EFFECTS OF PARATHYROID HORMONE ON HeLa CELL CULTURES

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Most investigations on the metabolic action of parathyroid hormone have suffered from an important limitation; *i.e.*, the heterogeneity of the cell population of the tissue under study. However, as it has been reported that parathyroid hormone also affects many tissues (1–4), it is

possible that the role of the hormone might not be restricted to the most often mentioned target organs; bone, kidney, or intestine. In an attempt to study parathyroid hormone in a homogeneous system, we investigated its effects in cultures of HeLa cells. The choice of this cell strain was mainly dictated by the fact that it has a high aerobic glycolysis (5), a characteristic of bone, kidney, and intestine, and also by the simplicity of its culture technique. This first report is concerned with the morphological aspects of the investigation.

MATERIAL AND METHODS

HeLa cells, both the wild strain and the S-3 clone of Puck, obtained from Microbiological Associates, Bethesda, were grown as monolayers in Leighton tubes or T flasks, in Eagle basal medium combined with Hanks' salt solution and supplemented with 10 per cent human serum. No antibiotics were used. A Coulter Electronic Cell Counter, Research Model, was used for cell count and cell size distribution. Polaroid photomicrographs were taken on a Zeiss microscope with brightfield only. Both Lilly parathyroid extract (PTE), Eli Lilly and Company, Indianapolis, Indiana, and the purified hormone (PTH), obtained from Dr. H. Rasmussen, were used in these experiments. The dosage was 1 unit/ml of medium, unless stated otherwise.

RESULTS

Effects of PTE and PTH on HeLa cell morpholgy

With 1 unit of PTE or PTH per ml of medium, the following modifications of the morphology of the cells and the colonies were observed: Instead of the classical polygonal, closed monolayer, the colonies assumed round, open in spot (cells separated), and heaped (cells in several layers) characteristics (Figs. 1 to 3). The cells themselves were round and their membrane was irregular, presenting many finger-like projections, blebs, and herniations (Figs. 3 to 6). In addition, there was an increased amount of multinucleated cells, sometimes with as many as 8 nuclei (Figs. 4 and 5). Whether the latter were the result of the fusion of single cells or lack of cell division could not be determined. All these general characteristics were predominant during the logarithmic phase of growth from the 3rd to the 6th day after harvesting and inoculation. They were obtained with both the crude extract and the purified hormone and consequently do not seem to be due to impurities of the crude extract.

Effects of PTE on Growth

To see whether the hormone had any toxic effect on their growth, cells were harvested in replicate cultures in Leighton tubes with or without 1 unit PTE/ml of medium. The cells were counted at various time intervals on a cell counter, after treatment with 0.025 per cent trypsin to put them in suspension. Fig. 7 *a* shows that there was no difference between the control and the treated group. As there was a possibility that the hormone might be inactivated quite rapidly at 37°C, counts were made on cultures fed every day with fresh medium and fresh hormone. Fig. 7 *b* shows that, despite a greater spread due to daily feeding, the slope of the growth curve is identical in both groups. Even when the original inoculate was different, their rate of growth proved to be the same (Fig. 7 *c*). Fig. 7 *d* shows that the log phase of growth and the stationary phase are equally unaffected. This seems to be a good evidence that the hormone does not have any toxic or growth-inhibiting effect in the concentration of 1 unit/ml of medium.

Effect of PTH on Mitosis

In observing these colonies, we had the feeling that, in the cultures treated with PTH, mitoses were more numerous than in controls. To try to quantitate this impression, four cultures were grown on small glass slides fitted in the bottom of Leighton tubes. After 4 days of growth in control medium, two cultures were exposed for 24 hours to 1 unit PTH/ml while the control cultures were fed only fresh medium. The 5th day, the slides were fixed and stained with hematoxylin and eosin, (Figs. 8 and 9). Only clearly visible mitotic figures were counted under magnification of 400 and expressed as mitoses per field. Twenty different fields were counted in each of the four slides. As seen in Table I, the number of mitoses was nearly doubled in the PTH group.

