THE INFLUENCE OF AGE AND OF DURATION OF TREAT-
MENT ON THE PRODUCTION AND REPAIR OF
BONE LESIONS IN EXPERIMENTAL HYPER-
PARATHYROIDISM*

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PLATES 7 TO 9

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We have pointed out that young guinea pigs weighing between 280 and 370 gm., when injected subcutaneously with a single dose of 20 units of parathormone per 100 gm. of body weight, develop severe and rapid decalcification of the skeleton, which becomes progressively more intense up to about the 48th hour. In contrast to these findings, the skeletons of adult guinea pigs weighing between 620 and 860 gm., and injected with the same doses, do not show histological changes. The serum calcium and phosphorus, however, indicate effects of parathormone on the adult as well as on the young guinea pigs, although they are pronounced in the latter (1, 2).

The striking difference in the histologic appearance of the skeletons of young and adult guinea pigs given single doses of parathormone led us to study in greater detail the effect of parathormone on guinea pig bones as influenced by the age of the animal. Furthermore, we studied the influence of increasing age on the effects of repeated or intermittent doses in young and old guinea pigs, as reflected in bone changes. This permitted us to make observations concerning the so called "immunity" to repeated doses of parathormone, which is supposed to develop. The details of our experimental methods are given in the papers mentioned above.

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Criteria of Age

In healthy stock the age of a growing guinea pig is well indicated by its weight. Our animals weigh between 70 and 90 gm. at birth; between 200 and 260 gm. at 1 month; between 300 and 400 gm. at 2 months; between 425 and 525 gm. at 3 months; between 500 and 600 gm. at 4 months. After this, increase in weight is much slower, our animals weighing between 600 and 700 gm. at the age of 6 months and between 800 and 1000 gm. at about 1 year. We consider a guinea pig fully adult when the epiphyseal cartilage plates of the upper end of the humerus, the lower end of the femur, and the upper end of the tibia are closed (they need not have disappeared). At this time the cells of the epiphyseal cartilage plate no longer retain their regular columnar arrangement, while the metaphyseal surface of the plate is becoming bridged by bone. From our casual observations, we believe that closure occurs at about the 10th month. As the animal becomes older, these closed epiphyseal cartilage plates become thinner and eventually disappear. While we have made no systematic study, our observations have led us to conclude that this does not take place in these plates until some time after the 1st year. This is quite in line with what Dawson showed for the albino rat (3)—that these epiphyseal cartilage plates do not disappear until senility. Guinea pigs between the 4th and 9th month of age may be considered young adults. Sexual maturity, however, occurs quite early in our stock. We have on several occasions observed pregnancy of approximately 1 to 2 weeks' duration in animals about 2 months of age.

EXPERIMENTAL

The Relative Absence of Parathormone Effects in the Bones of Guinea Pigs Treated Daily to the Age of 110 to 120 Days

In four guinea pigs 2 to 7 days of age, weighing between 78 and 128 gm., the injection of 1 or 2 units of parathormone daily was begun. This dose was gradually increased, the animals receiving 10 units a day after 16 days of treatment. They almost doubled their original weight during this period. The dose was then increased by steps to 20 units daily, one animal being given this dose on the 23rd day of the experiment, and the others on the 36th day. The animals then weighed 325, 296, 281, and 269 gm., as compared with 319, 290, 269, and 235 gm. respectively for their untreated litter mate controls. The dose of 20 units daily was continued for 65 days in one animal, 73 days in two, and 87 days in the fourth. The animals received total doses of 1500 to 1890 units of parathormone during the course of treatment. However, we do not consider the total dose as significant as the size and the gradation of the doses, and as the duration of treatment. The guinea pigs gained consistently in weight throughout the experiment.

The animal which had been injected with 20 units daily for 65 days weighed 508 gm. when its litter mate control reached 519 gm. Both were then fasted for 48 hours and killed to terminate the experiment. The animals receiving 20 units

of parathormone daily for 73 and 87 days, weighed 443, 560, and 497 gm., respectively; their controls 579, 553, and 482 gm. All were killed 5 days after the last injection of parathormone at which time they were 115 to 120 days old. They showed no hypercalcemia at the termination of the experiments, but on the contrary, a hypocalcemia, the significance of which is discussed elsewhere (4).

