was cut, resulting in a completely colorless thyroid gland.

The function of the transplanted thyroid was monitored after injecting the recipient mice intraperitoneally with approximately 0.25 μCi of ^{125}I (carrier-free) 19 to 21 days after transplantation. The recipient was killed 24 hours later, and both kidneys were removed and placed in counting tubes containing buffered formalin. Radioactivity in the kidney containing the thyroid transplant and that in the unoperated contralateral kidney were counted in a gamma counter. Test kidneys that showed ten times or more counts than did the control kidneys were considered to be carrying a functional transplant. All transplants were sectioned and examined histologically after counting. The histological appearance of the transplants was evaluated as described (*8*).

Survival on day 19 to day 21 of BALB/c allografts in C57BL/6 mice was increased by partial pressures of O_2 above 600 mm during the 4-day culture (Table 1, groups 2 and 3). No significant effects resulted from perfusion before culture (group 4) or the addition of serum during the culture (group 5). The addition of 10 μg of hydrocortisone acetate per milliliter of culture medium increased slightly but significantly the percentage of grafts lacking generalized infiltration (group 6).

These results indicate that mouse thyroid allografts survived as well after a 4-day culture period in hyperbaric oxygen and hydrocortisone as they did after a 4-week culture in 95 percent oxygen at atmospheric pressure (*8*). Both radioactive iodine uptake and histological appearance after 21 days correlated with a long-term survival graft (*8*).

The above results probably explain the failure of Raff *et al.* (*10*) to obtain prolonged survival of cultured rat thyroids when atmospheric O_2 (150 mm) was used. They also explain our failure (*11*) to obtain prolonged survival of thyroid grafts after retransplantation from a thymectomized, irradiated, allogeneic recipient to a normal animal of the same strain. The effect of hydrocortisone on these experiments may be similar to the prolongation of allogeneic rat skin graft survival after treatment of the grafts with fluocinolone acetonide (*12*).

The mechanism by which oxygen prolongs graft survival in an allogeneic host is not known. An attractive explanation is that oxygen selectively kills passenger leukocytes, which are efficient stimulators of allograft immunity (*8*). If this is the mechanism, further selective modifications of the culture conditions may further decrease the time of culture required, and should facilitate the application of the culture technique to the transplantation of other organs.

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The Essentiality of Vitamin D Metabolites for Embryonic Chick Development

Abstract. *Laying hens maintained on 1,25-dihydroxyvitamin D_3 as their sole source of vitamin D produce eggs which appear normal but which produce embryos having a defective upper mandible and which die at 18 to 19 days of embryonic life. Hens maintained on 25-hydroxyvitamin D_3, on the other hand, produce normal embryos. Hens fed a vitamin D deficient diet produce eggs which develop the same embryonic defect. Injection of the affected eggs from the 1,25-dihydroxyvitamin D_3 fed hens with vitamin D_3, 25-hydroxyvitamin D_3, or 1,25-dihydroxyvitamin D_3 greatly increases the percentage of normal embryos. It therefore appears that 1,25-dihydroxyvitamin D_3 is not transferred from hen to egg in sufficient amounts to support embryonic development and that vitamin D or its metabolites, or both, are necessary for normal chick embryo development.*

Although it is well accepted that vitamin D must undergo 25-hydroxylation followed by 1α-hydroxylation to be effective at physiologic doses, there is still considerable discussion of whether the resultant hormonal form of vitamin D_3, namely, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] can carry out all of the vitamin's functions (*1*). During the course of a study of egg production of hens maintained on 1,25-$(OH)_2D_3$ as their sole source of vitamin D, we learned that the embryos from these eggs failed to hatch and died prior to emergence from the shell. Examination of the affected embryos (Fig. 1) showed that the upper mandible failed to develop properly. In other respects the embryos appeared normal. An experiment was therefore carried out to determine if higher levels of 1,25-$(OH)_2D_3$ given to the hens could support normal embryonic emergence.

Forty-eight White Leghorn pullets, 40 weeks of age, from the University of Wisconsin flock were fed a basal diet composed (in grams of ingredient per kilogram of diet) of ground corn (752), alfalfa meal (20), meat scraps (30), soy-

Table 1. Effect of 1,25-$(OH)_2D_3$ on egg production, eggshell quality, body weight, and bone ash of laying hens.

