remains constant; and in the leaves (Petrie & Wood, 1938) the net rate of protein synthesis increases with increased water content.

SUMMARY

1. Pods of *Pisum sativum* at seven different stages of development have been analysed for total, protein, amino, amide and peptide nitrogen.

2. Protein is first formed, then broken down and transferred to the developing seeds in the form of amide and peptides.

3. The moisture content of the pods has no influence on protein hydrolysis, which, rather, is shown to be determined by the metabolic state of the seeds.

The author wishes to express special thanks to Professor Arne Tiselius, at whose suggestion the present series of investigations was carried out, for his interest and much valuable and friendly advice as well as for the privilege of

using the facilities of the Biochemical Institute. Sincere thanks are also due to Laborator Kai O. Pedersen for advice on ultracentrifuging, to Mr Ewald Hellman for the calculations of the ultracentrifuge data, and to Miss Gunnel Englund for the Kjeldahl determinations.

The author is indebted to Professors C. H. Li and E. W. Yemm, F.R.S., and to Dr R. L. M. Synge, F.R.S., for suggestions concerning the manuscripts.

REFERENCES

- Bisson, C. S. & Jones, H. A. (1932). *Plant Physiol.* **7**, 91.
 Emmerling, A. (1880). *Landw. Versuchsw.* **24**, 113.
 Mothes, K. (1926). *Planta*, **1**, 472.
 Petrie, A. H. K. & Wood, J. G. (1938). *Ann. Bot., N.S.*, **2**, 33.
 Pfenninger, U. (1909). *Ber. dtsch. bot. Ges.* **27**, 227.
 Prianischnikow, D. (1922). *Ber. dtsch. bot. Ges.* **40**, 242.
 Raacke, I. D. (1957). *Biochem. J.* **66**, 101.
 Schulze, E. & Winterstein, E. (1910). *Hoppe-Seyl. Z.* **65**, 431.
 Wassilieff, N. (1908). *Ber. dtsch. bot. Ges.* **26**, 454.

The Effect of Parathormone on the ^{32}P Uptake by the Bones of Rabbits and Cats

BY J. DAWSON, S. M. WEIDMANN AND H. G. JONES

Department of Biochemistry, University of Leeds, and The Biological Research Unit, Dental School and Hospital, University of Leeds

(Received 2 March 1956)

The two major hypotheses explaining the mechanism of action of parathormone on bones are those of Albright & Ellsworth (1929) and Thompson & Collip (1932). Albright & Ellsworth (1929) suggested that the main action of parathormone is on the kidney, inhibiting the reabsorption of phosphate by the renal tubules. This results in a tendency to a decreased serum inorganic phosphate level, which induces a withdrawal of phosphate from bone to restore the $[\text{Ca}] \times [\text{PO}_4]$ product in the serum to normal. The hypothesis of Thompson & Collip (1932) postulated a direct demineralizing effect of parathormone on bone. Subsequent investigations by Heller, McLean & Bloom (1950), on histological changes in bone after parathormone treatment, and by Barnicot (1948), on the local demineralization of bone with parathyroid tissue grafted in apposition to it, have increasingly supported the view that parathormone has a direct effect on bone, producing demineralization. Engel (1952) suggested that this demineralization was due to a destruction of osteoid matrix. The effect of parathormone on the renal tubules cannot be excluded, as Harrison & Harrison (1941) and Jacobs & Verbanck (1953)

have demonstrated a decreased tubular reabsorption of phosphate in normal animals. Other workers (Dent, 1953; Stoerck & Silber, 1949) found, however, that parathormone had no phosphate diuretic effect in normal subjects but did show this effect in parathyroidectomized preparations. This led to the concept that two parathormones may exist, one of which exerts a maximum effect on bone, whereas the other, by affecting the kidneys, produces a phosphate diuresis.

