haustion at its depots. The sharp fall in the tocopherol level in the liver against the background of activation of FRO may facilitate the disturbance of liver function, especially of synthetic processes. During exposure to cold, a combination of adaptive reactions thus develops, systemic in character and aimed at maintaining primarily oxidation–reduction reactions in the lungs, while they perform their respiratory and nonrespiratory (temperature-regulating) functions. This evidently is the explanation of the increased vulnerability of the lungs, both in animals and in man, during prolonged exposure to cold.

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DIRECT STIMULATING ACTION OF BLOOD SERUM AND OF VITAMIN D_3 AND ITS HYDROXY-ANALOGS ON CALCIUM TRANSPORT IN THE CHICKEN SMALL INTESTINE in vitro

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Stimulation of absorption of Ca^{++} in the animal intestine is one of the more important functions of vitamin D. In the modern view [1, 8], vitamin D_3 exerts its action through biochemical conversions in accordance with the following scheme: vitamin D_3 → 25-hydroxyvitamin D_3 (25-OHD_3) → 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) → transcription of DNA in cell nuclei of intestinal epithelium – de novo synthesis of calcium-binding protein (CaBP) → absorption of Ca^{++} in the intestine. This mechanisms of the action of the final active form of vitamin D_3, namely 1,25-$(OH)_2D_3$, is similar to the action of steroid hormones which induce in target organs the synthesis of specific proteins which perform a physiological function.

The role of CaBP, synthesized in the intestinal epithelium under the influence of vitamin D, in the process of Ca^{++} absorption has been demonstrated by many experiments [6, 17]. The present writers showed [2] that introduction of exogenous CaBP into the intestine of rachitic chicks restores their disturbed Ca^{++} transport.

However, recent evidence has been obtained [5, 14] that CaBP synthesis does not correlate with the increase in Ca^{++} transport under the influence of 1,25-$(OH)_2D_3$. It has been suggested that this steroid can directly influence the permeability of intestinal epithelial cells for the cation. The question of the action of vitamin D_3 and its analogs on Ca^{++} transport in vitro accordingly arises. One source of active metabolites of vitamin D_3, namely 25-OH_3 and 25-$(OH)_2D_3$, may be the blood serum of animals receiving vitamin D_3 [10].

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TABLE 1. Action of Blood Serum on Ca^{++} Transport in Nonreversed Intestinal Sac Depending on Dose and Time of Injection of Vitamin D_3 into Chickens (in nmoles/cm)

Time after injection of vitamin D_3, h	Dose, i.u.					
	0	50	250	500	1000	20 000
0	25,9±2,9	—	—	—	—	—
1	—	—	—	—	72,3±5,7	—
6	—	—	—	—	69,0±6,1	—
12	—	—	—	—	49,2±6,7	—
24	—	67,0±6,5	93,7±8,3	55,1±6,1	46,7±2,8	62,5±5,6
48	—	—	—	—	45,9±3,7	—
72	—	—	—	—	47,2±4,6	—

Legend. Blood serum was obtained from chickens into which vitamin D_3 had been injected intramuscularly in different doses and at different times before sacrifice. Investigations of transport were carried out on an intestinal sac from control chickens.

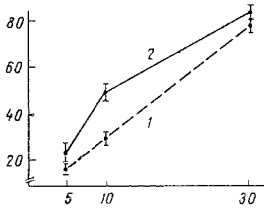

Fig. 1

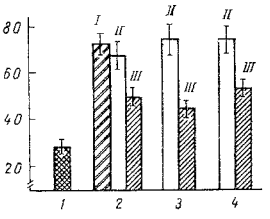

Fig. 2

Fig. 1. Dynamics of Ca^{++} transport after introduction of blood serum from chickens receiving and not receiving vitamin D_3 into small intestine of chickens with avitaminosis D. Abscissa, time of incubation (in min); ordinate, Ca^{++} transport in nmoles/cm); 1) control chickens, 2) experimental chickens.

Fig. 2. Action of vitamin D_3, 25-OHD_3, and 1α-OHD_3, introduced into intestinal lumen of chickens with avitaminosis D by addition to blood serum. Ordinate, Ca^{++} transport (in nmoles/cm). 1) Blood serum of control chickens, 2) 25-OHD_3, 3) 1α-OHD_3, 4) vitamin D_3; I) 6.25 ng; II) 25 ng; III) 250 ng.

In the investigations described below the action of chick blood serum on Ca^{++} transport in the small intestine was compared in vitro depending on the donors' vitamin D_3 intake, and the action of vitamin D_3 and its hydroxy-analogs (25-OHD_3, 1α-OHD_3), introduced into the intestinal lumen of chicks with avitaminosis D, also was studied.

