Selective Renal Involvement in the Early Development of Hypercalcemia and Hypophosphatemia in VX-2 Carcinoma-bearing Rabbits: Studies on Serum and Tissues Alkaline Phosphatase and Renal Handling of Phosphorus

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SUMMARY

Skeletal, renal, and intestinal effects of VX-2 carcinoma in rabbits were studied in relation to the pathogenesis of a hypercalcemic and hypophosphatemic state induced by the tumor. On the basis of an initial screening, alkaline phosphatase was chosen as a biochemical marker for monitoring any change in the cellular activity of bone, kidney, intestine, and liver in the carcinoma-bearing rabbits. Renal function was also evaluated by measuring the tubular reabsorption of phosphorus. No significant changes in bone, duodenal, or liver alkaline phosphatase levels were detected at any time throughout the experiment. However, kidney cortex alkaline phosphatase activity was markedly depressed on the second week posttransplantation, coinciding with hypophosphatasia of similar magnitude. No inhibitors of alkaline phosphatase were present in the circulation. Serum acid phosphatase as well as serum acid protease activities were unaffected by the tumor. Moderate hypercalcemia and hypophosphatemia developed on the third week posttransplantation and were associated with a significant depression of renal tubular reabsorption of phosphorus. Weight loss which accompanied the tumor growth was of minor importance in the development of hypophosphatasia and did not affect all the other parameters studied. The reported findings single out the kidney as an initially and primarily affected target organ in the carcinoma-bearing rabbits and indicate parathyroid-like effect of the tumor on renal function, namely, depressed tubular reabsorption of phosphorus. The metabolic consequences of the tumor action on its host in relation to calcium homeostasis are discussed, with special reference to the possibility of parathyroid-like hormone involvement.

INTRODUCTION

Hypercalcemia associated with nonosseous and nonparathyroid malignancies has become a recognized entity in recent years (35). Malignancy-induced hypercalcemia associated with hypophosphatemia constitutes a clinical syndrome termed "pseudohyperparathyroidism" (9, 28). This syndrome is most common in carcinoma of the lung, kidney, ovary, or pancreas and malignant lymphoma (6, 16).

Radioimmunoassay of tumor extracts from patients with malignant diseases associated with pseudohyperparathyroidism syndrome as well as sedimentation rate determinations of the immunologically active component in these extracts have recently provided direct evidence that certain malignant tumors contain significant quantities of material closely resembling parathyroid hormone (26). In addition, such tumor extracts were found to be capable of inducing radiocalcium release from prelabeled rat embryo bones grown in organ culture, a biologic effect characteristic of parathyroid hormone action (21). These findings strongly support the current concept that malignant cells of nonendocrine origin are sometimes capable of producing hormones and, in this specific case, parathyroid-like hormone. In this connection, therefore, recent studies on rabbits transplanted with VX-2 carcinoma are of particular interest since they indicate that these tumor-bearing animals may serve as an excellent experimental model for the study of pseudohyperparathyroidism syndrome in man (36, 37). When VX-2 carcinoma is implanted in rabbit muscle, a state of hypercalcemia and hypophosphatemia simulating hyperparathyroidism results, with no signs of parathyroid hyperplasia and no evidence of skeletal metastases. Excision of the primary tumor implant causes a dramatic reversal of the elevated plasma calcium concentration, whereas recurrence of the tumor at the primary site, or growth of metastatic tumor after removal of the primary VX-2 tumor, brings about reappearance of the hypercalcemic state. Further studies indicated that the parathyroid glands were not necessary to the development and maintenance of the hypercalcemia in these tumor-bearing animals (34). In a parathormone bioassay of the serum from these hypercalcemic VX-2 carcinoma rabbits, Gertner et al. (11) found that it had a phosphaturic effect, similar to exogenous parathormone when injected into mice. They conclud-
ed that parathormone-like substance was produced by the tumor. However, as pointed out by Watson (35), this conclusion should be accepted with some reservation until confirmed by other and more direct proofs. When the tumor is implanted in rabbit bone, localized, hyperparathyroid-like osteoclastic activity is observed, and the chemical alterations in the serum are the same as when the tumor is implanted in muscle (8).