TABLE I

Mitoses per Field

Control	4.97 ± 1.11	
PTE	8.80 ± 1.81	
Increase, *per cent*	77	$p < 0.001$

Effect of PTH on Cell Attachment

At that point, we were facing the paradox of similar growth curves with different mitotic rates. We had noticed, however, that, besides morphological changes, the cells treated with PTH seemed to show a decreased adhesiveness to glass, while clumping together to a greater extent (Figs. 2 and 9). We repeated the cell counts, this time counting separately the cells in suspension in the medium and the cells attached to the glass. Fig. 10 shows that the growth curve of attached cells is again identical in both groups but, as the PTH-treated cultures had more cells in suspension, the total number of cells is increased by that amount. This seems to reconcile the two previous observations of identical growth curves with different mitotic indices.

Cell Size

It was difficult to assess whether there was any difference in size between the treated and untreated cells because of their morphological differences. Cell size distribution obtained on the Coulter counter showed that there was no change between the two groups, (Fig. 11). The two curves were obtained on the two cultures shown in Figs. 1 and 2. Although the multinucleated cells were definitely larger than the other cells and more numerous in PTE-treated cultures, their number was probably too small as compared with other cells to be detected by the counter.

"Bone Resorption"

Finally we decided to test the behavior of these cells grown in monolayer in presence of bone powder. The cells were grown for 2 days in Leighton tubes and in control medium. On day 2, the cells were fed fresh medium containing a few crystals of autoclaved veal bone powder. The cultures were divided into three groups. The first one had fresh medium and bone powder alone. The second had, in addition, 1 unit PTE/ml of medium and the third 2 units/ml. Each group consisted of 4 Leighton tubes. Pictures were taken on the 3rd day (Figs. 12, 14, and 16). It is clear that the cultures accepted the bone powder quite well. The cells surrounded the crystals and attached to them. The PTE-treated colonies showed the same round and heaped characteristics a mentioned before (Figs. 14 and 16). Within 2 units/ml some cells were detached and some of them were loaded with microcrystals (Fig. 16). Pictures were taken again on day 6 (Figs. 13, 15, and 17). The control colonies seemed to have grown and spread normally. The cells were still attached to the bone particles. They even seemed to climb on the sides of them (Fig. 13). No significant reduction in the size and number of bone powder particles was noticeable. Occasionally, a few cells were loaded with crystals. The cultures treated with 1 unit PTE/ml also seemed healthy. There was, however, a significant reduction in the size and number of bone particles (Fig. 15). With 2 units PTE/ml the colonies seemed healthy, but not a single bone particle was present. Lacunae, with tiny crystals, were sometimes present in the center of some colonies, and many cells were loaded with microcrystals (Fig. 17).

DISCUSSION

It has never been determined whether the role of parathyroid hormone stops with the maintenance of a constant serum calcium level or whether it is further involved in calcium-dependent physiological activities such as, for instance, cell adhesion and membrane structure and properties. Furthermore, it is still difficult at the present time to envision a common mechanism accounting for the effects of the hormone on the membrane per-