EXPERIMENTAL METHOD

Chickens of the Leghorn breed were used. From the first days of life the total number of chickens was divided into two groups: the diet of group 1 contained no vitamin D (control), whereas vitamin D_3 was added to the diet of group 2 in a dose of 400 i.u./kg (experiment). At the age of 3 weeks the control chickens developed rickets, and various doses (50, 250, 500, 1000, and 20,000 i.u.) of vitamin D_3 were injected intramuscularly, in 0.1 ml propylene glycol, into separate subgroups (six to eight chickens in each subgroup) 24 h before sacrifice, and 1000 i.u. was given at different times (1, 6, 12, 24, 48, and 72 h) before sacrifice.

Ca^{++} transport was studied in vitro on a nonreversed intestinal pouch by a method developed by the writers previously. After decapitation of the chickens, a segment of their small intestine measuring 17 cm was removed, leaving 2 cm attached to the duodenum. The intestine was washed twice with cold buffer solution, divided into three segments each 5 cm long, from which intestinal pouches were made. Before the ligature was drawn tight, 0.5 ml blood serum or buffer solution, to which Ca^{++} was added to bring its final concentration up to 7.5 mM, was introduced into each pouch. Depending on the aims of the investigation, various quantities (6.25, 25, and 250 ng) of vitamin D_3 or its analogs (25-OHD_3, 1α-OHD_3), in alcoholic solution (0.02 ml/10 ml serum), were added to the blood serum from control chickens. The segment of intestine was placed in a tube containing 4.5 ml buffer solution of the following composition: mannitol 240 mM, Tris-HCl

20 mM, NaCl 40 mM, pH 7.4; the buffer did not contain Ca^{++}. Incubation was carried out at 37°C for different times (5-30 min) with constant oxygenation of the external solution. At the end of incubation the intestine was removed and 0.5 ml of a solution of murexide (80 μM) was added to the test tube. The Ca^{++} concentration was determined spectrophotometrically [13]. The intensity of Ca^{++} transport was assessed from the quantity of the cation which moved during incubation from the intestinal lumen into the external solution, and was expressed in nanomoles/cm intestine. For each variant of the experiments, from five to eight chickens were used.

Serum was obtained from chickens' blood after centrifugation (2000g, 20 min) and was used on the same day.

CaBP in the intestinal epithelium was determined by an immunochemical method [3]. Crystalline vitamin D_3 and alcoholic solutions of 25-OHD_3 and 1α-OHD_3 were obtained from the All-Union Vitamin Research Institute.

EXPERIMENTAL RESULTS

Transport of Ca^{++} from the unreversed intestinal sac of the control chickens, after introduction of buffer solution or blood serum from control chickens, was at a low level but rose considerably after injection of blood serum from the experimental chickens (receiving vitamin D_3 with the diet for 3 weeks). The curves in Fig. 1 show that the greatest difference (by 1.9 times; $P < 0.001$) between Ca^{++} transport from the blood serum of the experimental and control chickens was observed during the first 10 min of incubation, although after 5 min the difference in Ca^{++} transport between them was significant ($P < 0.05$); after 30 min, however, the differences were no longer significant ($P > 0.05$).

The data given in Table 1 show that the ability of blood serum to stimulate Ca^{++} transport in the small intestine of control chickens appeared very soon after intramuscular injection of the vitamin D_3: the maximal effect from a dose of 1000 i.u. occurred as early as after 1 h (2.8 times greater than the action of serum from control chickens; $P < 0.005$), it was maintained at a high level during the first 6 h, but after 12 h the effect gradually declined and thereafter remained stable for 2 days. Dependence of the stimulating action of serum on the injected dose of vitamin D_3 was less marked: the effects of 50 and 20,000 i.u. were the same. Some increase was observed if a dose of 250 i.u. was given.

These data show conclusively that after injection of vitamin D_3 into the chickens active substances capable of acting directly on the intestinal epithelium and stimulating absorption of Ca^{++} circulated in their blood. The main form of vitamin D which circulates in the blood is 25-OHD_3, the mean concentration of which in chicken blood serum is 30 ng/ml [10]. Direct injection of various doses of this steroid into the intestinal sac of control chickens together with blood serum from control chickens increased Ca^{++} transport similarly to blood serum from the experimental chickens. Injection of 1α-OHD_3 and vitamin D_3 had a similar stimulating action; in a dose of 6.25 ng, moreover, all three preparations had the same maximal effect. An increase in the concentration of steroids in the blood serum was not accompanied by any increase in Ca^{++} transport, and after a high dose (250 ng) it fell significantly.

