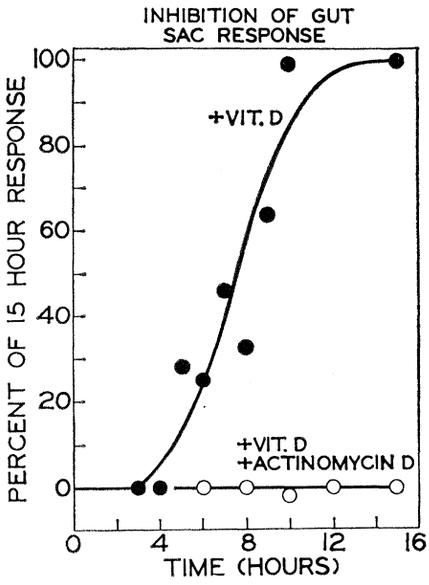

Fig. 1. Time course of the response of intestinal calcium transport to vitamin D in the presence and absence of actinomycin. The antibiotic was administered 1 hour before the vitamin. Each point on the graph represents the mean of values from five animals.

Conceivably, the vitamin may induce the synthesis of a calcium-translocating enzyme with which parathyroid hormone interacts to further stimulate transport of this ion. From this suggestion it would follow that without the vitamin-induced protein, as in extreme vitamin-D deficiency, no effect of parathyroid hormone on calcium metabolism would occur. That this is, in fact, the case has already been demonstrated (*12*). The blockage of hormone action by actinomycin observed by Rasmussen *et al.* might then be explained as an actual inhibition of vitamin-D function which renders these animals essentially vitamin-D deficient and thus insensitive to parathyroid hormone. This explanation would also account for the initial response to the hormone in actinomycin-treated animals, since loss of hormone action would only be seen once the protein synthesized by vitamin D and the messenger RNA for this protein were degraded.

This hypothesis would also explain the time lag between administration of the vitamin and the characteristic responses, the lack of intestinal-sac response to vitamin D added in vitro, the very low dose requirement, and the well-known inborn refractoriness to vitamin-D administration.

To explain the responses in vitro of subcellular systems to vitamin D, it may be necessary to suggest that the vitamin molecule does have a secondary effect on membrane permeability to calcium ions. This is consistent with data concerning the influence of vitamin D on calcium flux in the small intestine (*13*). However, this effect may be of a general nature, due mainly to hydrophobic interactions of the nonpolar vitamin with lipoid membrane systems, and may require large, nonphysiological amounts of the vitamin. In any case, this suggestion alone cannot account for all of the actions of vitamin D.

Finally, while much information must be forthcoming before a final answer concerning the mechanism of action of vitamin D is obtained, the suggestions presented here may stimulate investigations which can lead to this answer. Certainly, these ideas are all amenable to continued experimental examination, and it is in this spirit that they are now proposed.

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15. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported by a research grant No. AMO-5800-04 from NIH. We thank the Cancer Chemotherapy Center of the National Institutes of Health, U.S. Public Health Service, for the actinomycin, and Eli Lilly and Company for its supply of parathyroid extract.

25 March 1965

Actinomycin D and the Response to Vitamin D

Abstract. *The administration of low doses of actinomycin D to rachitic chicks inhibits the action of a subsequent dose of vitamin D_3 in promoting calcium absorption from the intestine.*

Many studies have demonstrated the essential role of vitamin D in mediating the absorption of calcium in the intact animal and in everted intestinal sacs or intestinal slices from the rat and chick (*1*, *2*). But despite intensive efforts the exact biochemical mechanism and loci of action of the vitamin remain unknown.

One of the characteristic features of the physiological expression of vitamin-D activity has been the time lag between the administration of vitamin D and the enhancement of calcium absorption across the intestinal mucosa. Although suboptimum enhancement of calcium absorption occurs in the rat in 3 to 5 hours after a massive dose [50,000 international units (I.U.)] of vitamin D, maximum expression of vitamin-D activity is not apparent until after 12 to 15 hours (*3*). In the chick very little calcium absorption enhanced by vitamin D_3 occurs until 12 to 16 hours after either an oral, intracardial, or intraperitoneal injection of 100 I.U. of vitamin D_3 (*2*, *4*).

