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A VITAMIN D-RELATED INHIBITION OF GROWTH OF AN EPITHELIOID CELL LINE DERIVED FROM RAT SMALL INTESTINE

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SUMMARY

A recently established epithelioid cell line derived from rat small intestinal crypt cells was tested for the effects on growth of rat serum obtained from normal, vitamin D-deficient and 25-$(OH)D_3$ repleted animals as compared to the growth curves established with fetal calf serum. The results suggest that there is a heat-labile growth-inhibiting factor(s) present in normal and 25-$(OH)D_3$ repleted animals which is not present in vitamin D deficient animals.

Introduction. Most of the recent research on vitamin D has been concerned with the discovery and isolation of the active metabolite, 1,25-$(OH)_2D_3$ [1,2], and of the effect of this metabolite on calcium absorption by the intestine and on calcium resorption by bone [3]. In earlier publications there were results suggesting that vitamin D may have effects other than those directly related to calcium homeostasis. Thomson and DeLuca [4] noted that vitamin D stimulated intestinal membrane phospholipid synthesis, a stimulation which could be detected 3 hours after giving vitamin D. Other structural membrane changes have been described which appear to be dependent on vitamin D. In addition to stimulating

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membrane synthesis, vitamin D has been reported to increase the rate of intestinal epithelial cell turnover, cell migration from crypt to villus, protein synthesis, DNA synthesis and cell size [5]. On the basis of this evidence a trophic action of vitamin D for the intestinal epithelium had been proposed but this was challenged by Spielvogel et al [6] who claim that vitamin D exerts its effect only on the mature absorptive cell in the upper half of the villus. We report here of anti-trophic factor(s) whose presence in rat serum is dependent on the vitamin D status of the rat.

With the availability of an epithelioid cell line in tissue culture derived from rat small intestinal crypt cells, we tested the effects of rat serum obtained from normal, vitamin D-deficient and 25-(OH)D_3 repleted animals as well as the direct effects of 1-25$(OH)_2D_3$ on its growth. The results suggest that there is a heat-labile growth-inhibiting factor(s) present in normal and 25-(OH)D_3 repleted animals which is not present in vitamin D deficient animals.

Methods. An epithelioid cell line, IEC-6, and a fibroblastic cell line, RIF, were both derived from rat small intestine [7]. These two cell lines were recently established and can be cultured serially. On the basis of morphological, ultrastructural and immunochemical data it was demonstrated that the IEC-6 cells are derived from the undifferentiated intestinal crypt cells [7]. Storage of cells, cell counting, determination of cell viability, and growth rates were done as previously described [7]. Weanling male albino rats were obtained from Holtzman Co. (Madison, Wi.) and either maintained on a complete pellet diet or made vitamin D deficient as previously described [8]. Serum was prepared from blood obtained by heart puncture; it was sterilized by microfiltration (0.45μ dia micropore filters) before adding to the culture medium.

Results and Discussion. The epithelioid cell line requires 5-10% fetal calf serum for optimum growth rate and exhibits a lag of 2 days before beginning the phase of logarithmic growth; the latter shows a doubling time of 20 to 24 hours and ceases when the cell density reaches 2.5×10^4 cells/cm^2. This growth curve is represented by the curve marked FCS in figure 1. If serum derived from normal, non-deficient rats was

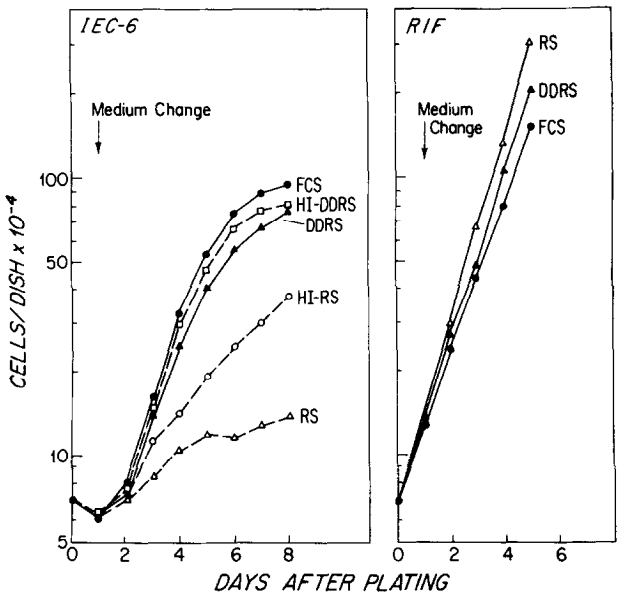

Fig. 1. Effect of sera from normal and vitamin D deficient rats on growth rates of IEC-6 (intestinal epithelial cells) and RIF (Intestinal fibroblasts) cell lines. The cells were grown in plastic dishes in Dulbecco's modified Eagle medium supplemented with 2mM glutamine, 4µg/ml insulin, 50 units/ml streptomycin and either 10% fetal calf serum (FCS), normal rat serum (RS) or vitamin D-deficient rat serum (DDRS). HI-RS, rat serum previously heated to 56° for 30'. HI-DDRS, vitamin D deficient rat serum previously heated to 56° for 30'.