The ^{32}P uptake by bone could depend on the operation of the following processes: an ion exchange between phosphate ions on the mineral crystal surface and ions in the surrounding fluid, a recrystallization of the bone crystal which would reduce the availability for re-solution of ions already deposited and also active growth and remodelling of bone which would, presumably, depend on cellular activity (Neuman & Weikel, 1954). The distribution of ^{32}P in bones of animals injected with the isotope has been investigated by two different techniques: autoradiography and radioactivity measurements. Autoradiography (Amprino & Engström, 1952) demonstrates in a qualitative way

the uneven distribution of the isotope, whereas with radioactivity measurements of bone extracts the tracer uptake can be determined quantitatively. In recent years various methods have been employed to demonstrate the anatomical distribution of tracers in bone, of which that of Weidmann (1956), with an improved technique of differential extraction, showed the largest regional difference in the magnitude of ^{32}P uptake by shaft bone. The endosteal layer was the most active region, the periosteal layer considerably less active, and the large middle portion of the shaft substantially less than the other two areas. The high activity of the endosteal layer, and to a lesser extent the periosteal layer, might be due to their being areas of active bone formation balanced by active remodelling of bone.

Engfeldt & Zetterström (1954), investigating the activity of parathormone on bone and using the techniques of radioactivity measurements of $(\text{NH}_4)_2\text{SO}_4$ extracts, autoradiography and micro-radiography, found that in the young rat parathormone decreased the uptake of ^{32}P into the bones, whereas in older animals the uptake of ^{32}P was increased. Microradiographs, prepared from bones of dogs, showed considerable bone destruction, but a marked increase of ^{32}P was seen with autoradiographs in areas of active bone formation. From this they concluded that the primary action of parathormone is an acceleration of bone destruction in all age groups, leading, eventually, to increased bone formation. This is more apparent in older animals where less new bone formation is occurring. The results of Engfeldt & Zetterström (1954) with the technique of radioactivity measurements of $(\text{NH}_4)_2\text{SO}_4$ extracts were only average results from bones in which marked regional differences of biological activity exist, as shown by autoradiography.

The present investigation was undertaken to obtain more specific information about the effect of parathormone on different areas of bone. To determine whether the kidneys were involved in any effects observed, some experiments were performed on nephrectomized preparations. By this means, it was thought, conclusions could be reached on the relative effect of ion exchange and active bone formation in connexion with the action of parathormone.

METHODS

Fully grown rabbits and cats were used for all experiments. The effect of parathormone on the turnover of ^{32}P in bones was investigated by the intraperitoneal injection of parathormone (Eli Lilly and Co.). Animals were injected with varying doses for different periods of time. Nine untreated rabbits and six untreated cats were examined some time previously, and these groups served as controls for all

further experiments. In nephrectomized animals, maintained under Nembutal anaesthesia, the parathormone injection was made immediately after nephrectomy and the ^{32}P injection 3 hr. later. After another 4 hr. the animal was sacrificed. Control animals were maintained under anaesthesia only (for 3 hr.) before the ^{32}P injection, or were nephrectomized and anaesthetized, but received no parathormone injection. To some nephrectomized preparations massive doses of inorganic phosphate were administered by repeated intravenous injections.

Blood for the serum calcium and inorganic phosphate determinations was obtained by heart puncture from anaesthetized cats and from the marginal vein of the rabbit's ear. Serum-calcium levels were determined by the Clark & Collip (1925) modification of the Kramer-Tisdall method, and serum inorganic phosphate was estimated as described previously (Rogers, Weidmann & Jones, 1953). Samples for bone investigations were prepared by the usual technique (Weidmann & Rogers, 1950; Rogers *et al.* 1953). The specific activity of phosphorus was measured in the cortical and cancellous parts of the shaft bone. In order to obtain the average specific activities of these bone types, representative bone-powder samples were collected from the diaphysis and metaphysis, dissolved in 20% trichloroacetic acid (TCA) and measurements carried out on samples of these solutions. The uptake of ^{32}P was also measured in the subperiosteal, 'middle' and endosteal areas of the diaphysis, from which differential extracts were obtained by the paraffin-protection method. This method and its application have been described elsewhere (Weidmann, 1956). By extracting bone fragments with saturated $(\text{NH}_4)_2\text{SO}_4$ solutions, with the above method highly active fractions can be obtained which are particularly suitable for study of the changes of phosphorus turnover in animals under pathological conditions. Ammonium sulphate extracts were also prepared from bone-powder samples of metaphyseal trabeculae.

The CO_2 -combining power of plasma in the nephrectomized rabbits was measured by the manometric procedure of Van Slyke.

