exposure of 13-day cells to interferon. It is unlikely that this transferable inhibitory substance is a protease which directly destroys interferon since anti-interferon activity was not reduced when the inhibitor was prepared in medium which contained an excess of protein in the form of calf serum. It has been difficult to characterize the repressor because of its very low activity, like the labile repressor of messenger function in mammalian cells postulated by Tomkins *et al.* (*19*).

The acquisition of viral susceptibility and interferon unresponsiveness by older chicken embryonic cells upon exposure to cells from younger embryos represents a regression to an earlier embryonic state, that is, dedifferentiation. The coding for interferon and the postulated antiviral polypeptide which interferon induces is thought to reside in the DNA of the host cell and therefore must be present after fertilization. The change observed during embryonic differentiation in the inherent cellular capacity to express interferon activity points to a regulatory mechanism involving repression at an early embryonic stage. Lymphocytes which dedifferentiate to blast forms become more susceptible to viral infection and insensitive to interferon (*20*). Other dedifferentiated cells, of cancerous origin, contain such a repressor which exerts an anti-interferon action (*21*). What function a repressor of interferon might have in the young, rapidly differentiating embryo is puzzling, if indeed it is specific for interferon action alone. On the one hand, it may be an abort system in the face of embryonic viral infection. On the other, it may represent a state of heightened receptivity to circulating chemical inducers or messengers essential to the early embryo for chemical and morphologic differentiation (*22*).

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Vitamin D: A Cholecalciferol Metabolite Highly Active in Promoting Intestinal Calcium Transport

Abstract. *A major polar metabolite of cholecalciferol (vitamin D_3) obtained from chick intestines is over four times as effective as cholecalciferol and over two times as effective as 25-hydroxycholecalciferol in stimulating intestinal calcium transport 24 hours after administration. Following a considerable lag, cholecalciferol and its 25-hydroxy derivative produce a maximum stimulation of the transport response at 24 to 48 hours. The polar intestinal metabolite greatly shortens this lag, stimulating maximum calcium transport by 9 hours. At 9 hours this metabolite is at least 13 times as active as the parent cholecalciferol and as such is a likely candidate for the biologically active form of cholecalciferol in the intestine.*

The results of numerous studies have led to the hypothesis (*1, 2*) that the steroid cholecalciferol (vitamin D_3) acts through the activation of the expression of genetic information, in a manner analogous to steroid hormones such as estrogen and testosterone. However, it seems certain that cholecalciferol must first be converted to a new polar form prior to the stimulation of intestinal calcium transport (*3, 4*). The search for this new active form is an area of intensive investigation. A major reason for this interest is the number of pathological conditions which might be due to abnormalities in cholecalciferol metabolism. Thus, the osteomalacia and hypocalcemia associated with uremia (*5*), vitamin D–resistant rickets (*6*), and glucocorticoid antagonism of vitamin D action (*7*) have all been related in some degree to abnormalities in cholecalciferol metabolism. Similarly, the apparent abnormal sensitivity to cholecalciferol found in sarcoidosis (*8*) could also be related to a defect in cholecalciferol metabolism.

DeLuca and co-workers have previously isolated a polar metabolite of cholecalciferol from porcine serum (*3*). This compound, identified as 25-hydroxycholecalciferol, has been reported to be the "active form" of cholecalciferol (*9*). This steroid is 1.4 times as active as cholecalciferol in curing rickets (*3*), as measured by the rat line test. In addition, 25-hydroxycholecalciferol was not only more active in

stimulating intestinal calcium transport and elevating serum calcium in the rat, but the response was elicited somewhat more rapidly. Most importantly, in the perfused rat intestine (*10*) moderate amounts of 25-hydroxycholecalciferol stimulated intestinal calcium transport, while large doses of cholecalciferol had little or no effect.

Concurrent with this work, Norman and co-workers found that in the intestinal mucosa of the chick a single, biologically active polar metabolite of cholecalciferol, designated metabolite 4B (*11–13*), was selectively localized within the nucleus (*14*) by way of a nuclear receptor protein (*15*). Moreover, 25-hydroxycholecalciferol isolated from the blood was shown to be an intermediate in the formation of this nuclear metabolite (*4*). Kodicek *et al.* (*16*) have reported similar findings. Recently, DeLuca *et al.* have also confirmed that 25-hydroxycholecalciferol is not the major form of cholecalciferol in the intestinal mucosa nucleus (*17*).