The bones of the test animals showed a surprising absence of severe changes. Those treated for 65 days showed slight to moderate resorption of the cortices of the ribs and long bones, indicated by enlargement of the vessel canals, which contained young connective tissue. Except in the metaphyses of the upper end of the tibia and the lower end of the femur there was no marrow fibrosis. These metaphyses showed numerous irregular trabeculae of cartilage with osteoid borders, surrounded by narrow zones of bone lined by osteoblasts. Some osteoid was present in the connective tissue between the trabeculae. The epiphyses showed nothing unusual; the trabeculae were thick, regular, and lined by osteoblasts. The epiphyseal cartilage plates and the costochondral junctions were as yet not closed.

The three animals treated for 73 and 87 days with 20 units of parathormone daily showed even fewer bone changes. There was at most a slight thinning of the cortices, especially of the ribs, and slight widening of some of the vessel canals, which contained young connective tissue. There was no metaphyseal marrow fibrosis, endosteal resorption, or abnormal phagocytosis by osteoclasts. The epiphyses showed a complete absence of resorption, the trabeculae being quite thick and lined by osteoblasts. The epiphyseal cartilage plates were not closed.

This group of guinea pigs brought out strikingly the relative absence of parathormone effects upon the bones as a result of daily injections to the age of 110 to 120 days. On the basis of our previous experience (1), we believe that after receiving 20 units of parathormone daily for 2 or 3 weeks, the bones of these animals had undergone severe decalcification, but when this treatment was continued, its effects, as indicated by bone changes, diminished. In spite of further continuance of the treatment with the same dose of parathormone, healing of the earlier lesions was possible (Figs. 1 and 2).

The Relative Absence of Parathormone Effects upon the Bones of Guinea Pigs Treated with Intermittent Injections of Large Doses of Parathormone to the Age of 95 to 145 Days

Three guinea pigs about one week old, weighing 100, 110, and 150 gm., were injected daily for 16 days with small, gradually increasing doses of parathormone. They were first given 2 units daily and finally received 10 units daily. At the end of this period the animals weighed 190, 220, and 240 gm., respectively. They were rested for 6 days, at the end of which time they weighed between 230, 250,

and 270 gm. Intermittent injections of large doses of parathormone were then begun, the animals being a month old. The injections were given at intervals of from 7 to 11 days; on one occasion, in two animals, 15 days elapsed between injections, during which period the animals had been bled from the heart to determine the serum calcium and phosphorus.

One guinea pig was given its first large subcutaneous injection of 60 units of parathormone when it weighed 250 gm. The intermittent injections covered a period of 66 days, and a total of 800 units were given. The animal was found dead 48 hours after the last dose (140 units). It was then 95 days of age, and weighed 370 gm. Death was due to acute hyperparathyroidism.

Autopsy showed beading at the costochondral junctions. The bones were soft. On histological examination, all of those examined showed moderate resorption; the Haversian canals were enlarged and contained young connective tissue with osteoclasts in Howship's lacunae. The marrow of the metaphyses was scarred, but osteoid tissue was absent here and elsewhere. Along the inner surface of the cortices, especially near the costochondral junctions and the epiphyseal cartilage plates, there were some Howship's lacunae and osteoclasts. At the costochondral junctions and in the metaphyses, areas of more acute decalcification were observed; these acute changes were probably due to the dose of 140 units of parathormone injected 48 hours before death. It should be noted that this animal was only 95 days of age, and that the epiphyseal cartilage plates were open. The effects of the previous intermittent doses were indicated by the numerous distinct longitudinal cement lines, and, where Haversian canals were present, by irregular cement lines, arranged in relation to the canals, giving a mosaic appearance to the bone.

Two guinea pigs weighed 270 and 230 gm., respectively, when intermittent injections were started. The first doses were 60 units and the final doses 140 units. The guinea pigs received 1420 units of parathormone each. 4 days after the last injection, the animals were killed to terminate the experiment. They weighed 675 and 540 gm. respectively and were about 20 weeks old.