On the basis of the experimental findings reviewed here, the present study was undertaken with the following objectives in mind: (a) to evaluate renal handling of phosphate in relation to the VX-2 carcinoma-induced hypercalcemia and hypophosphatemia in rabbits; (b) to detect some specific biochemical and physiological changes with the alterations in serum electrolytes and renal function in the VX-2 carcinoma-bearing rabbits; and (c) to relate the pattern of enzyme changes with the alterations in serum electrolytes and renal function in the VX-2 carcinoma-bearing rabbits; and (d) to compare the relevant chemical, biochemical, and functional changes occurring in the tumor-bearing animals with those observed in experimentally induced acute hyperparathyroidism in rabbits.

MATERIALS AND METHODS

Animals

The experiments were performed on New Zealand white male rabbits weighing 2.2 ± 0.3 kg. The animals were housed individually in stainless steel cages. Tap water and commercial Purina rabbit chow were fed ad libitum to all tumor-bearing rabbits (35 rabbits). Daily changes in body weight and food consumption were recorded. Pair-fed, sham-operated rabbits, closely following the changes in body weight of the tumor-bearing rabbits, served as control animals (21 rabbits).

VX-2 Carcinoma Transplantation

VX-2 tumor tissue from the anterior thigh muscle of a donor rabbit was obtained under aseptic conditions immediately upon sacrifice of the donor animal. A saline suspension (0.3 ml) of minced tumor which had been filtered through a 60-mesh copper screen was injected into the anterior thigh of each recipient animal.

Experimentally Induced Hyperparathyroidism

Parathyroid extract (Lilly) was injected in a single subcutaneous dose of 750 USP U/kg to each of a group of four rabbits. The animals were killed 20 hours later. Three control animals each received a single subcutaneous dose of the same volume of 0.85% saline containing 1.6% glycerol and 0.2% phenol.

Serum Samples

Ten-ml whole blood samples were collected into siliconized tubes under vacuum (Vacutainer, Becton, Dickinson and Co., Rutherford, New Jersey) by cardiac puncture. The tubes were immersed immediately in an ice bath at 2–4°C then placed in a refrigerated centrifuge; the serum was separated by centrifugation for 20 minutes at 2,000 rpm. Hemolyzed samples were discarded.

Preparation of Whole Tissue Homogenates

Immediately after sacrifice of the rabbits by cervical dislocation and bleeding, the right lobe of the liver, a section of the duodenum most proximal to the pylorus, the kidneys, and the calvaria were removed and chilled in crushed ice. The calvaria were cleaned free of any adhering soft tissue and all tissues were thoroughly washed with cold deionized distilled water to remove contaminating blood and, in the case of the intestine, to remove intestinal contents as well. The tissues were then blotted on filter paper, cut into small pieces (in the case of the kidneys, sections of the cortex and the medulla were removed), weighed, and homogenized in a VirTis 23 blender. Homogenizations were carried out at 0–3°C at maximum speed, using 20 ml of deionized distilled water per gm of tissue. Soft tissues were homogenized for 3 min, whereas bone tissues were homogenized for 15 min to insure their complete disintegration. Just prior to the enzymatic determinations, appropriate dilutions of the stock water homogenates were made with cold deionized distilled water.

Enzyme Assays

Alkaline Nonspecific Phosphomonoesterase (EC.3.1.3.1).
Determinations of alkaline phosphatase activity in serum and whole tissue homogenates were based on the method of Bessey et al. (4). Due to the limited buffering capacity of 0.05 M glycine-NaOH buffer in the presence of atmospheric CO₂ (13), and the considerable variation in alkaline phosphatase activity caused by small changes in the pH (18), assays were carried out in glass-stoppered tubes, and the incubation period was reduced to twenty minutes. In this way, the pH of the assay mixture was kept constant. For the enzymatic assay, 0.1-ml aliquots of serum or tissue homogenate were taken.

Acid Nonspecific Phosphomonoesterase (EC.3.1.3.2).
Serum acid phosphatase activity was determined according to Berger and Rudolph (3), employing 0.05-ml aliquots of serum. The pH of the assay mixture remained constant throughout the 30-min incubation period.