It has been suggested (*5*) that the vitamin must be converted by the adrenals to a metabolically active form before the increased absorption of calcium can be observed. However, little direct evidence for this hypothesis has been found. Experiments with tritium-labeled vitamins D_2 and D_3 revealed that, in both the chick and rat, the adrenals accumulated an insignificant amount of vitamin D in any time interval after the administration of the ^{3}H-vitamin D. Further experiments with the chick revealed that the intestinal mucosa accumulated 70 percent of the radioactivity found at 30 hours within 3 to 5 hours after either an intracardial or intraperitoneal dose of 500 I.U. of ^{3}H-vitamin D (*6*). Although vitamin D was present at 5 hours, the manifestation of this vitamin did not become apparent until after 12 to 16 hours.

A possible hypothesis for the delay in biological response to vitamin D may be that delay reflects some as yet undefined induction process. Perhaps one or several enzymes necessary for the mediation of calcium absorption are synthesized by the mucosa cells only

under the stimulus of the vitamin. Thus, actinomycin D, which blocks DNA-directed RNA synthesis and subsequent protein synthesis, would be expected to inhibit any protein synthesis, induced by vitamin D, essential to calcium absorption. There have been numerous reports documenting the potent inhibitory action of actinomycin D on RNA and protein synthesis (*7*).

The results (Fig. 1) record the effect of an oral dose of 100 I.U. of vitamin D_3 upon the intestinal absorption of calcium in rachitic chicks at varying intervals after administration of the vitamin. Intestinal absorption of calcium was measured by the appearance of ^{45}Ca in the serum prepared from whole blood obtained from the wing vein 60 minutes after oral intubation of 2.0 mg of ^{45}Ca-labeled calcium chloride. The concentration of ^{45}Ca in the serum increases approximately linearly with time from 15 minutes until 1½ hours after the oral dose. At this time the ^{45}Ca falls slowly, undoubtedly because of equilibration with rapidly exchangeable bone ^{40}Ca.

No significant enhancement by vitamin D of calcium absorption can be observed before 12 to 16 hours. But, if actinomycin D (10 μg/110 g of body weight of chick) is injected intraperitoneally 2 hours before the oral administration of 100 I.U. of vitamin D_3, the characteristic response of increased calcium absorption is abolished.

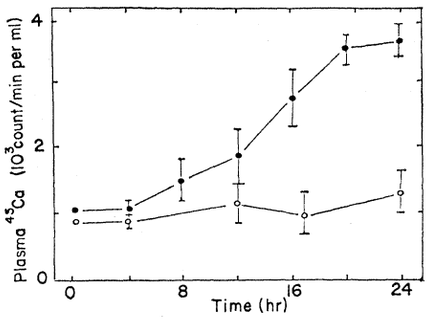

Fig. 1. The serum-calcium response of vitamin-D deficient chicks to an oral dose of 100 I.U. of vitamin D_3 in the absence (closed circles) and presence (open circles) of actinomycin D. The rachitic chicks received 10 μg actinomycin D per 110 grams of body weight in 0.20 ml of 0.9 percent NaCl intraperitoneally 2 hours before the dose of vitamin D_3 (in Wesson oil) was administered orally. At varying times after the vitamin D_3 treatment, 2.0 mg of ^{40}Ca + ^{45}Ca (1 to 3 × 10^6 count/min) in 0.20 ml was fed orally. Blood from wing veins was drawn with syringes containing heparin 30 and 60 minutes later. Each point represents the mean of the values obtained on the plasmas of 3 to 5 chicks (± s.d.).

Table 1. Inhibition by actinomycin D of calcium absorption mediated by vitamin D. Chicks were raised to the age of 4 to 5 weeks on a rachitogenic chick diet (commercial source) until the growth rate reached a plateau. Chicks in Experiment A, unless indicated otherwise, received 100 I.U. of vitamin D_3 2 hours after actinomycin D and 24 hours before ^{45}Ca. Chicks in Experiment B, unless indicated otherwise, received 20,000 I.U. of vitamin D_3 2 hours after actinomycin D and 24 hours before ^{45}Ca. Chicks in Experiment C received 4000 I.U. of vitamin D_3 daily for 4 days prior to actinomycin-D treatment, and ^{45}Ca was administered 26 hours after the actinomycin D. Actinomycin D was injected intraperitoneally in 0.20 ml 0.9 percent NaCl. Vitamin D_3 was injected intraperitoneally in 0.20 ml of 1,3-propanediol; 2.0 mg of ^{40}Ca + ^{45}Ca (1 to 3 × 10^6 count/min) in 0.20 ml was fed orally. Blood from wing veins was collected in syringes containing heparin 30 and 60 minutes after ^{45}Ca administration. Each number is the average of values from 7 to 12 chicks. One international unit (I.U.) of vitamin D_3 is equivalent to 0.025 μg.