substituted for FCS, growth was markedly inhibited (RS curve, figure 1). However, serum derived from vitamin D-deficient rats (DDRS) showed little inhibition of growth and closely approximated the rate of growth seen with FCS. If normal serum was heated for 30min at 56° partial restoration of a normal growth rate was observed (HI-RS curve, figure 1). Heat treatment of the D-deficient rat serum had only a slight effect on the growth rate (HI-DDRS curve, figure 1). These results suggest that there is a heat-labile factor (or factors) in normal rat serum which inhibits the growth of these intestinal-derived epithelial cells. The specificity of this effect of epithelial cells is also shown in figure 1 where it was demonstrated that normal rat serum did not inhibit the growth of the fibroblastic cell line derived from rat intestine (RIF cells) nor was there a difference with D-deficient rat serum.

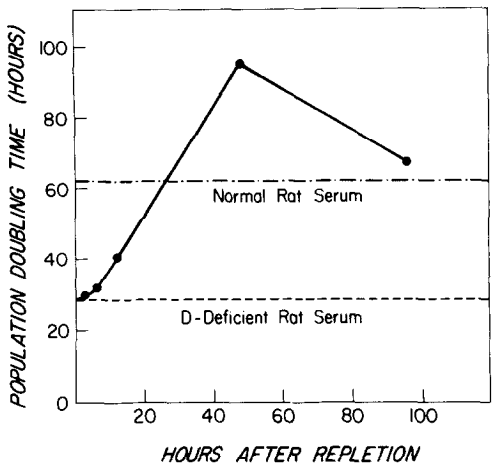

Fig. 2. Effect of repletion with 25-(OH)D_3 on the recovery of the IEC-6 cell growth inhibiting property in rat serum. 25-(OH)D_3 (250ng) was injected intrajugularly into vitamin D deficient rats, the rats sacrificed at different intervals thereafter and serum prepared from blood obtained by intracardiac puncture. (----) population doubling time of IEC-6 cells if medium is supplemented with 10% D-deficient rat serum. (·-·-·-·) population doubling time if medium is supplemented with 10% normal rat serum. Population doubling times were determined during the logarithmic phase of growth from growth curves of IEC-6 cells in standard medium supplemented with the different sera. Each point represents the average of 3 separate determinations differing by no more than 10%.

The vitamin D_3 metabolites, 1,25-$(OH)_2D_3$ and 25-(OH)D_3 added directly to the medium containing normal rat serum did not alter the inhibition of growth of IEC-6 cells, and there was no effect of vitamin D_3 or its metabolites on the growth rate of IEC-6 cells grown in FCS.

In order to determine how closely related the effects of D-deficient serum are to vitamin D mediated events, repletion studies were done. Serum was prepared from vitamin D deficient animals at different intervals after administrating 250ng of 25-(OH)D_3 intrajugular. An increase in population doubling time (measured during the logarithmic phase of growth) was detected as early as 6 hours but more definitely by 12 hours (40 hrs. doubling time as compared to 24 hrs) (Fig. 2). An apparent "excessive" inhibitory effect was seen at 48 hrs (doubling time 95 hrs) with a return to near normal rat serum inhibitory effect by 96 hrs after a single dose of 25-(OH)D_3. These results suggest that the production of the heat-labile inhibitory activity is closely related to an effect of vitamin D.

At the present time we have no data which would indicate the character of the inhibitory activity other than its heat lability and that it is non-dialyzable. Unpublished data (Andrea Quaroni) indicate that, as with other cell systems, glucocorticoids strikingly inhibit the growth of IEC-6 cells. Glucocorticoids have been shown to play a fundamental role in the post-natal development of the small intestinal mucosa [9]. Inhibition of growth rate of glucocorticoids may be indicative of an early control step in differentiation. However, if the vitamin D related inhibitory factor is a glucocorticoid and is related to a stress response to repletion, one might have expected an earlier, more sudden increase after 25-$(OH)D_3$ administration. The kinetics of growth inhibition induced by hydrocortisone (A. Quaroni, manuscript in preparation) are also quite different: a significant inhibition of IEC-6 cell growth is observed only 3-4 days after addition of the hormone and cell proliferation is, subsequently, completely blocked for at least a week. Other candidates that might respond to vitamin D are such polypeptide hormones as parathormone of calcitonin. However, 1,25-$(OH)_2D_3$ is postulated to inhibit the synthesis of parathormone [10]. Another explanation may be that 1,25-$(OH)_2D_3$ is, indeed, a promotor of intestinal differentiation but not, by itself, an inhibitor of growth. The resulting stimulus to differentiation would lead an increased number of differentiated cells which would produce a chalone-like substance. The presence of this chalone-like substance in the serum of the vitamin D treated animals would be inhibitory to growth of intestinal crypt-derived cells.

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