We now report further evidence that suggests that metabolite 4B, and not 25-hydroxycholecalciferol, is a likely candidate for the active form of cholecalciferol in the intestine.

White Leghorn cockerels (1 day old) were placed on a cholecalciferol-deficient diet (*13*) and were used during their fourth week. At this time they were severely rachitic. Groups of 100 to 600 rachitic chicks were dosed intracardially with generally labeled [^{3}H]-cholecalciferol [50 international units (I.U.)] (*12*). Twenty-four hours later, metabolites 4A and 4B were isolated from the blood and intestine, respectively (*4*). Metabolites 4A and 4B, along with standard amounts of crystalline cholecalciferol and 25-hydroxycholecalciferol, were then examined for biological activity by the in vivo intestinal calcium transport assay of Coates and Holdsworth (*18*), as modified by Hibberd and Norman (*13*). In this assay, 24 hours after oral or intracardiac administration of the compound being tested, 2.0 mg of a mixture of radioactive and nonradioactive Ca^{2+} (10 μc) is placed in an exposed duodenal loop. Thirty minutes later, the appearance of $^{45}Ca^{2+}$ in the plasma is determined (Table 1). All compounds examined were dissolved in 0.2 ml of 1,2-propanediol and administered orally. Controls received 0.2 ml of 1,2-propanediol. In each assay, standard amounts of cholecalciferol were also assayed. The radioactive metabolites 4A and 4B were administered at varying doses. The exact amount of metabolite in each dose was determined from the specific activity of the parent cholecalciferol from which they were derived and from the assumption that no tritium was lost in the metabolic conversion. For generally labeled [^{3}H]-cholecalciferol this assumption is valid (*4*). The 25-hydroxycholecalciferol was administered at various doses for determination of its intestinal biological activity by defining 1.0 I.U. as equivalent to 0.025 μg. Metabolites 4A and 4B and 25-hydroxycholecalciferol were considerably more active than cholecalciferol in stimulating intestinal