On histological examination, the cortices of the ribs and long bones were found to be compact. The vessel canals were, at most, only slightly enlarged, and contained no significant increase of connective tissue. The marrow of the long tubular bones, even in the metaphyses, was not fibrosed. The epiphyseal cartilage plates showed the earliest indications of closing. Many ribs were beaded at the costochondral junctions, due to new bone formation subsequent to fractures in these regions. The compact and spongy bone showed marked accentuation and increase of the cement lines. These ran parallel, but in the cortices of the long bones they were irregular, giving the bone a mosaic appearance. The innumerable cement lines were the only indication of the effects of the earlier injections of parathormone, the fractures at the costochondral junctions being probably due to the last one or two doses.

The age factor influencing the effect of parathormone on the bones of guinea pigs is also brought out in this group. The animal 95 days of age at autopsy, although it had received only slightly more than one-half of the amount of parathormone given to the others, succumbed to a dose of 140 units, and its bones showed some of the effects of acute hyperparathyroidism. The other two animals were 140 and 145 days of age. They showed increasing ability to withstand the injection of large doses of parathormone, and were not killed by several successive doses of 140 units. Their bones showed relatively very little decalcification (Figs. 3 to 8). All of these animals showed the effects on the bones of intermittent injections of parathormone. These were indicated by the numerous cement lines which gave a mosaic appearance to the bones (Fig. 6).

The Effect of Extremely Large Single or Pyramided Doses of Parathormone on the Bones of Adult Guinea Pigs

We have already shown that the subcutaneous injection of large doses of parathormone (20 units per 100 gm. of body weight) into adult guinea pigs weighing from 620 to 860 gm. resulted in no appreciable changes in their bones, which were examined up to 48 hours after the injection (1). In attempting to produce bone changes with parathormone in adult guinea pigs, it was therefore decided to use much larger doses (5).

A male guinea pig weighing 900 gm. was injected subcutaneously with 100 units of parathormone per 100 gm. of body weight. It received a total of 900 units of parathormone (45 cc.) given in two equal doses within a period of $2\frac{1}{2}$ hours. For 48 hours after the injection the animal did not appear ill. At the end of this period it was killed. The serum calcium and phosphorus were 15.7 and 4.9 mg. per 100 cc. respectively. Blood urea nitrogen was 9.2, and chlorides 489 mg. per cc. Hemoglobin was 14.8 gm. per 100 cc.

At autopsy the bones were dense and resisted cutting. The liver contained one large and several small areas of anemic necrosis. The heart showed some degeneration of the muscle fibers. When stained by the von Kossá method, considerable calcium was found in the necrotic areas of the liver. On histological examination the bones showed strikingly few changes. The cortices of the ribs and of the long tubular bones were compact; the vessel canals were of normal diameter. Two of the ribs showed small areas of acute decalcification in the vicinity of the costochondral junctions, and here a few osteoclasts were found.

The epiphyseal cartilage plates of the long tubular bones were closed and the marrow and trabeculae of the metaphyses showed nothing unusual. The trabeculae and marrow of the epiphyses also were normal. There was no osteoclastic resorption here.

The experience gained from this animal led us to investigate the effects of still larger single doses.

An adult female guinea pig weighing 1040 gm. was injected with 2080 units (104 cc.) of parathormone, on the basis of 200 units per 100 gm. of body weight. The parathormone was given subcutaneously in two equal doses at an interval of 3 hours. 24 hours later the animal was very ill; it died 40 hours after the injection.

Autopsy showed considerable edema of the fat and subcutaneous tissues. The lungs were congested; pneumonia was absent; the heart was whitish and contracted; the suprarenals were congested; the liver, spleen, and kidneys showed nothing unusual in the gross. Histological examination showed besides the edema scattered necrosis in the subcutaneous tissues. There was no degeneration of the kidney tubules, nor of the liver. Calcium was found on histological examination in greatest amounts in the lung, and to a less extent in the other tissues.