Acid Proteolytic Activity.
Hemoglobin-splitting activity of serum was assayed at pH 3.6 in a manner similar to that described by Anson (1). Serum aliquots of 0.3 ml were incubated in a total volume of 3.0 ml of 0.14 M acetate buffer containing 0.66% (w/v) denatured bovine hemoglobin (Type II, Sigma Chemical Co., St. Louis, Mo.), at pH 3.6. The pH of the assay mixtures remained constant throughout the incubation period.

pH measurements of the assay mixtures at the start and at the end of the incubation periods were done with a combined calomel and glass electrode using a Radiometer pH-meter 25 (Copenhagen) with a scale expander.

All enzymatic assays were carried out at 37° ± 0.5°C, under linear kinetic conditions, in duplicate (phosphatases) or triplicate (acid protease).
Serum acid and alkaline phosphatase activities are given in international units, namely, micromoles of substrate transformed per min per liter. Serum acid protease activity is expressed in terms of micromoles of free tyrosine liberated per 1 ml of serum per 1 hr incubation. Alkaline phosphatase specific activity in various tissues is expressed on the physiologic basis of units per gm wet weight of tissue, one unit referring to the transformation of one micromole of substrate per min.

Chemical Analyses

Calcium and Magnesium. Total serum calcium and magnesium were determined by direct recorded titration with ethylenediaminetetraacetate (19).

Phosphorus. Serum and urine inorganic phosphorus was determined by the method of Tausky et al. (30).

Creatinine. Serum and urine creatinine were determined with picric acid by the standard Autoanalyzer method (Technicon Autoanalyzer Methodology, N-11b).

Serum Total Protein Content. This was determined by refractometry using the Goldberg refractometer (American Optical Co., Buffalo, N. Y.).

All chemical determinations were conducted in duplicate.

Tubular Reabsorption of Phosphate (TRP). This was calculated by the formula:

\[
\% \text{ TRP} = \left( \frac{1}{\text{Serum Creatinine} \times \text{Urine Pi}} - \frac{1}{\text{Urine Creatinine} \times \text{serum Pi}} \right) \times 100
\]

on the assumption that the creatinine clearance represents the glomerular filtration rate (22). Urine samples were collected overnight, and blood samples were consistently obtained in the early morning hours thereafter.

All chemical, biochemical, and functional studies were carried out in the basal fasting state (overnight fasting, but with free access to water).

In view of the relatively small number of observations, the data for each variable were examined by a nonparametric test (the Mann-Whitney U test) (27) at each point. The trend of the change in any of the variables studied was examined by comparing the end points of the interval of interest using the same test. The level of significance, \( P \leq 0.05 \), was used.

Histologic Preparations (17).

Necropsy was performed on selected animals immediately after sacrifice. Representative sections of various bone tissues were fixed in a 10% aqueous solution of buffered (pH 7.0) formalin. Soft tissues were first fixed in Bouin’s solution for a day and then in buffered formalin. Bony tissues were demineralized under vacuum, in 10% formic acid buffered to pH 4.5 with sodium citrate. Paraffin sections were stained with hematoxylin and eosin. Von Kossa’s stain was used for detection of soft tissue calcification.

RESULTS

In relation to calcium homeostasis, it is noteworthy that, as compared to human sera, rabbits are normally hypercalcemic (13.80–14.50 mg %) and hyperphosphatemic (7.00–7.90 mg %).

Valid assay conditions for acid and alkaline phosphatases, as well as for acid protease, were established by a series of kinetic investigations exploring such variables as enzyme concentration, incubation time, and pH. The measured activities of each of the three enzymes were strictly linear with respect to both enzyme concentration and duration of incubation. Alkaline phosphatase activity in the serum as well as in the various tissue homogenates was stable for at least two days at 0°C. Under the specific experimental conditions employed in this study, acid phosphatase activity in normal rabbit serum (94.8–134.4 IU) was found to be considerably higher than that of alkaline phosphatase (46.2–67.5 IU). Alkaline phosphatase activity in the normal kidney medulla (0.15–0.21 U/gm), was found to be too low as compared to that of kidney cortex or the other tissues studied; therefore, this tissue was omitted from further investigation.

