blood pressure and a subsequent slow increase of renin content in the remaining unclamped kidney. A unifying hypothesis to explain these changes is presented, involving a concept of renin inhibition or inactivation.

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Effect of Water-Miscible Preparation of Vitamin D_2 on Metamorphosis of Thiourea and Citrate Treated *Rana pipiens* Larvae. (27130)

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Vitamin D_2 (calciferol) in moderate doses has been shown to enhance metamorphosis in tadpoles(1,2). Since calciferol has been demonstrated to have a thyroid-enhancing effect(3,4,5) it was thought interesting to study its effect when combined with other substances which may affect thyroid function.

Immersion in thiourea inhibits tadpole metamorphosis(6) presumably by interfering with the oxidation of iodide in synthesis of thyroxine(7). Therefore, it was decided to determine the effect of calciferol on the metamorphosis of thiourea-treated tadpoles.

Furthermore, an increase in serum and tissue citrate levels with Vit. D_2 has been demonstrated in mammals(8). Although the thyroid has an unusually high citrate concentration(9,10), it does not synthesize citrate at all, but does degrade it to a moderate degree(11). Since the decrease in thyroidal citrate can be correlated with an increase in the gland's oxidative metabolism(9,12) the possibility exists that citrate is captured by the thyroid, perhaps adventitiously with calcium, and is either utilized as fuel or released in the course of metabolic activity. Therefore, it was decided to determine the effect of immersion in citrate plus Vit. D_2 on tadpole metamorphosis.

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Methods. *R. pipiens* tadpoles obtained by induced ovulation were immersed at the hind-limb bud stage in thiourea solution (.033%), sodium citrate (.05%), thiourea (.033%) and calciferol (1000 I.U./l), sodium citrate (.05%) and calciferol (1000 I.U./l). Control tadpoles were also reared. Hind-limb lengths were measured prior to immersion in experimental media and after 63 days. The larvae were placed in fresh solutions on alternate days. On the 35th day of experiment the level of calciferol was increased to 2500 I.U./l, when preliminary observations indicated an incipient difference between control and experimental groups.

Results. The results summarized in Table I, subjected to a "t" analysis showed a significant difference between Groups I and II (95-98% level) and II and V (90-95% level).

One may conclude that Vit. D_2 has a metamorphosis-enhancing effect; it does not counteract the action of thiourea; the effect of Vit. D_2 is greater than that of Vit. D_2 plus citrate.

Discussion. Although these results cannot be fully explained there are several speculative possibilities. The enhancing effect of Vit. D_2 on thyroid function may be explained: by its citrate effect (reference to

TABLE I. Hind Limb Lengths.

		Initial rdg. (mm)			Final rdg. (mm)		
		Mean	S.E.	No.*	Mean	S.E.	No.†
I	Control	3.1	.52	15	6.5	2.20	9
II	Vit. D_2	3.0	.45	15	13.6	1.90	8
III	Vit. D_2, thiourea	3.0	.48	15	4.1	1.20	10
IV	Thiourea	2.5	.20	15	3.1	.24	12
V	Vit. D_2, citrate	2.9	.31	15	7.8	2.00	9
VI	Citrate	2.9	.30	15	10.7	3.40	10

S.E. is stand. error of mean.

* These groups were made up of tadpoles chosen at random from a much larger number, many of which failed to reach hind limb bud stage.

† The difference between final and initial numbers was due to deaths.

which has been made above) in providing a presumed fuel for thyroid function; by the role of its isomer, tachysterol (with which it can be in active equilibrium)(13) in oxidizing iodide(14); by the role of its bipolar free radical intermediate in the above equilibrium in stabilizing the presumed free radical intermediate in thyroxine synthesis(15). Since the thiourea effect was not counteracted, at least with the concentrations used, it is less likely that the second and third possibilities were operative.

The reasons for the lesser activity of Vit. D_2 plus citrate are also unknown but one can speculate that since either Vit. D_2 or citrate alone increases calcium assimilation(16,17), by combining them, a relative hypercalcemia resulted. It should be noted that the animals were fed spinach which is high in calcium oxalate, which salt is solubilized by citrate. A high assimilated Ca:I is known to have a goitrogenic effect(18). Further, the possibly high citrate concentrations resulting from the combination may have reached an *in vivo* level capable of interfering with the deiodination of thyroxine to the more active triiodothyronine(19). This would be, in effect, a mild peripheral goitrogenic mechanism.

The study of citrate metabolism in relation to thyroid function is being continued.

Summary. An immersion experiment using hind-limb length increase of *R. pipiens* tadpoles has shown that although Vit. D_2 enhances metamorphosis it does not counteract the effect of thiourea. Addition of citrate reduces the Vit. D_2 effect.

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