The same amount of tracer was injected into all animals, i.e. $33 \mu\text{C}$ of carrier-free $\text{H}_3^{32}\text{PO}_4/\text{kg. body wt.}$ Technique of tracer administration and calculation of specific activity has been described previously (Weidmann, 1956). Radioactivity measurements were always made on animals killed 4 hr. after the administration of the tracer.

RESULTS

Effect of parathormone on the turnover of inorganic phosphorus in various bones and parts of bones of rabbits and cats

Engfeldt & Zetterström (1954) concluded from radioactivity measurements and microradiographic evidence that the primary action of parathormone is an enhanced bone destruction, which eventually leads to increased new bone formation. Since the rate of mineral-salt replacements varies to a large extent within the bone (Weidmann, 1956), the above measurements of radioactivity in $(\text{NH}_4)_2\text{SO}_4$ extracts from average bone-powder samples are probably not sufficiently specific to establish the

characteristic effects of parathormone action. In the present investigation the effect of parathormone on the specific activity of phosphorus was tested in average samples of cortical and cancellous bone, and in the easily exchangeable fraction of bone salts from various layers of the diaphyses of rabbits and cats.

Table 1 demonstrates the changes observed in serum-calcium and inorganic-phosphorus levels after parathormone treatment. In rabbits the serum values show no significant alteration. In cats treated for a long time with high parathormone dosage the serum-calcium level increased to a mean of 17.5 mg. % as compared with 11.3 mg. % in the control animals, and serum-inorganic phosphorus to a mean of 9.8 from 6.7 mg. % in the normals. Seven hours after a single injection of parathormone neither rabbits nor cats show any significant change in the serum values.

The only change in the specific activity of serum phosphorus, as a consequence of parathormone treatment, was found in the 4 hr. experiment with rabbits [Table 1, column (3)]. The comparatively high concentration of ^{32}P in the serum of these animals may indicate a decrease in the rate of bone-salt deposition from blood, although other factors such as rate of urinary phosphate excretion may play a part.

Rabbit. In Table 2 average specific activities of ^{32}P in bone are given for control animals and for those subjected to parathormone treatment. The values for the three areas of cortical bone represent averages of two different estimations, one for the femur and the other for the tibia. The agreement between the two values was satisfactory.

The effects of parathormone were only shown in short-term experiments after a single injection of 200 u.s.p. units of parathormone. In experiments of longer duration, such as 8 and 20–28 days of parathormone administration, no statistically significant bone changes were observed. In short-term experiments a large reduction in the specific activity of the endosteal layer occurred: as compared with the control value (t 2.2, $P < 0.05$; Table 2, column 4). The ratio between the inner and middle layers was not significantly reduced (25.0–16.5). No effect of parathormone is observed on the sub-periosteal layer. No changes were observed in the specific activity of the cancellous bone either. There are, apparently, differences in the mechanism of calcification between the two bone types, and the one operating in the cancellous bones is perhaps less affected by parathormone.

Cat. The cat appears to be a much more suitable animal in which to examine the long-term effects of parathormone than the rabbit. Whereas there was no deterioration in the general health of the rabbit, most cats became severely ill after a few days of hormone administration. This is an interesting difference in the susceptibility of the two species to parathormone. Several animals lost weight considerably, two died, and others were on the brink of death after 7 days of treatment. A diminution of dosage from 200 to 100 u.s.p. units/day decreased the mortality, but the ill-effect was still noticeable, particularly in the youngest of the mature animals.

The cat differs from the rabbit in that the effect is only shown after some days of hormone administration (Table 3). After 9 days the specific activity in

Table 1. *Effect of parathormone on the percentage concentration of inorganic calcium and phosphorus, and the specific activity of serum in rabbits and cats, measured 4 hr. after the injection of ^{32}P*

For definition of specific activities see Methods. In columns (1) and (2) mean values and ranges are given.