Phosphatase activity in normal rabbit serum exhibited a sharp pH optimum at the acid range: 5.40 ± 0.15 (5 x 10^{-3} M p-nitrophenyl phosphate in 5 x 10^{-2} M citrate buffer) and a rather broad pH optimum peak at the alkaline range extending over the pH values of 10.5–11.5 (5 x 10^{-3} M p-nitrophenyl phosphate in 5 x 10^{-2} M glycine-NaOH buffer) (Chart 1). The pH activity profile for serum acid protease in normal young rabbits (Chart 2) showed a broad peak extending over the pH values of 1.75–2.75 (1.0 x 10^{-4} M denatured hemoglobin, 1.40 x 10^{-1} M acetate buffer). At pH 3.15, however, only half of the peak activity was detected. From pH value of 4.50, and towards neutrality, the acid protease activity increased slowly, but even at pH 6.60 this activity was only about 70% of the peak activity. Owing to the lack of solubility of denatured hemoglobin, hemoglobin-splitting activity could not be assayed at neutral and alkaline pH values.

VX-2 Carcinoma-bearing Rabbits. Tumor growth in the thigh muscle was confirmed in every transplanted rabbit. Gross metastatic lesions were consistently detected in the iliac lymph node.
"Pseudohyperparathyroidism" in VX-2 Carcinoma-bearing Rabbits

3.0

2.0

1.0

0.0

Chart 2. Effect of pH on serum acid proteolytic activity in normal young rabbits. Denatured bovine hemoglobin (1.0 \times 10^{-4} M), served as the substrate.

node draining the implantation site; in two cases lung metastases were also detectable 23 days posttransplantation. Between the second and third week following the tumor transplantation, a gradual depression of food intake accompanied by gradual weight loss (up to 10–15% of maximal body weight) were observed. The effects of the tumor growth on serum chemistry, tubular reabsorption of phosphate, and serum and tissue alkaline phosphatase levels are depicted in Charts 3 and 4. The previously reported hypercalcemic and hypophosphatemic state was confirmed in this study and was found to occur simultaneously with depressed renal tubular reabsorption of phosphate (68–73% vs 88–98% in the controls). This "pseudohyperparathyroidism" syndrome was evident at about the sixteenth day posttransplantation, and its magnitude did not change over the following week. Serum levels of magnesium and creatinine, as well as serum total protein content, remained normal throughout the experiment. Of the three serum enzymes studied during tumor development, alkaline phosphatase activity was found to be considerably depressed, whereas the activities of acid phosphatase and acid protease remained unchanged (Table 1). The decrease in serum alkaline phosphatase level (approx. 60%) coincided with a decrease in kidney cortex alkaline phosphatase activity of similar magnitude while no significant changes in bone or duodenal or liver alkaline phosphatase levels were detected at any time during the experiment. The simultaneous depression of serum and kidney cortex alkaline phosphatase activities occurred at about the 11th day posttransplantation, was not progressive as compared to control values, and preceded the alterations in serum levels of calcium and phosphorus by approximately five days. Alkaline phosphatase activity in serum mixtures from VX-2 rabbits with hypophosphatasia and control rabbits with normophosphatasia was the sum of the individual serum activities, indicating the absence of alkaline phosphatase inhibitors in the circulation of the tumor-bearing animals. Organ-specific alkaline phosphatase isoenzymes in the sera of the tumor-bearing rabbits could not be detected by starch-gel electrophoresis. Weight loss per se in the control animals induced a small but significant decrease in serum alkaline phosphatase activity (approx. 20%), but had no effect on the other parameters studied.

With the exception of leukocytosis (16,000 ± 2,000 cu mm on the third week posttransplantation vs 6,800 ± 1,000 cu mm in the controls), the hematologic profile in the tumor-bearing rabbits was normal.

Roentgenograms and histologic examination of various bone specimens failed to reveal metastatic lesions. Kidney sections appeared normal under light microscopy, with the exception of moderate interstitial nephritis which is a normal incidental finding in laboratory animals. No indication of nephrocalcinosis was present.

Experimentally Induced Hyperparathyroidism. The effects of acute parathyroid hormone (PTH) treatment on serum
Chart 4. Serum, kidney-cortex, liver, bone (calvaria), and intestine (duodenum) alkaline phosphatase activity in control and VX-2 rabbits during the early stages of tumor development and appearance of the "pseudohyperparathyroidism" syndrome. The solid line represents the values for the VX-2 carcinoma-bearing animals. The broken line represents the values for the control animals. Vertical bars indicate the standard deviation wherever significant differences in the various parameters between the control and the tumor-bearing rabbits occurred. The pairs of points for each time interval represent the mean values obtained from the analysis of five tumor-bearing rabbits and three controls.

chemistry, renal tubular function, and serum and tissues alkaline phosphatase levels in rabbits are summarized in Table 2. Whereas the magnitude of hypercalcemia, hypophosphatemia, and TRP depression closely resembled that found in the VX-2 rabbits, no effect on serum and kidney cortex alkaline phosphatase levels was detected. Bone alkaline phosphatase activity, however, was markedly depressed.