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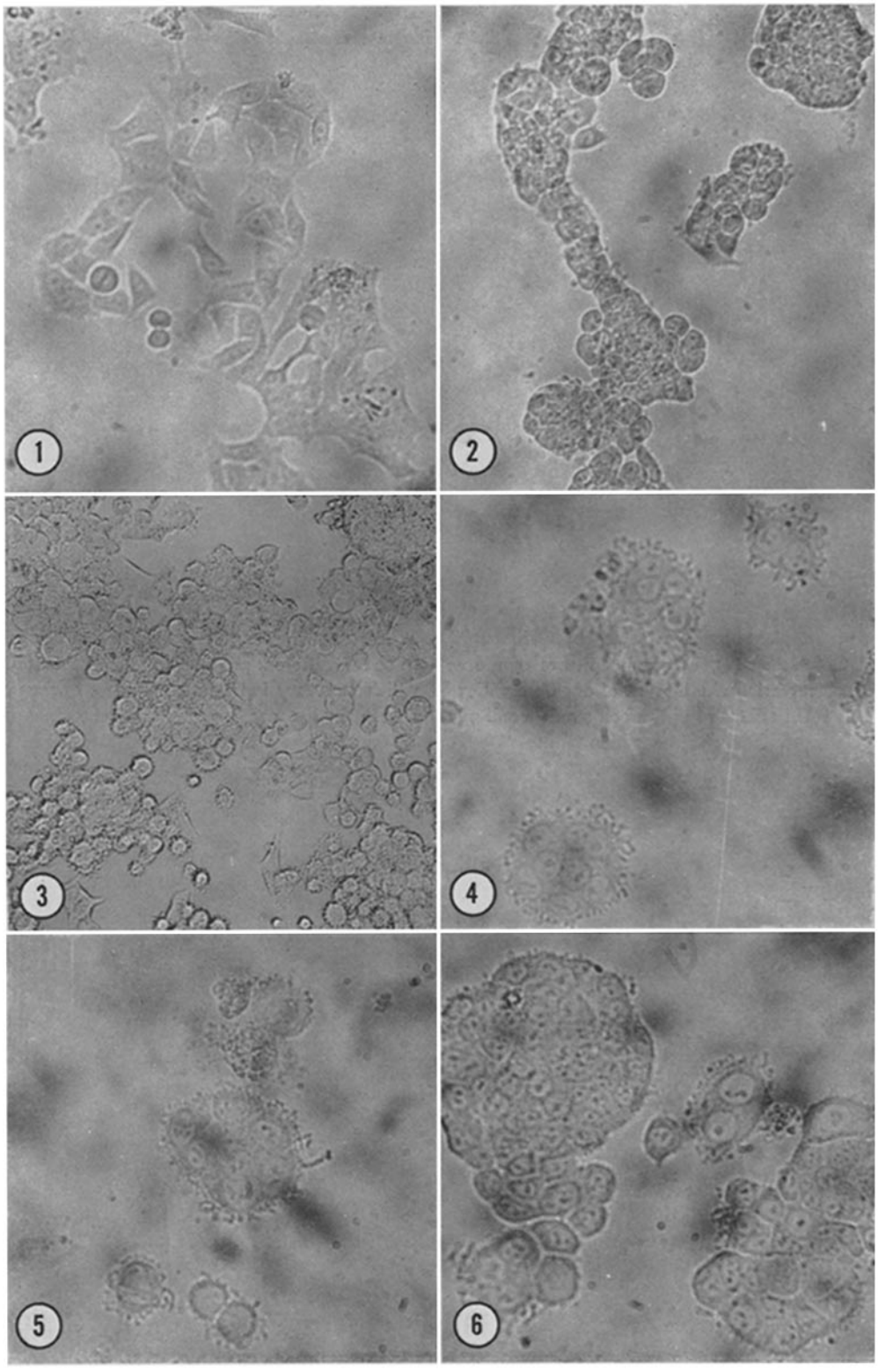
Magnification of all figures approximately 200.

FIGURE 1 HeLa cells, control culture, 3rd day.

FIGURE 2 HeLa cells, culture with 1 unit PTE (Eli Lilly and Company)/ml of medium, 3rd day.

FIGURE 3 HeLa cells, culture with 1 unit PTH (purified hormone)/ml medium, 3rd day.

FIGURES 4 to 6 HeLa cells, cultures with 1 unit PTE/ml of medium, 3rd day.



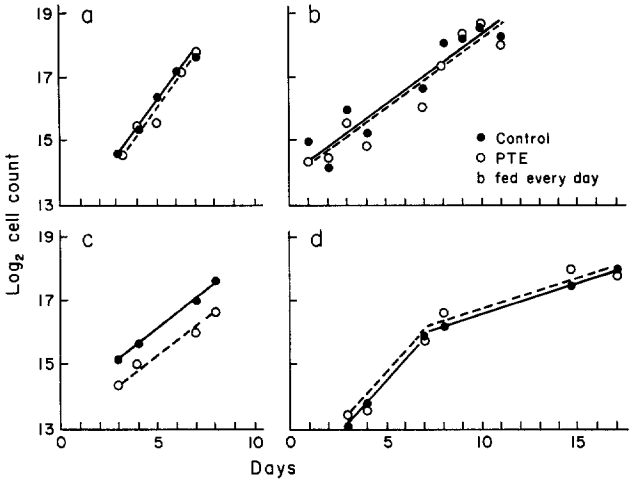


FIGURE 7 Effect of 1 unit/ml of PTE on growth rate of HeLa cells.

meability of the renal tubule and on bone resorption. It is, therefore, of interest to see in our model system two effects of PTH: an effect on the cell membrane and what we would like to call "bone resorption." Induction of bone resorption by PTH *in vitro* has already been reported by Gaillard (6) and Goldhaber (7), in organ cultures of mouse radii or calvaria. The interest of our observations is that PTH affects as well a cell strain seemingly unrelated to parathyroid function.