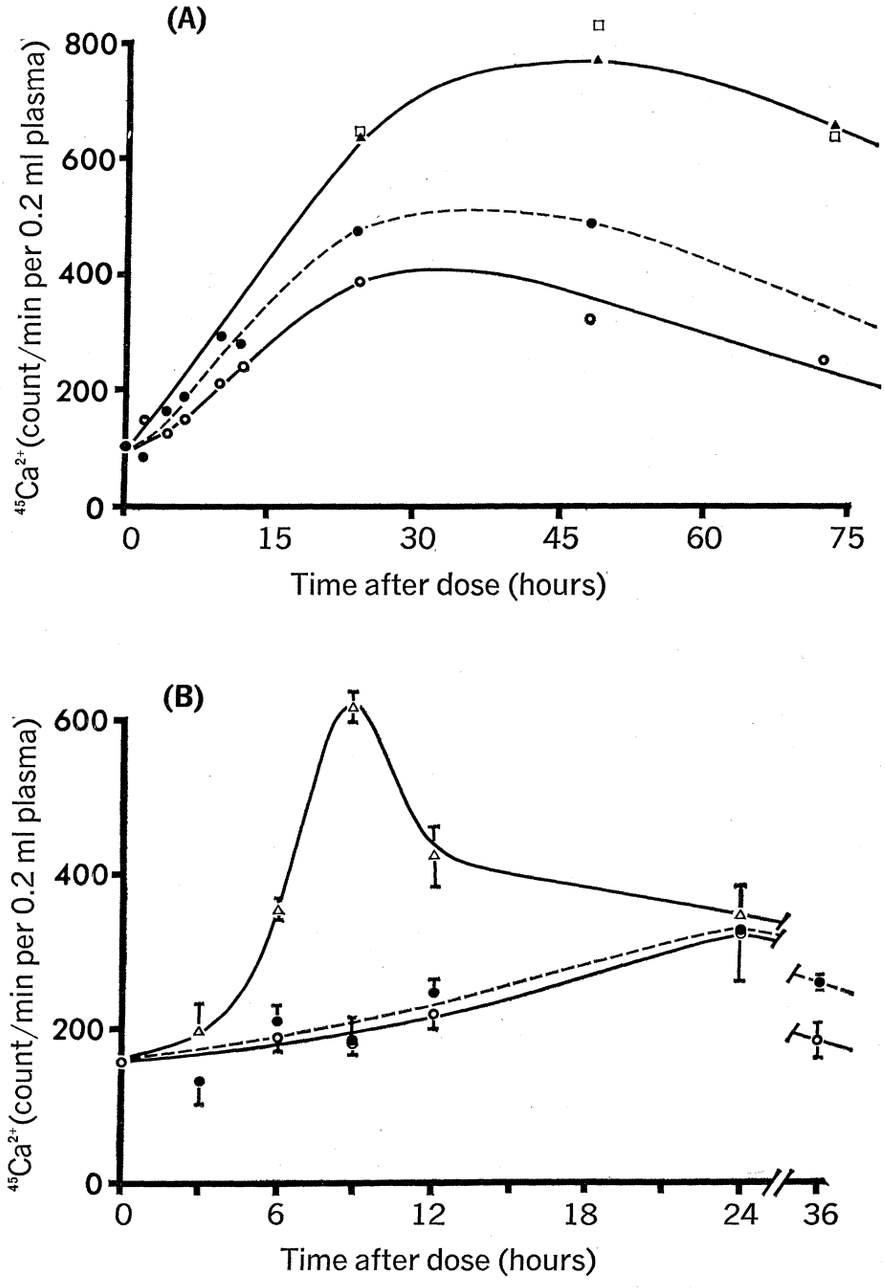

Fig. 1. Time course of the intestinal calcium transport response. Each point represents the average of four to six chicks. Transport was assayed as described in the text. In (A), the compounds assayed were dissolved in 1,2-propanediol and administered intracardially. Open circles, 10 I.U. of cholecalciferol; closed circles, 10 I.U. of 25-hydroxycholecalciferol; open squares, 50 I.U. of cholecalciferol; closed triangles, 500 I.U. of cholecalciferol. In (B), the compounds were administered orally. Open circles, 5 I.U. of cholecalciferol; closed circles, 5 I.U. of 25-hydroxycholecalciferol; open triangles, 1.8 I.U. of metabolite 4B. Each point represents the average ± S.E. for $^{45}Ca^{2+}$ absorption of three to five chicks.

calcium transport. If the response obtained from a known amount of metabolite is compared with the amount of cholecalciferol required to elicit an equivalent transport response, the result can be expressed in terms of a relative biological activity (Table 2). Metabolite 4A and 25-hydroxycholecalciferol are both approximately two times as active as cholecalciferol in stimulating intestinal calcium transport. However, metabolite 4B is even more active, averaging 4.5 times the activity of cholecalciferol.

Clearly, metabolite 4B is highly active in initiating intestinal calcium transport but so is 25-hydroxycholecalciferol. If metabolite 4B is the active form of cholecalciferol in the intestine and 25-hydroxycholecalciferol an intermediate in its formation, then metabolite 4B should act considerably faster than either cholecalciferol or its 25-hydroxy derivative, since the latter steroids would have to undergo an obligatory metabolic conversion before becoming biologically effective. For comparison of rates of action of these compounds, the ability of 10 I.U. of cholecalciferol and 25-hydroxycholecalciferol to stimulate intestinal calcium transport was measured as a function of time after intracardiac administration (Fig. 1A). In addition, 50 and 500 I.U. of cholecalciferol were measured at three time periods. After an initial lag, 10 I.U. of cholecalciferol produced a maximum stimulation between 24 and 48 hours after administration. The 25-hydroxy derivative acted only slightly faster than the parent cholecalciferol, and the maximum increase attained was only somewhat greater. Cholecalciferol (50 or 500 I.U.) reduced the lag slightly further and produced maximum stimulation around 48 hours.

Table 1. Intestinal calcium transport response to cholecalciferol and its metabolites. Transport was assayed 24 hours after oral administration of the compound tested. One international unit (I.U.) of cholecalciferol is equivalent to 0.025 μg. The numbers in parentheses indicate the number of birds per group. Values given are the mean ± standard error of the mean.