Expt.	No. of animals	Ca (mg. %) (1)	P (mg. %) (2)	Specific activity (Mean $\pm$ s.e.m.) (3)
Rabbit				
Control animals	9	13.1 11.1–14.1	5.4 3.4–7.2	20 300 $\pm$ 2230
200 u.s.p. units daily for 28 days	6	13.8 13.1–14.5	4.9 4.3–5.5	22 030 $\pm$ 670
100 u.s.p. units daily for 8 days	6	12.6 12.3–12.9	5.5 3.1–8.4	25 800 $\pm$ 980
200 u.s.p. units, single injection	4	12.3 11.5–13.5	5.8 3.5–7.8	37 080 $\pm$ 1550
Cat				
Control animals	6	11.3 9.4–12.5	6.7 5.4–9.5	33 600 $\pm$ 3080
100 u.s.p. units daily for 9 days	5	17.5 13.8–23.9	9.8 5.6–22	29 860 $\pm$ 2840
200 u.s.p. units, single injection	4	11.4 10.3–12.4	6.3 5.8–7.2	29 270 $\pm$ 2575

Table 2. *Effect of parathormone on the specific activities of phosphorus in bones and parts of bones of rabbits, measured 4 hr. after the injection of ³²P*

Results represent mean specific activities $\pm$ s.e.m. For the definition of specific activities see Methods.

Expt.	No. of animals	Cortical bone					Cancellous bone			
		TCA extract of shaft (average)	(NH ₄) ₂ SO ₄ extract of layers of bone shaft			Ratio of specific activity	TCA extract of metaphysis (average)	(NH ₄) ₂ SO ₄ extract of bone powder from metaphysis		
			(NH ₄) ₂ SO ₄ extract of layers of bone shaft							
			Outer (1)	Outer (2)	Middle (3)	Inner (4)				Outer Middle (5)
Control animals	9	46 ± 6.0	656 ± 133	149 ± 30	3910 ± 620	4.7 ± 0.7	25.0 ± 3.9	336 ± 53	2100 ± 570	
200 u.s.p. units daily for 28 days	6	57 ± 7.7	637 ± 83	129 ± 26	3650 ± 579	6.7 ± 2.8	32.4 ± 4.0	439 ± 58	2125 ± 670	
100 u.s.p. units daily for 8 days	6	53 ± 3.7	934 ± 115	204 ± 12	5780 ± 990	4.7 ± 0.7	28.5 ± 5.6	415 ± 47	3390 ± 687	
200 u.s.p. units, single injection	4	44 ± 5.0	448 ± 29	118 ± 17	1820 ± 230	4.1 ± 0.6	16.5 ± 2.9	380 ± 44	1996 ± 422	

Table 3. *Effect of parathormone on the specific activities of phosphorus in bones and parts of bones of cats, measured 4 hr. after the administration of ³²P*

Results represent mean values $\pm$ s.e.m. For the definition of specific activities see Methods.

Expt.	No. of animals	Cortical bone					Cancellous bone		
		TCA extract of shaft (average)	(NH ₄) ₂ SO ₄ extract of layers of bone shaft			Ratio of specific activity		TCA extract of metaphysis (average)	(NH ₄) ₂ SO ₄ extract of bone powder from metaphysis
			Outer	Middle	Inner	Outer	Inner		
Control animals	6	19.0 ± 2.6	477 ± 70	80 ± 14	2180 ± 313	5.8 ± 0.8	31.3 ± 5.1	273 ± 86	2160 ± 309
100 u.s.p. units daily for 9 days	5	26.3 ± 3.3	431 ± 68	63 ± 9	959 ± 87	6.9 ± 1.0	16.6 ± 4.0	392 ± 88	—
200 u.s.p. units, single injection	4	31.0 ± 9.0	500 ± 156	72 ± 16	1750 ± 710	7.0 ± 0.8	22.4 ± 1.1	348 ± 96	1683 ± 338

the endosteum is markedly reduced as compared with the control group (t 3.4, $P < 0.01$; Table 3, column 4), whereas the activity of the sub-periosteum is not affected. The ratio between the endosteum and the middle layer fell from 31.3 to 16.6. In both species the overall specific activities of the shaft are not altered after parathormone administration; the effect seems to be restricted to the endosteum. The cancellous bones, like those of the rabbit, showed no sign of parathormone influence.

Effect of parathormone in nephrectomized animals

According to Albright & Ellsworth (1929) the increase in bone resorption during hyperparathyroidism is due to a lowering of the serum-phosphate level owing to phosphate diuresis. In order to test this hypothesis the kidneys were ligated and the action of parathormone was examined under these conditions (Table 4).