**DISCUSSION**

The previously reported state of hypercalcemia and hypophosphatemia in VX-2 carcinoma-bearing rabbits prompted us to further investigate the nature and magnitude of skeletal, renal, and intestinal effects contributing to this phenomenon. The present study clearly demonstrated the depression of tubular reabsorption of phosphorus, occurring simultaneously with the hypophosphatemia and hypercalcemia, induced by the tumor. This renal effect is one of the well-established actions of parathyroid hormone (2).

Direct skeletal effect of the tumor leading to bone resorption and hypercalcemia was also sought. To this end, bone alkaline phosphatase activity, as well as serum levels of alkaline phosphatase, acid phosphatase, and acid protease were continuously determined during the early stage of tumor development and appearance of the hypercalcemic state. The rationale behind the selection of these three enzymes was as follows: Alkaline phosphatase is intimately associated with various metabolic processes of bone, and its blood level provides valuable means in the diagnosis of bone disease (15). Acid phosphatase (29, 32) and acid protease (32, 33) actively participate in the process of bone resorption. It was hoped that enhanced bone resorption, if it did indeed occur, would lead to an increased serum levels of the two acid hydrolases and would also affect alkaline phosphatase activity. However, preliminary studies of the three enzymes in the serum revealed that only alkaline phosphatase activity was markedly affected by the tumor, whereas the acid hydrolases levels remained unchanged. Subsequent enzymatic studies, therefore, concentrated on alkaline phosphatase. In addition to serum, bone, kidney, intestine, and liver alkaline phosphatase activities in the tumor-bearing rabbits were also determined in relation to the chemical changes in serum levels of calcium and phosphorus and the alterations in renal handling of phosphate. The liver, though unrelated directly to calcium homeostasis, was included in this study since a certain fraction of serum alkaline phosphatase is of hepatic origin.

Under the specific experimental conditions employed in this study, serum acid phosphatase activity was found to be considerably higher than that of alkaline phosphatase. This finding, though not relevant to the main theme of the study, is emphasized only because it contradicts the usual findings in both humans and rodents, whereby serum alkaline phosphatase activity is many times higher than that of acid phosphatase (e.g., 20). The only explanation of this discrepancy lies in the magnitude of platelet acid phosphatase released into the serum during the coagulation process (38).

The biochemical findings in the VX-2 rabbits, and in particular the selective depression of kidney alkaline phosphatase activity, coupled with the observed depression of TRP, point to

**Table 1**

<table>
<thead>
<tr>
<th>Determination</th>
<th>Control</th>
<th>VX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase (IU)</td>
<td>110.3 ± 14.3</td>
<td>113.5 ± 11.0</td>
</tr>
<tr>
<td>Acid protease (µM tyrosine/ml</td>
<td>1.29 ± 0.15</td>
<td>1.19 ± 0.20</td>
</tr>
<tr>
<td>serum/hr)</td>
<td>18)</td>
<td>(30) N.S.</td>
</tr>
<tr>
<td>Total protein (gm %)</td>
<td>5.25 ± 0.38</td>
<td>5.45 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(30) N.S.</td>
</tr>
</tbody>
</table>

Mean serum values in control and VX-2 rabbits compounded from consecutive determinations at six time intervals during the early development of the tumor. Values are means ± S.D. Figures in parentheses represent the number of observations. N.S., no significant change.
an exclusive renal involvement at an early stage of the tumor growth in relation to the development of hypercalcemic and hypophosphememic state. This conclusion is based on two quite reasonable assumptions: (a) due to its particularly high concentration in a few selected organs (23), alkaline phosphatase activity in kidney, bone, intestine, and liver provides a sensitive biochemical index for monitoring any change in the cellular activities of these organs; (b) the calvaria truly represent the skeletal system in its response to the regulatory mechanisms of calcium in the body fluids. The significant depression of alkaline phosphatase activity observed in the calvaria of the PTH-treated rabbits supports this second assumption.