Blood serum, it must be noted, is not a specific medium essential for manifestations of the action of vitamin D_3 and its analogs on Ca^{++} transport in a system in vitro. Introduction of 25 ng of 25-OHD_3 in the composition of a buffer solution (NaCl 0.15 M, Tris-HCl 0.013 M, fructose 0.02 M, Ca^{++} 7.5 mM; pH 7.4) also caused significant stimulation of Ca^{++} transport from the intestinal sac of the control chickens (49.6 ± 6.0 compared with 26.3 ± 2.9 nmoles/cm; $P < 0.05$).

TABLE 2. Action of Blood Serum, 25-OHD_3, and 1α-OHD_3 on Ca^{++} Transport in Non-reversed Intestinal Sac from Chickens Depending on Their Vitamin D_3 Intake

Chickens receiving the vitamin	Ca^{2+} transport (in nmoles/cm) after introduction of blood serum from chickens receiving			
	+D	−D	−D+25 ng 25-OHD_3	−D+25 ng 1 α-OHD_3
+	88,5±5,6	91,5±6,1	86,5±4,9	87,5±5,2
−	48,7±3,4	25,9±2,9	66,0±6,8	70,6±9,9

Legend. Vitamin D_3 injected intramuscularly into control chickens 72 h before sacrifice.

Characteristically the stimulating action of blood serum from the experimental chickens or of vitamin D preparations on Ca^{++} transport was observed only in intestinal pouches from rachitic chickens, i.e., against the background of a low level of absorption of the cation (25.9 nmoles/cm). In chickens receiving vitamin D, Ca^{++} transport was increased more than threefold 72 h before sacrifice (91 nmoles/cm) and remained substantially unchanged by injection of vitamin D_3 preparations into the intestine (Table 2).

One feature which distinguished the intestinal epithelium of the experimental chickens from that of the controls was that specific CaBP was present in the mucous membrane of chickens receiving 1000 i.u. of vitamin D 72 h before sacrifice; it was present in a concentration of 93.3 ± 5.1 μg/kg total protein, but was absent in the control chickens. The presence of this protein in the intestinal epithelium of the experimental chickens was evidently responsible for intensive Ca^{++} transport in these birds, against the background of which the vitamin D preparations did not give any additional effect. The action of the steroids on permeability for Ca^{++} in the in vitro system could thus be detected only in an intestine not containing CaBP. As the results show, it differed significantly from the known physiological action of vitamin D which is mediated through CaBP: it is manifested almost instantaneously, there are not specific structural differences associated with individual forms of the vitamin, and the effect depends only a little on dose. This action of vitamin D_3 and its hydroxy-analogs in physiological concentrations on Ca^{++} was demonstrated for the first time. In previous investigations [15] stimulation of Ca^{++} transport could not be detected after introduction of $1,25\text{-}(OH)_2D_3$ and $1\alpha\text{-}OHD_3$ into the isolated intestine, although their intravenous injection into rachitic rats did stimulate absorption of Ca^{++}. In another experiment [9] increased incorporation of ^{45}Ca was observed into isolated intestinal epithelial cells after preliminary incubation of the cells for 90 min with $1,25\text{-}(OH)_2D_3$, but $25\text{-}OHD_3$ had no such effect. The discovery of a direct effect of vitamin D on the intestine in the present experiments can be partly explained on the grounds that a more adequate model was used for the investigation of transport: an unreversed intestinal pouch, in which the one-way flow of the cation through the wall took place on account of the concentration gradient.

The mechanism of the rapid effect of vitamin D_3 and its hydroxy-analogs on the ability of Ca^{++} to pass through the intestinal epithelium is not clear. This effect is evidently connected with the direct effect of the steroids on the plasma membranes of epithelial cells, in the same way as has been observed for the polyene antibiotic filipin in experiments on stimulation of Ca^{++} absorption in chickens [4]. The "polyene-like" action of vitamin D in increasing permeability for Ca^{++} also was observed in model experiments with artificial double membranes. On the basis of these findings the original hypothesis was put forward [12] that the membranophilic effect of vitamin D and its hydroxy-analogs is linked with the ability of their molecules to condense with the formation of a structure similar to filipin.

Other model experiments [11] have shown that the transport of Ca^{++} in the zone of partition between the aqueous phase and the CCl_4 phase is considerably increased in the presence of vitamin D. This property may be very important as an explanation of the effect of vitamin D on intensification of the flow of Ca^{++} through membranes and cells.

These observations all suggest that besides its main physiological action, which in vivo follows the classical scheme for steroid hormones, vitamin D and its analogs can directly affect cell membranes.

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