Form of vitamin added	Amount per kilogram of diet (μg)	Egg production (%)	Eggshells with 0.001-mm deformation (%)	Body weight gain* (g)	Bone ash (%)†
None	0	35.7	21.9	16	55
Vitamin D_3	8	76.4	16.7	189	62
Vitamin D_3	12	68.0	15.2	216	62
1,25-$(OH)_2D_3$	1	76.0	15.1	386	61
1,25-$(OH)_2D_3$	2	70.5	17.9	273	65
1,25-$(OH)_2D_3$	4	73.5	15.8	198	67
1,25-$(OH)_2D_3$	8	64.1	16.0	57	64

*The hens averaged 1637 to 1708 g at 34 weeks and the weight gain is given in grams for the 34- to 61-week period. †Percentage of dry, fat-free weight.

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Fig. 1. Short upper mandible in an embryo from an unhatched egg produced by a hen fed on a diet low in vitamin D_3. The same condition occurs in embryos from hens provided with $1,25\text{-}(OH)_2D_3$.

bean meal (165), dicalcium phosphate (10), calcium carbonate (8), and sodium chloride (5). Minor ingredients added included (in milligrams per kilogram) riboflavin (2.2), MnO (300), vitamin A (5000 IU), erythromycin (20), and DL-methionine (500). Oystershell and water were provided ad libitum. The hens were maintained in individual wire cages. They were divided into groups of six hens and given the amount of vitamin D metabolites shown in Table 1 for 28 weeks. Egg production was calculated by dividing the number of eggs produced per day by the number of hens times 100. Shell deformation is a measure of eggshell strength (*2*). Percentage bone ash was measured in the tibia of each bird at the conclusion of the 28-week period. The purity of crystalline 25-hydroxyvitamin D_3 (25-OH-D_3) and $1,25\text{-}(OH)_2D_3$ (*3*) and of crystalline vitamin D_3 (*4*) was assessed by high-pressure liquid chromatography and by examination of their ultraviolet absorption spectra (*5*, *6*). Using a molar extinction coefficient of 18,200, we prepared appropriate solutions in ethanol (*6*). Portions were diluted in diethyl ether and added to cottonseed-soybean oil (Wesson). The ether and ethanol were removed by a stream of N_2 at room temperature, and the appropriate amount of metabolite was incorporated into the diet. Additions of Wesson Oil were made to give identical concentrations of the oil in all diets.

The hens in each group were inseminated weekly and the eggs collected were then incubated. In Fig. 2, the percentage of eggs from the hens in each group which produced normal chicks is plotted against the number of weeks on the diet. Virtually all of the eggs from hens given the 8 or 12 μg of vitamin D_3 per kilogram of diet hatched, giving normal chicks. Three weeks of vitamin D depletion resulted in 40 to 50 percent of the eggs failing to hatch. Most surprising, however, was the observation that as many as 80 to 90 percent of the eggs from hens given 1 to 8 μg of $1,25\text{-}(OH)_2D_3$ per kilogram of diet produced embryos which failed to hatch (Fig. 1). The embryos from the vitamin D deficient group which failed to hatch also showed the upper mandible defect. It was surprising that the hatching defect appeared much earlier in the $1,25\text{-}(OH)_2D_3$ fed hens than in the vitamin D deficient group and was not corrected by giving the hens higher doses of $1,25\text{-}(OH)_2D_3$. Although there are several possible explanations, one worthy of investigation is the possibility that $1,25\text{-}(OH)_2D_3$ or a metabolite might antagonize the transfer of either vitamin D or 25-OH-D_3 to the egg yolk thereby precipitating a deficiency at an earlier point in the depletion period than would be the case for vitamin D deficiency alone.

Table 2. Increase in the number of normal embryos in eggs from $1,25\text{-}(OH)_2D_3$ fed hens as a result of injection with vitamin D compounds. The values are presented as the mean ± standard error.

Injection	Eggs tested (No.)	Normal embryos (%)
None	36	15 ± 5
Acetone (0.05 ml)	26	11 ± 8
Vitamin D_3 (1.2 μg)	8	100 ± 15*
25-OH-D_3 (0.5 μg)	44	61 ± 15*
$1,25\text{-}(OH)_2D_3$ (0.5 μg)	43	46 ± 16*

*Significantly different from the group given 0.05 ml of acetone, $P < .001$.

To rule out the possibility that the failure of $1,25\text{-}(OH)_2D_3$ to support the development of normal embryos was due to instability or a lack of availability of the compound, we collected the data shown in Table 1. Unlike the vitamin D deficient group, the hens receiving $1,25\text{-}(OH)_2D_3$ had normal tibia ash, normal or better than normal body weight gain, normal egg production, and normal eggshell strength. The highest dose of $1,25\text{-}(OH)_2D_3$ actually suppressed weight gain and egg production—as might be expected because this is a rather high dose of this form of vitamin D. Even at this dose level, $1,25\text{-}(OH)_2D_3$ did not support normal embryonic development, however.