Sections of the bones (Figs. 9 and 10) showed a striking absence of parathormone effects. The cortices of the ribs and long tubular bones were compact. The vessel canals were small, and there was no pathological subperiosteal or sub-endosteal osteoclastic resorption. The epiphyseal cartilage plates of the long tubular bones were closed, as were the costochondral junctions of the ribs. The rib cartilages showed beginning ossification. The skull and jaw bones also showed no pathological resorption or fibrosis. The marrow, especially near the costochondral junctions and in the vicinity of the closed epiphyseal cartilage plates, was congested, but there was no marrow necrosis. The trabeculae and marrow of the epiphyses were normal. This animal was older than the last one discussed, and twice the dose was therefore ineffective in producing bone changes, although the animal was killed by the general toxic effects of the parathormone.

Still testing the possibility of altering seriously the histological structure of the bones of an adult guinea pig, we injected an animal with a total of 300 units of parathormone per 100 gm. of body weight, in doses pyramided over a period of 48 hours. An adult male guinea pig weighing 860 gm. was injected with three doses of parathormone (860 units each), at intervals of 24 hours between the doses. The animal thus received a total of 2580 units (129 cc.) of parathormone within 48 hours. The sites of injection were varied to assure absorption.

24 hours after the last injection, blood was withdrawn from the heart, under ether anesthesia, for chemical analyses. The serum calcium and phosphorus were 19.6 and 4.7 mg. per 100 cc., respectively. About 48 hours after the last injection, and at the end of the 4th day of the experiment, the animal was killed when moribund. It weighed 860 gm. The serum calcium and phosphorus were 18.9 and 8.4 mg. per 100 cc., respectively, the urea nitrogen 40 mg. and sodium chloride 430 mg. per 100 cc. The hemoglobin was 14.6 gm. per 100 cc.

At autopsy the liver showed several patches of necrosis, all within an area of 8 mm. The bones resisted cutting. On histological examination, the liver cells showed cloudy swelling; nothing unusual was seen in the kidneys aside from necrosis of a few tubules. The other organs showed nothing unusual and contained no large deposits of calcium. A section from tissues at one of the sites of injection showed edema and necrosis of both the connective tissue and muscle, and calcium was demonstrated by the von Kossá method.

On section the cortices of the long tubular bones were quite compact, the vessel canals showing only slight enlargement. Underneath the periosteum and endosteum there were numerous Howship's lacunae filled with osteoclasts. The epiphyseal cartilage plates of the long bones were not completely closed, the cartilage cells still preserving, to a great degree, the columnar arrangement. So severe was the decalcification that epiphyseal separation had occurred in all the long tubular bones. The metaphyses showed, as would be expected, a larger number of Howship's lacunae with osteoclasts on the surfaces of the bony trabeculae. There was in addition slight scarring of the metaphyseal marrow. On the surface of the cortices of the ribs were Howship's lacunae with osteoclasts, more abundant on the subendosteal than on the subperiosteal surface. The vessel canals of the ribs were slightly enlarged. Some acute decalcification and marrow fibrosis was observed at the costochondral junctions. The skull and jaw bones also showed resorption, with Howship's lacunae and osteoclasts in abnormal numbers. Some resorption of the trabeculae of the epiphyses with osteoclasts and Howship's lacunae was observed.

This guinea pig, from the appearance of its epiphyseal cartilage plates, and from the fact that epiphyseal separation had occurred, was younger than the first two. It is therefore all the more significant that in this animal, probably not fully adult, (see page 140) 2580 units of parathormone, pyramided within a period of 48 hours, were required to produce moderately severe bone resorption. However, the bone changes were less than those produced by much smaller doses in younger animals (Figs. 10 and 12).

The Effect of Continued Daily Injections of Parathormone on the Adult Guinea Pigs

We have demonstrated that the injection of large single doses of parathormone into two adult guinea pigs produced no effects on the bones of the animals and that in one case when 2580 units were administered, some changes had been produced. We decided to ascertain the effects of repeated doses of parathormone on adult animals.