Since nephrectomized animals were maintained under Nembutal anaesthesia for the whole experiment, the effect of anaesthesia on the ^{32}P turnover of bone was first investigated. Anaesthesia has no effect on the overall uptake of tracer into the cortical part of the diaphysis, whereas nephrectomy causes a marked reduction (t 2.6, $P < 0.05$; Table 4, column 1), which is due to a decrease in endosteal activity. Anaesthesia and nephrectomy together decrease the ^{32}P -uptake of the endosteal layer, whereas anaesthesia alone does not affect it. A comparison between the two groups shows a significant decrease in the former preparation (t 2.8, $P < 0.05$). The activity in the middle layer is not significantly decreased in either nephrectomized or anaesthetized preparations (t 2.15, P 0.05 and t 1.5, $P < 0.2$ respectively; Table 4, column 3). The ratio of the ^{32}P -uptake into the inner and middle areas is significantly increased above control values in both the anaesthetized and anaesthetized nephrectomized preparations (t 3.8, $P < 0.01$ and t 2.5, $P < 0.05$ respectively; Table 4, column 6).

The decrease in specific activity may be due to increased bone resorption, or to a decreased rate of equilibration with ^{32}P in the blood, in which the serum-calcium level has increased from 12.8 to 20.8 mg. % (mean values), without alteration in the serum inorganic phosphorus concentrations. An increase in the serum-calcium concentration, together with an increased bone resorption, could be caused by development of massive acidosis, but this would not explain the constant phosphorus concentrations in the serum. Acidosis is, however, not likely to have developed, because the blood CO_2 -combining power of these animals is normal. In nephrectomy the activity of the endosteal layer is decreased relative to the values found for anaesthetized preparations. Nephrectomy can there-

Table 4. *Effect of anaesthesia, nephrectomy, parathormone and phosphate infusion on the specific activity of ^{32}P in bones and parts of bones of rabbits, measured 4 hr. after the administration of ^{32}P*

Results represent specific activities $\pm$ s.e.m. For the definition of specific activities see Methods.

Expt.	No. of animals	Cortical bone					Cancellous bone		
		TCA extract of shaft (average) (1)	(NH ₄) ₂ SO ₄ extract of layers of bone shaft			Ratio of specific activity		TCA extract of metaphysis (average) (7)	(NH ₄) ₂ SO ₄ extract of bone powder from metaphysis (8)
			Outer (2)	Middle (3)	Inner (4)	Outer Middle (5)	Inner Middle (6)		
Control animals	9	46 ± 6.0	656 ± 133	149 ± 30	3910 ± 620	4.7 ± 0.7	25.0 ± 3.9	336 ± 53	2100 ± 570
Anaesthesia	3	41 ± 0.2	483 ± 10	72 ± 14	4071 ± 535	7.6 ± 1.4	65.6 ± 16.5	—	—
Anaesthesia and nephrectomy	7	27 ± 3.4	367 ± 47	71 ± 15	2720 ± 208	5.6 ± 1.5	41.6 ± 5.4	231 ± 26	1150 ± 173
Anaesthesia, nephrectomy and parathormone	3	16 ± 1.9	255 ± 11	75 ± 24	985 ± 80	3.6 ± 1.2	17.3 ± 4.6	183 ± 32	1050 ± 203
Anaesthesia, nephrectomy and PO ₄ infusion	7	109 ± 23	1991 ± 468	236 ± 43	4810 ± 1060	8.1 ± 0.9	20.4 ± 3.1	626 ± 93	2571 ± 350

fore affect the activity of the endosteal layer, by some mechanism other than production of acidosis.

Effect of parathormone on nephrectomized rabbits

The effect of parathormone was tested on nephrectomized rabbits only, since the cat does not respond to parathormone action in the short time interval (7 hr.) available for these experiments, whereas in the rabbit immediate responses were seen to parathormone injection.

The effect of parathormone on nephrectomized rabbits was tested by injecting 200 U.S.P. units of parathyroid extract immediately after nephrectomy. The results are contained in Table 4. There is no significant decrease of specific activity in whole-bone samples from cortical and cancellous bones (t 2.0, $P < 0.1$ and t 1.1, $P < 0.3$ respectively; Table 4, columns 1 and 7). The outstanding effect of parathormone on nephrectomized rabbits appears to be the big fall in the specific activity of phosphorus in the endosteum (t 5.17, $P < 0.001$; Table 4, column 4). Apart from this difference, there is also a drop in the endosteal activity in relation to the middle area when anaesthetized and nephrectomized preparations are compared with similar animals treated with parathormone (t 2.71, $P < 0.05$). Since in intact and nephrectomized animals treated with parathormone a decreased ratio of specific activities of endosteal:middle layer is observed, it seems likely that this is a characteristic of parathormone activity and is, presumably, caused by a direct effect on bone. The kidneys were eliminated in this experimental set-up, consequently the reduction in the endosteal area cannot be initiated by a primary lesion of the kidneys.