In studies not shown here, hens fed 25-OH-D_3 produced eggs which hatched normally. Therefore, the most likely explanation for the defective embryos from the $1,25\text{-}(OH_2)D_3$ fed hens is that the $1,25\text{-}(OH)_2D_3$ present in the diet, and thus in the hen's circulation, was not transferred to the egg. To test this hypothesis, eggs collected from hens given $1,25\text{-}(OH)_2D_3$ for 17 to 28 weeks were injected with vitamin D_3, 25-OH-D_3, or $1,25\text{-}(OH)_2D_3$ prior to incubation. For this study crystalline vitamin D compounds were dissolved in acetone (analytical reagent grade). The eggs were wiped with 80 percent ethanol and a hole was drilled aseptically into the air cell. Each egg received 0.05 ml of acetone containing the appropriate amount of vitamin D or metabolite. In preliminary tests this amount of acetone and this

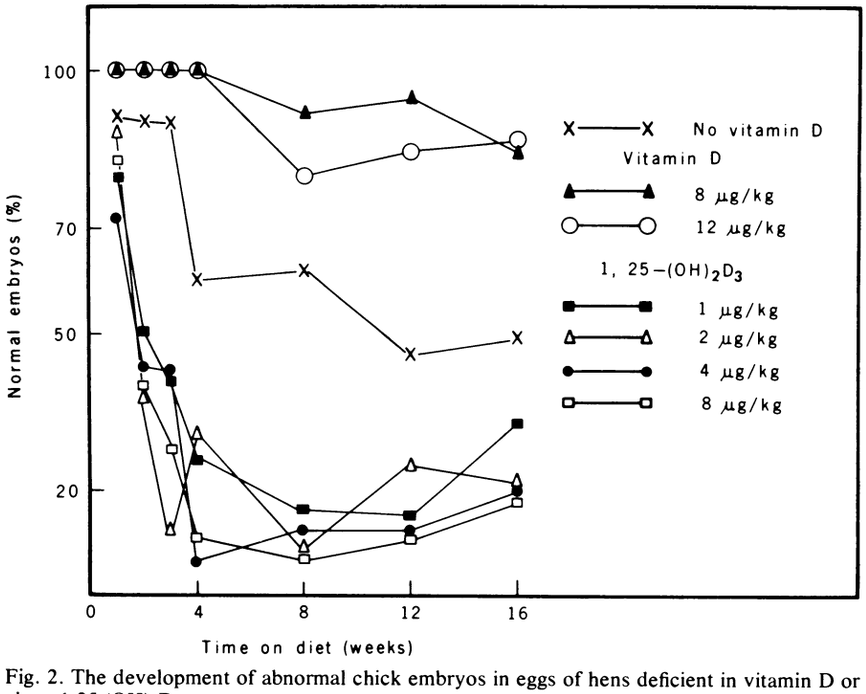

Fig. 2. The development of abnormal chick embryos in eggs of hens deficient in vitamin D or given $1,25\text{-}(OH)_2D_3$.

treatment did not affect the development of the embryos or their hatching from eggs of normal hens. In addition, the acetone alone did not affect the poor hatchability of the eggs under study (Table 2). The acetone solutions were aseptically placed near the yolk with a Hamilton syringe and the eggs sealed with collodion or Duco cement. All eggs were placed in the same incubator and the results are shown in Table 2. Injection of vitamin D_3 itself markedly improved the hatchability of the eggs. Although injection of either 25-OH-D_3 or 1,25-$(OH)_2D_3$ improved hatchability of the embryos, at the doses used, hatchability did not return to normal. Nevertheless there is no doubt that injection of all the vitamin D compounds markedly improved embryo development and hatching. These results demonstrate the importance of vitamin D and its metabolites in chick embryonic life and development. They also strongly suggest that 1,25-$(OH)_2D_3$ is not transferred in adequate amounts to the yolk from the maternal circulation.

Exactly why vitamin D deficiency causes a failure in mandible development is unknown, but improper calcium transport or defective collagen synthesis may be involved. In addition, chick embryos from eggs produced by hens maintained on 1,25-$(OH)_2D_3$ may provide an important experimental approach to some of the functions of vitamin D. It is also likely that hens maintained on 1,25-$(OH)_2D_3$ may be used to provide vitamin D deficient embryonic tissue for tissue culture experiments. Certainly these experiments demonstrate that 1,25-$(OH)_2D_3$ cannot satisfy all of the functions of vitamin D in the laying hen.