TABLE I
Summary of Daily Treatment of Adult Guinea Pigs

Day of experiment	No. 1206, M.		No. 1195, M.		No. 1135, F.		No. 959, F.		No. 1136, F.		No. 1196, M.		No. 1194, M.	
	Weight		Weight		Weight		Weight		Weight		Weight		Weight	
	gm.	units	gm.	units	gm.	units	gm.	units	gm.	units	gm.	units	gm.	units
1	890	20	950	20	900	20	1015	20	1000	20	820	20	950	20
2		20		20		20		20		20		20		20
3		20		40		40		40		40		20		40
4		20		40	820	40		40	990	40		40		40
5		40	960	60		60	965	60		60	770	40	860	60
6	830	40		60		60		60		60		60		60
7	830	40	880	60		60		60		60		60		60
8					750	60		60		60		60		60
9							840	60	990	60		60		60
10									970	60		60		60
11											620	60	750	60
12												0		
13											580	0		
Total units. . .	200		300		360		420		480		500		540	

Seven adult guinea pigs received daily injections of parathormone for 7 to 11 days. They received a total of from 200 to 540 units as shown in Table I. All died from acute hyperparathyroidism.

The histological examination showed that with such treatment bone and marrow changes could be produced in adults; the degree varied directly with the total dosage of parathormone given, but the adults which were younger showed relatively more bone and marrow change, on a given dosage, than those that were older. For example, No. 1206, killed in 7 days by 200 units, showed some marrow necrosis, and shallow subendosteal Howship's lacunae, containing a few osteoclasts; while No. 1196 killed in 11 days by 500 units showed very extensive mar-

row necrosis, considerable subendosteal resorption, and fractures at some of the costochondral junctions. Some of these animals showed enlargement of the Haversian canals which contained connective tissue and osteoclasts in Howship's lacunae, and some also showed slight degrees of marrow fibrosis.

These experiments show that parathormone, given in separate doses over a period of several days, produces bone changes and kills adult guinea pigs which would not be affected by the same amount of the hormone given at a single injection. However, if care is taken not to

TABLE II

Dose	Duration	No. 1204 Weight	No. 1207 Weight
<i>units</i>	<i>days</i>	<i>gm.</i>	<i>gm.</i>
20	4	950	810
40	4		
0	2	900	750
20	2	900	750
40	2	900	780
60	1		
0	1	900	770
20	1		
40	1		
60	3	880	723
0	3	850	715
20	1	855	705
40	3	875	710
60	1	855	700
40	8	860	725
60	13	820	700
0	1	875	720
60	18	875	725

increase the dose too rapidly, or if the treatment is intermitted when the animals appear sick from hyperparathyroidism, the injections can be continued for a long time, with increasing dosage. Two adult guinea pigs, weighing 950 and 810 gm., respectively, received 3040 units of parathormone over a period of 69 days, as shown in Table II.

Guinea Pig 1207 was the younger of the two animals, and showed the greater number of changes. There was very extensive enlargement of the Haversian canals, which contained much young connective tissue. Some of the enlarged resorption spaces showed deep Howship's lacunae and numerous osteoclasts.

This animal also showed a slight amount of subperiosteal and subendosteal resorption, with some subendosteal fibrosis. However, in Guinea Pig 1204 considerable evidence of healing was encountered. There was practically no subperiosteal or subendosteal resorption, and the enlarged Haversian canals, although filled with young connective tissue, showed smooth surfaces, and lining of the surfaces by osteoblasts as another indication of the healing (Fig. 13).

An adult guinea pig, weighing 960 gm., received a total of 4020 units of parathormone in the same period. The dosage was the same as for Guinea Pigs 1204 and 1207 until the 52nd day, after which the following amounts were given daily: 60 units for 5 days; 80 units for 2 days; 100 units for 2 days; 120 units for 2 days; 140 units for 2 days; 160 units for 2 days; 180 units for 2 days; and 200 units for 1 day.

At autopsy this animal showed beaded ribs and bones that were definitely softened. Microscopical examination showed marked enlargement of the Haversian canals. So extensive was the resorption within these canals that in places the cortex was almost completely replaced by young connective tissue. Some of the long tubular bones and the ribs showed subendosteal resorption, with Howship's lacunae and osteoclasts.