Vitamin D_3 treatment (I.U.)	Actinomycin treatment (μg/110g chick)	^{45}Ca incorporation (count/min) per ml	
		30 min	60 min
	Experiment A		
None	None	840 ± 230	1200 ± 240
100	10	780 ± 320*	1280 ± 360*
100	1	1290 ± 500†	1500 ± 430†
100	None	2440 ± 430*†	2750 ± 370*†
	Experiment B		
None	None	350 ± 70	600 ± 150
20,000	10	690 ± 420‡	750 ± 250*
20,000	None	1770 ± 390‡	2100 ± 490*
	Experiment C		
4000 24 hr before actinomycin D	None	2310 ± 320	2920 ± 290
4000 24 hr before actinomycin D	10	2500 ± 450	3100 ± 150

* Difference between ± actinomycin D treatment is significant $P < .001$. † Difference between ± actinomycin D (1 μg/110 g chick) is significant $P < .02$. ‡ Difference between ± actinomycin D (10 μg/110 g chick) treatment is significant $P < .01$.

A more detailed examination of the relation between vitamin D and actinomycin D is shown in Table 1. In these experiments the vitamin D_3 was administered intraperitoneally rather than orally to avoid any possible effect of actinomycin D upon intestinal absorption of the vitamin. In confirmation of the results of Fig. 1, actinomycin D (10 μg per 110 grams of body weight) effectively blocked a dose of 100 I.U. of vitamin D_3. Moreover, when as little as 1 μg of actinomycin D per 110 grams of body weight was administered, an approximate 50-percent inhibition of the calcium absorption response to a dose of 100 I.U. of vitamin D_3 was observed. This is one of the lowest effective dosages for actinomycin reported. But if chicks deficient in vitamin D were given an oral dose of 4000 I.U. of vitamin D_3 daily (Experiment C) for 4 days before the intraperitoneal injection of actinomycin D (10 μg/110 g chick), no impairment of calcium absorption was observed. This finding tends to exclude the likelihood that the inhibitory effects are due to blocking of parathyroid-hormone synthesis by actinomycin. If parathyroid hormone were obligatory for calcium absorption, then actinomycin D should have blocked calcium absorption in the chicks which received 4000 I.U. of vitamin D_3 4 days before actinomycin D.

Perhaps more significant is the actinomycin inhibition of an intraperitoneally injected dose of 20,000 I.U. of vitamin D_3 (Experiment B). If vitamin D functioned strictly as a coenzyme, this amount of the vitamin should be adequate to saturate any receptor sites crucial for calcium absorption that would be present. Yet apparently any manifestation of the vitamin activity is strictly dependent upon the sequence of actinomycin-sensitive events involved in the conversion from a deficient to a normal state.

Not all lags or latent metabolic events are *a priori* sensitive to actinomycin D. Ray *et al.* have reported (*8*) that glucocorticoids can effectively stimulate gluconeogenesis (*9*) in adrenalectomized or normal rats even though the rats have been treated with sufficient actinomycin D to block induced synthesis of enzymes. Also, Greengard and Gordon (*10*) observed a lag in the pyridoxine-induced rise of tyrosine transaminase, in rat liver, that was sensitive to puromycin but insensitive to actinomycin D. The possibility remains, however, that other vitamins may act on enzyme synthesis by way of DNA-directed RNA synthesis.

One cannot decide from the foregoing experiments whether the biochemical role of vitamin D is to promote the induction of the appropriate enzyme systems or the alteration of membrane structure necessary for calcium absorp-

tion, or whether vitamin D in addition to participating in such an induction process can also function as an obligatory catalyst in the induced system. However, the promotion of calcium absorption by vitamin D probably requires an unimpaired RNA-synthesizing system.