It has been suggested that in nephrectomized animals there is a rise of serum inorganic phosphorus (which was found only in cats in these experiments), and that this rise may act as a stimulus to parathyroid activity (Crawford, Osborne, Talbot, Terry & Morrill (1950)). Varying amounts of sodium phosphate buffer, pH 7.4 (120–420 mg. of P), were injected into nephrectomized rabbits, to investigate whether increases in serum inorganic phosphorus levels would result in a further decrease in the turnover rate of the inner layer. The results (Table 4) show that phosphate injection considerably increased the magnitude of ^{32}P uptake of the whole bone. This would be expected if precipitation was occurring in the bone without any biological activity being responsible. The interesting feature is that the deposition in the endosteal layer is less than in the other two. This results in the ratio of the specific activities of the endosteal and middle layers being reduced as compared with the nephrectomized preparation (t 3.4, $P < 0.01$), which would

support the hypothesis that the serum inorganic phosphorus level can act as a stimulus to increased parathyroid activity.

DISCUSSION

The results presented show that in both the cat and the rabbit there is no effect on the ^{32}P uptake in whole-bone determinations, when the animals are treated with either single or multiple doses of parathormone. Lack of effect of parathormone on the renewal rates of bone phosphate in whole bones was also observed by Tweedy & Campbell (1944) in rats. If, however, different parts of the shaft of cortical bone are analysed, a significant reduction is observed in the activity of the endosteal layer of the cat after multiple dosage, and in that of the rabbit after a single injection. It may be significant in this connexion that in the cat multiple dosage produces a rise in serum-calcium level, an effect typical for parathormone treatment. This effect is not observed in the rabbit, which appears more resistant to multiple dosage with parathormone. It would appear that only the more active areas of bone are susceptible to parathormone action, whereas in whole bones the effect is masked by the large areas of comparatively inactive bone. This finding seems to be in agreement with that of Engfeldt & Zetterström (1954), who found a decrease of ^{32}P uptake in the biologically active diaphyses and epiphyses of young rats, and an increase in the more static bones of mature specimens. In cancellous bones no alteration in the ^{32}P uptake due to parathormone was detected, and in this respect the present results differ from those of Engfeldt & Zetterström (1954). The effect of parathormone may be antagonized by strains and stresses in bone tending to increase osteoblastic activity, which may be more pronounced in cancellous than in cortical bones.

In cats an absolute decrease in ^{32}P uptake into the endosteal layer was detected in spite of the absence of the expected decrease in serum phosphate after parathormone administration. It was also shown that a rise in serum-phosphate level in rabbits, brought about by phosphate infusion, caused an absolute increase in endosteal activity, as would be expected if the solubility-product principle operated to produce increased bone-salt precipitation. Therefore the decrease of turnover rate in the endosteal layer is not related to changes in the inorganic-phosphate level, but is a direct effect of parathormone. Stewart & Bowen (1951), using the injection of oxalate to lower the serum-calcium-ion level and thus affect the ionic equilibrium between blood and bone, also found that calcium could only be withdrawn from bone if the parathyroids were intact. This is further evidence that parathormone has a direct effect on bone.