These experiments show that the adult animal can tolerate great amounts of parathormone if the earlier injections are graded so as not to produce fatal hyperparathyroidism. The bone changes produced during a long course of parathormone injections in adults have a tendency to heal as the treatment is continued. However, when the dose of parathormone is further increased, as during the final 13 days in the guinea pig last discussed, indications of more acute hyperparathyroidism reappear, and manifest themselves by extensive subendosteal resorption.

SUMMARY AND DISCUSSION

These studies have shown that the bones of guinea pigs given daily injections of parathormone from the age of 2 to 7 days to the age of 110 to 120 days, show relatively very little effect after receiving 20 units daily during the last 65 to 87 days of treatment. But it is probable that their bones underwent decalcification early in the treatment and that subsequently the parathormone, continued at the same dosage, did not maintain the effects on the bones. Healing finally occurred despite it.

The bones of guinea pigs treated with intermittent injections of large doses of parathormone from the time they were 1 week old to

the age of 95 to 145 days also showed relatively few changes at the end of the treatment. The injections were given at intervals of 7 to 11 days, and were stepped up from 60 units to 140 units. From our previous experience (1) we infer that the earlier injections of parathormone produced very extensive bone changes which healed in the intervals between the injections. As the guinea pigs became older the injections of parathormone did not produce as severe effects.

We have found in our studies of experimental hyperparathyroidism that the bone changes after a single large dose of parathormone in young guinea pigs are soon healed. The study of a series of animals shows that healing begins at about the 48th hour after injection, and proceeds rapidly. Between the 8th and 14 days, callus may be observed at the costochondral junctions, where fractures had occurred. Now the endosteum may be lined by osteoblasts and the vessel canals by new formed bone.

In adult guinea pigs extremely large single doses had little effect on the bones in 48 hours, even though the dose killed the animal. It was only when three doses pyramided over a period of 48 hours and totaling 2580 units of parathormone were given, that moderately severe bone resorption could be demonstrated in the adult.

The elevation of serum calcium may be considered as one of the indices of calcium mobilization in experimental hyperparathyroidism. When the rate of calcium excretion exceeds the rate of its mobilization, or when the animal is on a low calcium diet, hypercalcemia may be absent. It is possible to raise the serum calcium of adult guinea pigs by large single doses of parathormone, but the resulting rise is not as great as in the young (2). This is confirmatory evidence of the fact that calcium is mobilized much less rapidly from the bones of old animals than from those of young ones. Collip pointed out that young normal dogs are more susceptible to parathormone (6). This observation was corroborated by Morgan and Garrison (7). We found that the same difference held also in experimental hyperparathyroidism produced in dogs by repeated doses of parathormone (8). In man, clinical experience likewise indicates the necessity of using relatively large doses of parathormone to raise the serum calcium of adults. The serum calcium of middle-aged or old adults does not rise significantly unless as much as 100 units or more of parathormone are

given daily for a number of days. Charts VI and VII, in a recent paper by Merritt and Bauer (9), support our findings of the relative difficulty of obtaining a significant elevation of serum calcium in adults.

If adult guinea pigs are given daily injections of parathormone which are rapidly stepped up, the animals may be killed by the ensuing acute hyperparathyroidism, only slight bone changes being produced. However, a careful avoidance of the induction of acute hyperparathyroidism by gradual stepping up of the parathormone dose permits the employment of doses continued over a long period of time that could not possibly have been tolerated otherwise. Furthermore, healing of the lesions thus produced may occur, in spite of the continuance of parathormone at this level.

It seems likely that the difference in response of young and old guinea pigs to single doses of parathormone, as indicated by the bone changes, as well as by the serum calcium and phosphorus, is related to the more rapid rate of mineral metabolism in the young, actively growing animals. The calcium mobilizing effect of parathormone is most prominent in actively growing young animals, the calcium being withdrawn from the most readily available stores—the regions of most active new bone formation and most active bone reconstruction (10).

In the adult animal the calcium reserves (in the formed bone) are less susceptible to the calcium mobilizing effect of parathormone. The adult guinea pig will show relatively slight bone changes even as a result of extremely large, fatal doses of parathormone. Repeated doses, as is well known, will produce, by pyramiding, greater effects than the entire amount administered at one time. In this type of experiment the young again show greater susceptibility of the bone than the adult. In time, however, some compensation takes place, and the effects of the same doses are decreased until finally healing may occur in spite of the continued treatment. Increase of the dose, however, again elicits the parathormone effects upon the bone, as well as upon the serum calcium and phosphorus, without toxic changes (1, 8). It would seem that some compensation sets in which may be overcome by increasing the dose. This compensation is especially evident in the experiments in which the parathormone doses were stepped up gradually from small amounts.