Decreased adhesiveness to glass and clumping of cells have already been studied in cell cultures. The mechanisms involved, however, are still unclear and controverisal. Adhesiveness to a foreign substrate and mutual adhesiveness between cells have been shown by Coman and coworkers (8, 9) to be two different properties of the cell membrane which can vary independently from one another as seen, for instance, in our experiments. In a paper on the role of calcium in cellular attachment to a

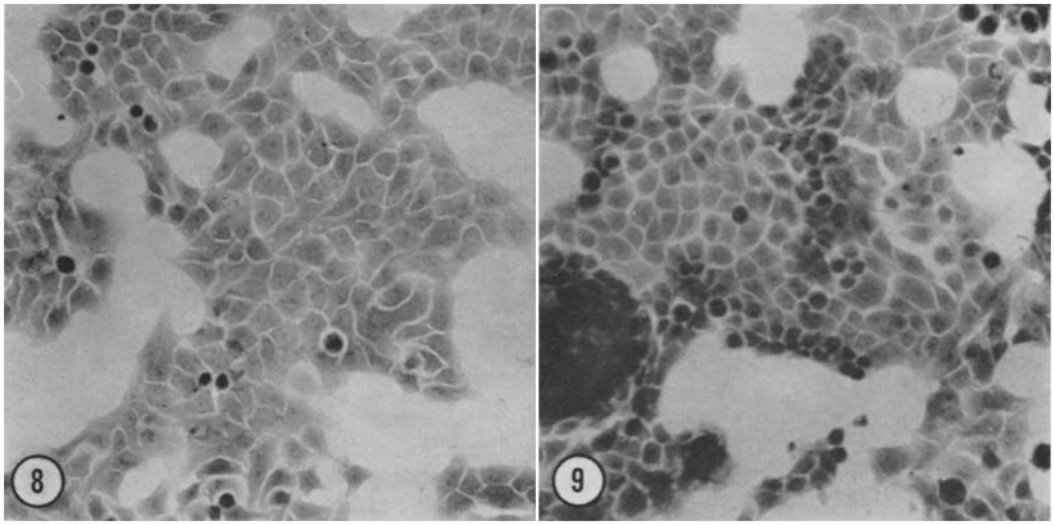


FIGURE 8 HeLa cells, control culture, 4th day, hematoxylin and eosin.

FIGURE 9 HeLa cells, culture exposed for 24 hours to 1 unit PTH/ml of medium, 4th day, hematoxylin and eosin.

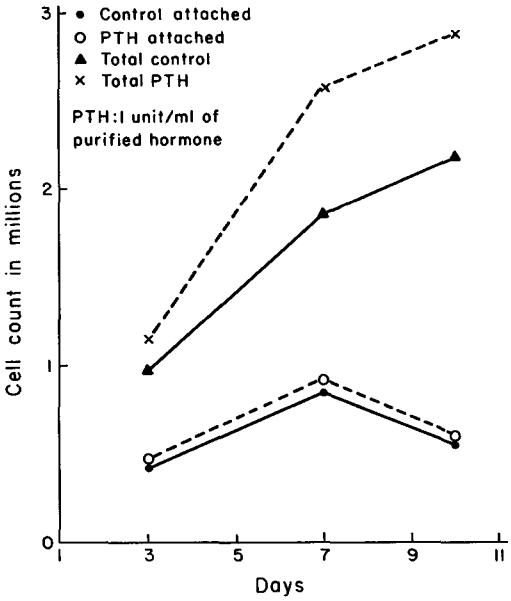


FIGURE 10 PTH effect on HeLa cell adhesion.

glass surface, Weiss (10) has shown that there is an optimal calcium concentration favoring cell adhesion at about 3 mg/100 ml, below and above which glass adhesiveness is decreased. If calcium was involved in our experiments, we could not decide, by this parameter alone, whether the decreased adhesiveness of PTE-treated cells is due to a higher or lower calcium concentration at the cell surface, because both could have the same effect. However, we observed also that the treated cells were clumping together, suggesting an increased adhesion between them. Coman (8), noting that calcium is essential for cell adhesion, reports that cancer cells are deficient in adhesiveness to each other and at the same time seem to be incapable of binding an adequate amount of calcium at their surface (11). Our results, if comparable, would then suggest a higher calcium binding capacity in the PTE-treated cells. But, without speculating on mechanism, it might be worthwhile to mention the many other factors, reviewed by Taylor (12), Weiss (13), and Curtis (14), which could be implicated in cellular adhesion: calcium, proteins, mucopolysaccharides, surface charges, surface projections and microvilli, pH, and, of course, several

