in their cortical tubular basement membranes. The identical, even fatal, disease may be produced in normal guinea pigs by a single intraperitoneal injection of serums obtained from guinea pigs with this tubulointerstitial disease, provided such serums contain sufficient amounts of autoantibodies against tubular basement membranes.*

Guinea pigs injected with heterologous renal basement membranes and complete Freund's adjuvant develop renal cortical tubular disease and form antibodies, some of which react with their own cortical tubular basement membranes (TBM) (*1*). Severe cortical tubulointerstitial disease is correlated with extensive linear fixation of antibodies along the cortical TBM and high titers of serum autoantibodies to TBM (*1, 2*). We now report the induction of progressive and fatal renal tubulointerstitial disease in normal guinea pigs by intraperitoneal injection of serum containing large amounts of autoantibodies to TBM.

Preparations rich in rabbit TBM were emulsified in complete Freund's adjuvant (*1*). On days 1 and 14 donor guinea pigs (*3*) were injected with 0.1 ml of emulsion [15 mg of TBM (wet weight) per milliliter of emulsion] intradermally in six sites on the back. Control donors were either injected with an emulsion of complete Freund's adjuvant alone or were untreated. All donors were exsanguinated on day 21. The titers of antibodies against guinea pig TBM or glomerular basement membrane (GBM) in the serums of 125 donors with tubular disease were determined by indirect immunofluorescence (*4*). Three pools of serums were formed; in each pool the ratio of the titer of antibody to TBM to the titer of antibody to GBM was different (Table 1). The pooled serums were sterilized by passage through an autoclaved 0.45-μm membrane filter.

A total of nine recipients was injected intraperitoneally with donor serum. Prior to the injections of donor serums, one kidney was removed from each of two recipients to serve as a control for the remaining kidney in pathologic and immunologic studies. The progression of disease was studied in five recipients, after injection of donor serums, by removing one kidney from each of two recipients on day 2 and from each of the other recipients on days 3, 7, and 8 and comparing it with the contralateral kidney subsequently obtained at autopsy. Donor serum was injected into two recipients with both kidneys intact. Blood urea nitrogen (BUN) tests and urinalyses were performed daily (*5*). Four uremic recipients were killed when moribund on days 10 to 14 (Table 1). The remaining recipients were killed on day 14. Renal tissue from nephrectomy or autopsy was prepared by conventional methods for light and fluorescence microscopy. Cryostat sections (4 μm) of recipient and donor kidneys were studied by direct immunofluorescence for deposition of immunoglobulin G (IgG) (*6*) and by indirect immunofluorescence for detection of complement (C3) (*7*). Recipient kidneys were eluted with citric acid buffer at *p*H 3.2 (*1*). These eluates and daily serum samples were layered over cryostat sections of homologous kidney or of those kidneys that were removed before injection of the recipient and stained with fluorescein-conjugated rabbit antiserum to guinea pig IgG to identify donor antibodies which can react with TBM or GBM.

All nine recipients developed progressive cortical tubulointerstitial disease (Table 1). Fourteen kidneys were examined 2 to 14 days after injection of donor serum. The development of disease was correlated directly with extensive deposition of IgG along the TBM in vivo (*8*). The autoantibodies to TBM injected were in large excess, since they were still detected in the serums of all nine recipients for 10 to 14 days after transfer. Control recipients had no evidence of autoantibodies to