When ^{32}P is incorporated into bone it becomes diluted by a large mass of ^{31}P already present; tracer losses due to bone resorption are therefore not noticeable. The relatively low ^{32}P concentrations found in the endosteum of animals treated with parathormone are more likely to be due to a reduction in ^{32}P -uptake than to losses through bone resorption. It is suggested therefore that the decrease in the specific activity of the endosteal layer is due to a decreased rate of ^{32}P incorporation and not to an increased withdrawal from bone. The injection of parathormone into anaesthetized nephrectomized rabbits demonstrates that the main effect of parathormone on the bones is on the endosteal layer, a finding similar to that seen with parathormone injections in intact animals. The injection of a buffered phosphate solution into a nephrectomized rabbit produces a marked decrease in the serum-calcium level and an increase in the serum inorganic phosphate level. The overall uptake of ^{32}P by the whole bone is increased, as would be expected from an increased deposition of tricalcium phosphate, owing to the markedly increased serum inorganic phosphate level. There is a substantially increased uptake by all three layers but the increase is much smaller in the inner layer. The ratio between the middle and inner layers is therefore reduced, an effect which is also seen with parathormone. That the phosphate effect could be due to a decreased availability of the inner layer for precipitation as compared with the middle layer is unlikely, as the blood supply to the inner layer is likely to be at least as good as that to the middle layer. The decreased serum-calcium level, or the increased serum inorganic phosphate level, may have stimulated the animals' parathyroids. This would result in an inhibition of osteoblastic activity, an effect mainly seen in the inner layer, leading to the relatively smaller ^{32}P uptake in this area. Similarly, nephrectomy alone will decrease the activity of the inner layer which may also be due to a stimulation of the animals' own parathyroids.

Whilst there seems no reason to dispute that exchange and recrystallization of bone minerals may play some part in the maintenance of normal adult-bone structure, it seems likely that none of the effects of parathormone on the uptake of ^{32}P by bone can be explained on this basis. In their histological investigation Heller *et al.* (1950) observed a marked decline in the number of osteoblasts after parathormone treatment, and also a marked increase in osteoclasts. They suggested that osteoclasts were the same cells as osteoblasts but in a different functional state. The present results are

interpreted as indicating that the primary effect of parathormone is an inhibition of osteoblastic activity. This decreases the ^{32}P -uptake into the area of bone where osteoblastic activity is maximal, namely the endosteal layer. Some of the osteoblasts become degenerate and others become osteoclasts. These cells are either non-functioning osteoblasts or are cells whose synthetic ability has been inhibited and which now demonstrate their catabolic function in the dissolution of the bone matrix.

SUMMARY

1. The major effect of parathormone on bones in both cats and rabbits is to decrease the uptake of phosphates into the endosteal layer of the diaphyseal shaft, as shown by tracer experiments with ^{32}P .

2. This effect of parathormone can be observed in nephrectomized rabbits.

3. The relation of parathormone to active processes maintaining bone structure is discussed.

We would like to acknowledge the technical assistance given to us by Miss E. B. Morley and Mr R. W. Lofthouse.

REFERENCES

- Albright, F. & Ellsworth, R. (1929). *J. clin. Invest.* **7**, 183.
 Amprino, R. & Engström, A. (1952). *Acta anat.* **15**, 1.
 Barnicot, N. A. (1948). *J. anat.* **82**, 233.
 Clark, E. B. & Collip, J. B. (1925). *J. biol. Chem.* **63**, 461.
 Crawford, J. D., Osborne, M. M., Talbot, N. B., Terry, M. L. & Morrill, M. F. (1950). *J. clin. Invest.* **29**, 1448.
 Dent, C. E. (1953). *Proc. R. Soc. Med.* **46**, 291.
 Engel, M. B. (1952). *Arch. Path.* **53**, 339.
 Engfeldt, B. & Zetterström, R. (1954). *Endocrinology*, **54**, 506.
 Harrison, H. E. & Harrison, H. C. (1941). *J. clin. Invest.* **20**, 47.
 Heller, M., McLean, F. C. & Bloom, W. (1950). *Amer. J. Anat.* **87**, 315.
 Jacobs, E. & Verbanck, M. (1953). *Acta med. scand.* **145**, 143.
 Neuman, W. F. & Weikel, J. H. (1954). *Atomic Energy Report*, UR-314.
 Rogers, H. J., Weidmann, S. M. & Jones, G. H. (1953). *Biochem. J.* **54**, 37.
 Stewart, G. S. & Bowen, H. F. (1951). *Endocrinology*, **48**, 568.
 Stoerck, H. C. & Silber, R. H. (1949). *Fed. Proc.* **8**, 371.
 Thompson, D. L. & Collip, J. B. (1932). *Physiol. Rev.* **12**, 309.
 Tweedy, W. R. & Campbell, W. W. (1944). *J. biol. Chem.* **154**, 339.
 Weidmann, S. M. (1956). *Biochem. J.* **62**, 593.
 Weidmann, S. M. & Rogers, H. J. (1950). *Biochem. J.* **47**, 493.