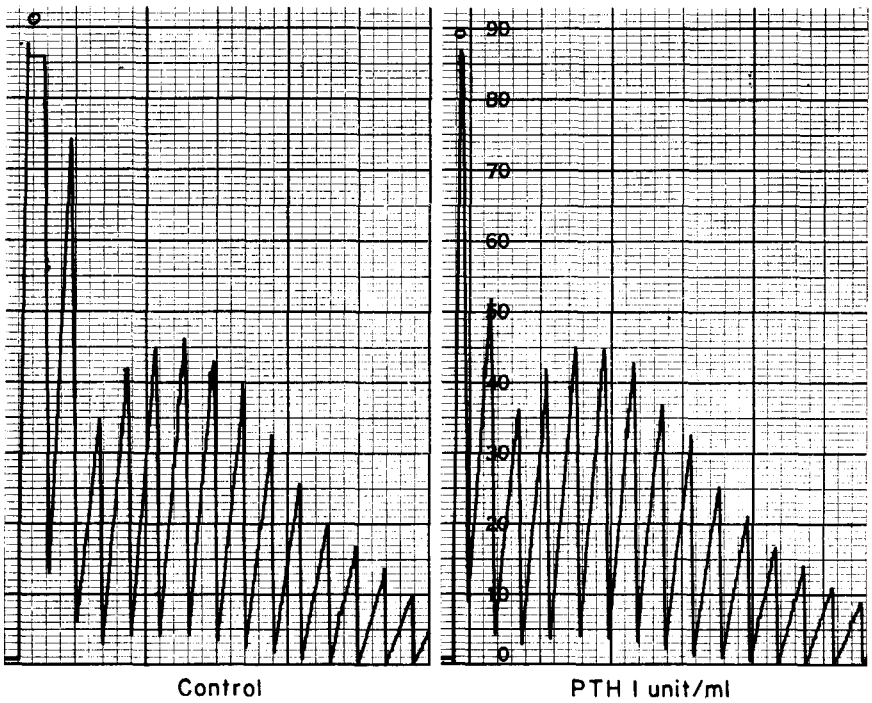


FIGURE 11 Cell size distribution. Abcissa; number of cells. Ordinate; cell size. The first two peaks are noise and cell debris. Third peak, smaller cell size. Cell size increasing from left to right.

combinations of those. Which of these factors was or were affected by PTH is presently under study. Whatever the mechanism, it is worth mentioning now that our cells did show surface projections and microvilli, bringing further evidence that PTH affects the cell membrane. It has already been reported by Roth and Munger (15) that the chief cells of human parathyroid adenoma presented marked interdigitation of cell membranes and folds of microvilli. This observation has been further substantiated by Roth and Raisz (16) with rat parathyroid glands cultivated *in vitro*: electronmicrographs show tortuous cell membranes with numerous microvilli interdigitating between adjacent cells in a state of hyperfunction, while the cell membranes are straight, parallel, and with fewer microvilli in cells in a state of hypofunction. In further support of the role of PTH on cell membrane and adhesion is a report by Roizman (17) that PTH decreases adsorption of *herpes simplex* virus on HEp-2 cells in tissue cultures, and slows down the "transport" of the virus through the cell membrane.

The increased mitotic rate observed in our treated cultures would suggest that PTH belongs to the group of hormones stimulating mitotic activity, as reviewed by Bullough (18). It is of further interest to note with Abercrombie (19) that during mitosis of fibroblasts there is a decrease in adhesiveness to the culture flask.

Finally the presence of multinucleated cells in one set of experiments and dissolution of bone crystals after exposure to PTE suggest that our artificial model system might not be irrelevant to the problem of bone resorption. We think it might be suitable to help further clarify the mode of action of PTH in later investigations.

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FIGURE 12 Control culture + bone, day 3.

FIGURE 13 Control culture + bone, day 6.

FIGURE 14 Culture with 1 unit PTE + bone, day 3.

FIGURE 15 Culture with 1 unit PTE + bone, day 6.

FIGURE 16 Culture with 2 units PTE + bone, day 3.

FIGURE 17 Culture with 2 units PTE + bone, day 6.