In addition to the compensation observed in young and adult animals as a result of repeated injections of parathormone, we must also consider the possibility that there is a compensating mechanism in adult animals more effective than in the young. That compensation occurs is unquestionable but its nature is not clear. Apparently it is less effective during pregnancy, doses of parathormone which produce only slight bone changes in ordinary adults causing very severe lesions in advanced pregnancy (11).

Parathormone has been shown to produce only one primary effect on bone, and that is decalcification. This may come about as the result of a change in the circulating tissue fluids, the salts being dissolved out of the organic matrix, and the latter disappearing secondarily. The process is most rapid in the vicinity of most active bone formation. The osteoblasts disappear from the surfaces of bone where dissolution is occurring, and at the same time the marrow connective tissue proliferates. Fusion of cells produces osteoclasts (12), which then proceed to remove the decalcified organic matrix, with the production of the deep lacunae of Howship. Frequently leucocytes are also observed actively phagocytizing the decalcified organic matrix, and often leucocytes are observed within the osteoclasts (12). Healing is associated with the complete reversal of the process. The osteoclasts disappear, the connective tissue diminishes, osteoblasts reappear, and bone formation is resumed.

As we have previously stated (13), parathormone produces a more continuous effect than experimental acidosis and greater changes than are seen in experimental osteoporosis. A pronounced decalcification results from it which, with its sequelae, simulates von Recklinghausen's disease. The emphasis which the older pathologists laid on osteoclasts as a special feature of *ostitis fibrosa cystica* is justified, for in the experimental condition the appearance of great numbers of osteoclasts is a constant feature, whenever decalcification occurs (13). There seems to be no doubt that the giant cell tumors found in *ostitis fibrosa cystica* are expressions of the same pathological response.

The other features of the bone changes of hyperparathyroidism—marrow hemorrhage, cysts, fractures, and osteoid proliferation—are secondary to the primary decalcification. Progress of the pathological changes leads to circulatory stasis and cyst formation. Stresses and

strains exerted on the progressively weakening bone may result in microscopical or gross fractures. Osteoid tissue is, as we have previously pointed out (13), merely reparative in nature, being laid down as support to the weakened or fractured bone, or as a part of healing. Osteoid borders appear on bone surfaces 48 hours after one large dose of parathormone.

The mosaic picture which we have observed in the bones of some of our animals is produced by short and irregularly disposed cement lines resulting from rapid bone transformation. Schmorl (14) recently emphasized the mosaic-like appearance of the newly formed lamellar bone in Paget's disease (ostitis fibrosa deformans). The mosaic-like appearance of bone has also been described in local bone conditions, as *e.g.* syphilitic periostitis, and in bone in the vicinity of cysts and giant cell tumors in von Recklinghausen's disease (ostitis fibrosa cystica). However, Schmorl claims that in no disease is the mosaic appearance so constant and the arrangement of the cement lines so irregular as in Paget's disease. In chronic experimental hyperparathyroidism (von Recklinghausen's disease), the mosaic structure is not a prominent feature because of the progressive decalcification. But the bones of our young guinea pigs which received intermittent injections showed a mosaic-like appearance indicative of the periodic decalcifications and restorations which they had undergone.

CONCLUSIONS

1. Young guinea pigs are more susceptible than adult guinea pigs to the effects of single or repeated doses of parathormone, as shown by the bone changes.
2. Several successive daily doses of parathormone, in rapidly increasing amount, result in an accentuation of the effects.
3. In young and adult guinea pigs a compensation is established during prolonged parathormone treatment, which enables them to tolerate repeated large doses and which permits considerable repair of bone lesions produced earlier in the treatment.