Compound	Dose (I.U.)	$^{45}Ca^{2+}$ (count/min per 0.2 ml of plasma)
	Assay 1	
Control	0	157 ± 9 (3)
Cholecalciferol	2.3	185 ± 15 (3)
Cholecalciferol	5.7	217 ± 14 (4)
Metabolite 4B	4.1	228 ± 21 (4)
	Assay 2	
Control	0	70 ± 10 (5)
Cholecalciferol	2.6	97 ± 10 (5)
Cholecalciferol	5.2	142 ± 33 (5)
Cholecalciferol	10.4	205 ± 31 (4)
Cholecalciferol	20.8	297 ± 66 (5)
Metabolite 4B	3.1	302 ± 10 (3)
Metabolite 4A	5.2	208 ± 47 (5)
	Assay 3	
Control	0	110 ± 21 (8)
Cholecalciferol	1.3	142 ± 20 (4)
Cholecalciferol	2.6	214 ± 17 (5)
Cholecalciferol	5.2	167 ± 13 (5)
Cholecalciferol	10.4	279 ± 14 (4)
Cholecalciferol	20.8	538 ± 72 (5)
Metabolite 4B	1.9	258 ± 21 (4)
Metabolite 4A	1.5	181 ± 36 (5)
Metabolite 4A	3.0	225 ± 23 (6)
Metabolite 4A	6.1	358 ± 65 (5)
25-Hydroxycholecalciferol	1.7	198 ± 40 (5)
	Assay 4	
Control	0	163 ± 18 (6)
Cholecalciferol	1.3	158 ± 28 (5)
Cholecalciferol	2.6	213 ± 18 (5)
Cholecalciferol	5.2	329 ± 94 (4)
Cholecalciferol	10.3	311 ± 34 (5)
Cholecalciferol	20.7	555 ± 49 (4)
Cholecalciferol	500	791 ± 99 (5)
Metabolite 4B	1.8	345 ± 42 (4)
25-Hydroxycholecalciferol	5.0	328 ± 53 (4)

Table 2. The biological activities of metabolites 4B and 4A and 25-hydroxycholecalciferol, relative to an equal amount of cholecalciferol. These results are calculated from the data given in Table 1 which reports the intestinal transport response obtained 24 hours after oral administration of the metabolites. For each experiment in Table 1, a standard curve of response versus amount of administered cholecalciferol was prepared. Then the responses of the test compound and cholecalciferol were compared to determine a relative biological activity. Values are the means ± the standard error of the mean. Numbers in parentheses indicate the total number of separate determinations.

Compound tested	Relative activity
Metabolite 4B (intestine)	4.74 ± 1.09 (4)
Metabolite 4A (blood)	2.04 ± 0.13* (5)
25-Hydroxycholecalciferol	1.78 ± 0.23† (5)

* Includes one additional assay not included in Table 1. † Includes three additional assays not included in Table 1.

The steroids were also administered orally, and the action of 1.8 I.U. of metabolite 4B was compared with 5 I.U. of both cholecalciferol and its 25-hydroxy derivative (Fig. 1B). After a considerable lag, both cholecalciferol and 25-hydroxycholecalciferol produced a maximum increase in calcium transport at around 24 hours. The response to metabolite 4B was remarkably different. The lag in stimulation of Ca^{2+} transport, while still present, was considerably shorter, and maximum stimulation occurred at approximately 9 hours. Moreover, the maximum Ca^{2+} transport response produced by 1.8 I.U. of metabolite 4B far exceeded the maximum response obtained with 5 I.U. of either cholecalciferol or 25-hydroxycholecalciferol. The stimulation of 1.8 I.U. of metabolite 4B attained after 9 hours is approximately 78 percent of the total response obtained from 500 I.U. of cholecalciferol after 24 hours. Comparison of the response of metabolite 4B at 9 hours with the response obtained from standard amounts of cholecalciferol at 24 hours indicates that metabolite 4B is over 13 times as active as cholecalciferol.

These results demonstrate the occurrence of an extremely potent polar form of cholecalciferol, metabolite 4B. At the time of the initiation of the physiological response, this is the only metabolite of cholecalciferol found in the nucleus (*4*) of the chick intestinal mucosa cell. This nuclear-bound metabolite is found in the intestine when either radioactive cholecalciferol or 25-hydroxycholecalciferol is administered (*4*). Metabolite 4B is also present in the intestine of the rat, pig, and several other species (*19*). The fact that metabolite 4B acts considerably faster than 25-hydroxycholecalciferol in stimulating calcium transport is consistent with the finding that 25-hydroxycholecalciferol is an intermediate in the formation of intestinal metabolite 4B. Because 25-hydroxycholecalciferol stimulated perfused intestinal calcium transport, but cholecalciferol did not (*10*), it seems likely that 25-hydroxycholecalciferol is converted to metabolite 4B at the target tissue. The time course of the appearance of 25-hydroxycholecalciferol and metabolite 4B in the intestine (*4*) is consistent with this view. The rapid conversion of 25-hydroxycholecalciferol to more polar intestinal nuclear metabolites has also recently been reported by DeLuca *et al.* (*17*). Although it is not known definitely, it is likely that one of their more polar metabolites, possibly peak V, is identical with metabolite 4B.