Since the physiologic role as well as the biologic significance of alkaline phosphatase are still obscure, it is hard to interpret the observed depression of its activity in the kidney cortex in relation to the “pseudohyperparathyroidism” syndrome. In this connection, however, it is interesting to note that experimentally induced hyperparathyroidism in rats also caused a considerable decrease of alkaline phosphatase activity in the region of the proximal convoluted tubules of the kidney. The lowered enzyme activity was associated with anatomic changes in this region, as revealed by electron microscopy (7). Our attempt to reproduce the biochemical changes observed in the VX-2 rabbits, by acute treatment of rabbits with parathyroid extract (PTE) was unsuccessful though the chemical changes in the serum as well as the depression of TRP were quite similar in magnitude. No effect on serum and kidney cortex alkaline phosphatase activity was observed, whereas its activity in bone was markedly lowered. This last finding is in line with the current concept that bone alkaline phosphatase level mainly reflects osteoblastic activity (31) and that this activity is decreased by parathyroid hormone (10). The failure to register any change in serum and kidney alkaline phosphatase activities in the PTE-treated rabbits may be due to such parameters as dose and timing. In the VX-2 rabbits, the simultaneous decrease in serum and kidney alkaline phosphatase levels, as well as the absence of alkaline phosphatase inhibitors in the serum, strongly suggest that the hypophosphatasia was, at least in part, of renal origin. The small decrease in serum alkaline phosphatase activity in the pair-fed controls towards the end of the experiment was due to depressed food intake. It has been shown before, that decreased food intake reduced the serum levels of alkaline phosphatase (5, 14). The finding of normomagnesemia in the face of moderate hypercalcemia, induced either by the tumor or by the PTE treatment, is consistent with the recent view that the relationship between parathyroid hormone and magnesium metabolism is different, at least in intensity, from the direct way in which calcium and PTH are related (12). Considering the observed phosphaturic effect of the tumor, the possibility that increased tubular reabsorption of calcium might contribute to the hypercalcemia should be mentioned. Increased tubular reabsorption of calcium is another renal effect of parathyroid hormone (25). One possible explanation to the lack of experimental evidence for bone participation in the early development of the hypercalcemic and hypophosphememic state in the VX-2 rabbits could be thyrocalcitonin antagonism which, however, does not interfere with the renal effects of parathyroid hormone and might even enhance the phosphaturic response (24).

Finally, it may be concluded that in our attempt to characterize the biochemical effects of VX-2 carcinoma in rabbits leading to a state of hypercalcemia and hypophosphatism: (a) parathyroid-like effect of the tumor on renal function, namely, depressed tubular reabsorption of phosphorus was established; (b) though selective renal involvement was observed in this study, skeletal effects leading to concurrent calcium mobilization from bone cannot be excluded; and (c) the biochemical effects of one super dose of PTH may not provide an adequate basis for comparison with the biochemical changes induced by the tumor.

Table 2

<table>
<thead>
<tr>
<th>Tissue-alkaline phosphatase (U/gm wet wt.)</th>
<th>Controla</th>
<th>PTE-treatedb</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>3.35 ± 0.25</td>
<td>2.05 ± 0.18</td>
<td>-37.6</td>
</tr>
<tr>
<td>Intestine</td>
<td>1.89 ± 0.25</td>
<td>1.62 ± 0.20</td>
<td>0</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>24.68 ± 1.85</td>
<td>26.58 ± 2.10</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>7.99 ± 0.60</td>
<td>8.47 ± 0.53</td>
<td>0</td>
</tr>
</tbody>
</table>

Effect of parathyroid extract on renal function, serum chemistry, and serum and tissues levels of alkaline phosphatase. Values are means ± S.D. TRP, tubular reabsorption of phosphorus.

*Three rabbits serving as controls each received a single subcutaneous dose of the same volume of 0.85% saline containing 1.6% glycerol and 0.2% phenol as the parathyroid dose in the experimental animals.

Four rabbits were injected with a single subcutaneous dose of 750 USP U/kg of parathyroid extract (PTE) (Lilly) and killed 20 hr later.

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