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Viremia in Experimental Creutzfeldt-Jakob Disease

Abstract. *Inoculation of the buffy coat of blood from guinea pigs infected with Creutzfeldt-Jakob disease resulted in passage of this disease to recipient animals. This demonstrates that there is a viremia in experimental Creutzfeldt-Jakob disease. These findings suggest that the hematogenous route may be implicated in the human infection and that the disease may possibly be transmitted by blood transfusions.*

The occurrence of viremia in encephalitides caused by conventional viruses is a well-recognized event, and its importance in the pathogenesis of these diseases has been emphasized (*1*). However, in spongiform virus encephalopathies, which include scrapie of the sheep, transmissible mink encephalopathy, and kuru and Creutzfeldt-Jakob disease of man (*2*), no viremia has been reported in the diseases affecting humans (*3*). Similarly, no stages of viremia have been detected in large-scale timed experiments dealing with the pathogenesis of scrapie. Thus, in experiments on the distribution of the scrapie virus in tissues and body fluids during the course of the infection in goats (*4, 5*) and in mice (*6*), no virus was found at any time in the circulating blood. However, in a few isolated instances and contrary to these pathogenesis studies, it has been claimed that blood or serum did contain the agent. Thus, serums and blood from mice taken up to 18 hours after inoculation (*7*), serum of a ram with natural scrapie (*8*), and serums of mice and rats (*9*) in the terminal stages of scrapie have been reported to be infective. In one study, the presence of scrapie agent in the blood in a small percentage of mice was thought most likely to result from tissue contamination (*10*).

We have transmitted Creutzfeldt-Jakob disease to guinea pigs and serially propagated it (*11, 12*). Using the guinea pig model, we undertook a series of timed experiments in order to study the pathogenesis of experimental Creutzfeldt-Jakob disease by virological and light and electron microscopic techniques. Our findings indicate the presence of viremia in this disease.

We inoculated 140 approximately 3-month-old guinea pigs (Hartley strain) with 0.1 ml intracerebrally of a 10^{-2} suspension of brain in normal saline from two guinea pigs that developed Creutzfeldt-Jakob disease during the fifth serial passage. Starting with the 1st week and at weekly intervals, up to the 28th week after inoculation, two inoculated guinea pigs (donors) were anesthetized with ether and the thorax was opened sterilely; then 8 ml of blood was removed, with a heparinized syringe, from the heart of each animal. After the blood was removed, the animals were killed and, in addition to the blood, various tissues including the central nervous system (CNS) were removed for virological and microscopic examinations. In our attempt to demonstrate the presence of viremia, we used the technique described by Horstmann (*13*). The blood removed from each animal was placed in a sterile polyallomer tube within a larger sterile polypropylene tube, centrifuged at 1000 rev/min for 15 to 20 minutes and frozen at −90°C overnight. The following day, each frozen tube was cut with a sterile blade approximately 0.5 cm beyond each side of the buffy coat. The section of the blood containing the entire

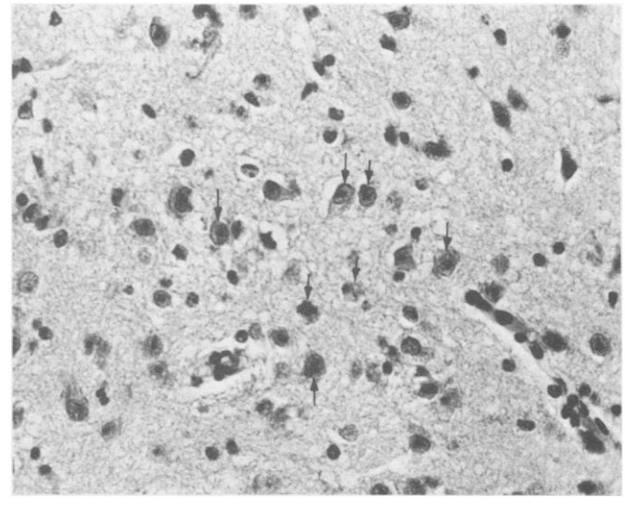

Fig. 1. Thalamus from animal inoculated with blood drawn at week 25. Several disintegrating neurons are seen (arrows) in a field showing spongiform changes of the surrounding neuropil. Hematoxylin-eosin (× 640).

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