To date, metabolite 4B is the most potent form of cholecalciferol, in terms of stimulating intestinal calcium transport. On the basis of these results and other studies (*4, 14*), it seems probable that metabolite 4B is the active form of cholecalciferol in the intestine. However, confirmation of this through further biological and chemical characterization is rendered very difficult due to

an apparent control of the amount of metabolite 4B found in the intestine. Administration of massive amounts of cholecalciferol does not increase the amount of metabolite 4B found in the intestine above approximately 0.2 I.U. (0.05 μg) per chick intestine (*4, 16*). In addition, it remains to be determined whether metabolite 4B is active in other cholecalciferol target tissues such as bone or whether it will be effective in alleviating any of the abnormalities in calcium metabolism discussed earlier. Until metabolite 4B is characterized and synthesized, these questions will probably remain unanswered.

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Superior Colliculus Cell Responses Related to Eye Movements in Awake Monkeys

Abstract. *Single cell responses were recorded from the superior colliculus of awake monkeys trained to move their eyes. A class of cells that discharged before eye movements was found in the intermediate and deep layers of the colliculus. The response of the cells was most vigorous before saccadic eye movements within a particular range of directions. These cells had no visual receptive fields, and visually guided eye movements were not necessary for their discharge, since they responded in total darkness before spontaneous eye movements and vestibular nystagmus.*

The superior colliculus has long been suspected to be associated with eye movements (*1*). Electrical and chemical stimulation experiments (*2*) have substantiated this point of view. Ablation of the superior colliculus has shown lasting effects on eye movements in some cases (*3*) but not in others (*4*). However, studies of single cells in the superior colliculus have dealt not with the effects of eye movements but with the determination of the visual receptive fields (*5*) or of proprioceptive input (*6*). We have now recorded responses of single cells in the superior colliculus of awake monkeys trained to make eye movements, and we have found neurons related specifically to eye movements rather than to any visual stimuli.

Rhesus monkeys (*Macaca mulatta*) were trained to look at a spot of light for periods of 1 to 3 seconds (*7*). The spot (width, 0.5 degree of arc) was projected on a tangent screen at a distance of 58 cm in front of the monkey. The spot could be held stationary or could be moved continuously with speeds ranging from 5 to 125 degrees of arc per second. This fixation point could also be made to jump instantaneously from one point to another by turning off the first spot as a second one at a different point came on. These methods could reproducibly elicit fixation, smooth pursuit eye movements, or saccadic eye movements.

During recording experiments the monkey's head was held rigidly according to the method developed by Evarts (*8*). Eye movements were measured by using vertical and horizontal electrooculograms (*7*), and lateral rectus electromyograms were obtained from ball electrodes implanted on the capsule overlying the lateral recti. The activity of single cells in the superior colliculus was recorded extracellularly using platinum-iridium microelectrodes insulated with glass that were positioned stereotaxically by a movable microdrive (*8*). The amplified signals from the neurons, electrooculograms, and electromyograms, together with information about the monkey's behavioral responses, were displayed on oscilloscopes and a penwriter and were stored on magnetic tape. At the end of the experiment, monkeys were killed, perfused with 0.95 percent saline and then 10 percent formalin, and 50-μm sections through the superior colliculus were stained alternately with Weil and cresyl violet stains.

We studied 332 cells in the superior colliculus of three monkeys. The anatomical location of 157 cells within the colliculus was determined histologically by proximity to electrolytic lesions made at the time of recording: 50 percent of the cells were in two of the upper layers of the superior colliculus (stratum opticum and stratum griseum superficiale), 40 percent in the intermediate layers (stratum griseum intermedium and stratum album intermedium), and 10 percent in the deep layers (stratum griseum profundum and stratum album profundum). The cells in the two upper layers had visual receptive fields, as has been reported previously (*5*). In the four deeper layers a variety of cell types were found, but in this report we consider only those cells related exclusively to eye movements. Of the histologically identified cells in the intermediate and deep layers, 20 percent were of this type; in the superficial layers, there were none. We studied 26 of these cells in detail.

The change in discharge rate of each cell preceded the eye movement, as is