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EXPLANATION OF PLATES

PLATE 7

FIG. 1. Cortex of the shaft of rib of a guinea pig given 20 units of parathormone for 87 days after some preliminary treatment with smaller doses. The animal weighed 269 gm. when the injection of 20 units was begun. Note that the bone is compact and no subperiosteal resorption is present. Compare with Fig. 2. Magnification 80 $\times$. Stained with hematoxylin and eosin.

FIG. 2. Cortex of the shaft of rib of a guinea pig given 10 units of parathormone for 16 days, shown for comparison with Fig. 1. The animal weighed 260 gm. when treatment was begun. The cortex is thin; there is marked subperiosteal resorption; the vessel canals are enlarged and filled with cellular connective tissue; the marrow is congested. Magnification 65 $\times$. Stained with hematoxylin and eosin.

FIG. 3. The costochondral junction of a rib of a guinea pig injected daily for 16 days with small, gradually increasing doses of parathormone. This was followed by intermittent injections begun when the animal was 1 month old, and weighing 272 gm. These were given at intervals of 7 to 11 days, and stepped up from 60 to 140 units. Note fractures of the cortex and the scarifying of the marrow. The only severe lesions in this animal were observed at the costochondral junctions. Magnification 65 $\times$. Stained with hematoxylin and eosin.

FIG. 4. The cortex of the shaft of the rib shown in Fig. 3. Note the compactness of the bone and the absence of subperiosteal and subendosteal resorption. Magnification 80 $\times$. Stained with hematoxylin and eosin.

FIG. 5. The epiphysis and metaphysis of the upper end of the tibia of the same guinea pig as in Fig. 3. Note the complete absence of resorptive changes in the metaphysis. Magnification 80 $\times$. Stained with hematoxylin and eosin.

PLATE 8

FIG. 6. The skull of the guinea pig described in Fig. 3 legend. Note the numerous cement lines which give the bone a mosaic appearance. These lines show the effect on the bone of the intermittent parathormone injections. Magnification 125 $\times$. Stained with hematoxylin and eosin.

FIG. 7. The costochondral junction of a rib of a young guinea pig weighing 300 gm., shown for comparison with Figs. 3 and 5. The animal was killed 48 hours after the injection of 60 units of parathormone. The marrow is degenerated; the cortex is fractured in numerous places; the columns of endochondral bone are splintered. Magnification 65 $\times$. Stained with hematoxylin and eosin.

FIG. 8. Cortex of the shaft of the rib shown in Fig. 7. The lymphoid marrow has disappeared, and the marrow cavity is filled with blood, the result of free hemorrhage. The cortex shows extensive resorption and enlargement of the vessel canals, which contain connective tissue. Compare with Fig. 4. Magnification 65 $\times$. Stained with hematoxylin and eosin.

FIG. 9. The costochondral junction of the rib of an adult guinea pig weighing 1040 gm., injected with 2080 units of parathormone. The animal died 40 hours later showing no changes in the bone, but some congestion of the marrow. Magnification 85 $\times$. Stained with hematoxylin and eosin.

PLATE 9

FIG. 10. The cortex of the shaft of the rib shown in Fig. 9. Note the complete absence of subperiosteal and subendosteal resorption. The bone is compact. Magnification 85 $\times$. Stained with hematoxylin and eosin.

FIG. 11. The cortex of the shaft of a rib of an adult guinea pig injected with 2580 units of parathormone in 48 hours. About 48 hours after the last injection the animal was killed. Only with such large, pyramided doses was it possible to induce subendosteal resorption. Magnification 85 $\times$. Stained with hematoxylin and eosin.

FIG. 12. Part of the epiphysis and metaphysis of the lower end of the femur of the animal described in the legend of Fig. 11. Note evidences of resorption of the trabeculae of the epiphysis and metaphysis. Magnification 85 $\times$. Stained with hematoxylin and eosin.

FIG. 13. Cortex of the shaft at the middle of the femur of an adult guinea pig receiving 3040 units of parathormone over a period of 69 days. Care had been taken not to increase the dose too rapidly during the early critical periods. Note the marked enlargement of the vessel canals which are filled with cellular connective tissue and contain osteoclasts in Howship's lacunae. Magnification 40 $\times$. Stained with hematoxylin and eosin.

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