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9 April 1965

Red-Light Thresholds in Heterozygote Carriers of Protanopia: Genetic Implications

Abstract. *Absolute thresholds in response to red light were compared in nine normal subjects, six female carriers of protanopia (heterozygotes), and six male subjects with protanopia. The fovea and four peripheral retinal areas were tested, and all data were obtained before the occurrence of the rod–cone break. Elevated thresholds were found in all retinal areas tested in protanopic males, at the fovea in all carriers, and in some peripheral retinal areas in two carriers. The thresholds for carriers were far below those for the protanopic males, and no greater variability of threshold was found in the carriers when they were compared with the normal control group. The findings do not substantiate the occurrence of inactivation at the locus on the X chromosome for protanopia.*

Protanopia and protanomaly are defects of color vision characterized by the partial or complete absence of the ability to discriminate hues in the red-yellow-green region of the spectrum and by a decrease in sensitivity to brightness at the red end of the spectrum. The protanomalous subject can match all colors of the spectrum with mixtures of three hues but requires more red in each mixture than the normal subject. The protanope, on the other hand, can match all colors of the spectrum with only two hues. The genes for these defects are carried at the same locus on the X chromosome. Males, therefore, always show a color-vision defect, whereas females with only one defective gene (heterozygotes) are considered to be carriers.

In a previous study in which we used five males with protanopia and protanomaly and eight female carriers of these defects, we determined absolute thresholds in response to red light at numerous retinal areas in order to test the X-chromosomal inactivation hypothesis of sex-linked inheritance (*1*). Elevated threshold responses to red light of the same magnitude were noted in four of the five color-defective males and in seven of the eight carriers, for a one-degree target located five degrees from the fovea. There was no evidence of greater variability of thresholds in female carriers than in a control group with normal vision.

Since fully dark-adapted eyes were tested in the previous study, the rods probably functioned as red-light detectors at retinal areas tested outside of the macula (*2*). In the study described here, the eyes were adapted to such a level of cone activity that the participation of rods in the detection of red light in peripheral retinal areas was eliminated. Absolute thresholds to red light were compared in normal subjects, female carriers of protanopia defects (heterozygotes), and males with protanopia.

Six protanopes, previously identified with the Nagel anomaloscope, and six female carriers of the defect were tested. All carriers were either mothers or daughters of males known to have protanopia. Carriers Ia and Ib were related to protanope I, carriers IIa and IIb to protanope II, and carrier III to protanope III. Carrier IV was related to a subject with protanopia who was tested in a previous study (*1*). All subjects were normal on routine ocular examination. A control group of nine subjects with normal vision was used.

The method of testing eliminated or minimized the participation of rods in the detection of red light. At the fovea, threshold determinations reflect mainly cone activity, and a monophasic curve (*3*) is obtained if a target of sufficiently small size is used (0.25 degree in this study). However, outside of the fovea, threshold measurements reflect both cone and rod activity and a biphasic curve is obtained. The first portion of the curve reflects cone activity and the second portion rod activity. At the onset of dark adaptation a rapid drop in threshold occurs, and in a few minutes a constant value called the cone plateau is reached. A further drop in threshold occurs after 5 minutes or more (depending on how much light the subject is exposed to before the test begins) and this point is called the rod–cone break. The change in the threshold after the rod–cone break reflects rod activity. We attempted to obtain all our data before the occurrence of the rod–cone break.

Studies of dark adaptation were performed after a period of careful in-

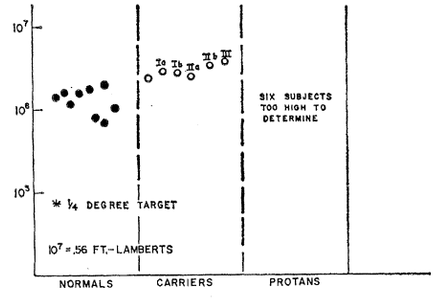

Fig. 1. Foveal thresholds of the three groups, a one-fourth degree target being used. Thresholds could not be obtained in any of the six protanopes. The six carriers have thresholds above the highest threshold in the control group. The five carriers with tested male relatives are identified.