Effect of Parathyroid Extract Administration in Sheep.* (20858)

W. E. Lotz, Roy V. Talmage,† and C. L. Comar.

From the University of Tennessee Atomic Energy Commission Agricultural Research Program, Oak Ridge, Tenn.

Studies of parathyroid function and the effects of administration of parathyroid extracts have been confined primarily to 3 species of mammals, the rat, dog, and man. However, there is evidence to indicate that these glands function similarly in most mammals. Fleischmann(1) has reviewed the comparative physiology of the parathyroid glands. The availability of radioisotopes of phosphorus and calcium has facilitated investigation of parathyroid function in relation to blood levels, kidney function and particularly bone physiology. Minor differences have been noted in the bone uptake of Ca^{45} and P^{32} following parathyroidectomy or administration of parathyroid extract(2,3). Recently Talmage *et al.*(4) have shown that parathyroid extract administration to rats was able to remove previously deposited radiophosphorus to a greater extent than similarly deposited radiocalcium. Studies on the effect of parathyroid extract in sheep were therefore undertaken to determine species differences. In addition, the size of the animal permitted daily collection of blood and urine samples for analysis, and the large bones of the animal facilitated detailed autoradiographic observations of the isotope distribution pattern in the bone. It was hoped, therefore, that the locus of action of the parathyroid hormone could be more clearly elucidated.

Materials and methods. For this work, 19 ewes weighing approximately 75 lb were used. The radioactivity was injected either intravenously or intraperitoneally and at least one control was run concurrently with each group of experimental animals. The radioactivity was administered from one to 10 days prior to parathyroid extract‡ administration. The extract was injected subcutaneously at levels of 1000 i.u. and 3000 i.u. daily which corresponds to 30 and 90 i.u. per kg body weight. At the higher level the experiment was concluded at the end of 24 or 48 hours. At the lower levels animals were killed after 2, 5, and 7 days of treatment. Examination of the data showed little or no differences due to variation in dose or time of sacrifice and therefore all results are averaged for clarity of presentation. In some cases the animals were placed in metabolism cages for the collection of urine. Due to the comparatively large blood volume of the animals, frequent sampling was possible for serum calcium and phosphate determinations. At the conclusion of the experiment, the animals were sacrificed and the bones prepared for autoradiographs as described previously(5). Radioactivity measurements for P^{32} and Ca^{45} were made by standard procedures, serum calcium determinations by the method of Clark and Collip, and serum phosphate determinations by the method of Fiske and SubbaRow.

Results. The data from these experiments are tabulated in Tables I and II and the autoradiographs are pictured in Fig. 1. The data indicate that the sheep did not follow the pattern of other animals studied in its response to parathyroid extract and the various effects are summarized below.

Effect on serum phosphate and calcium. In all experiments the serum phosphate level was elevated by the injection of parathyroid

TABLE I. Inorganic Calcium and Phosphorus Serum Values for Controls and Sheep Treated with Parathyroid Extract.

	Ca, mg/100 ml —Mean and S.D.—	PO_4, mg/100 ml —Mean and S.D.—	No. of samples	No. of animals
Control	11.7 ± .15	6.1 ± .16	24	7
Treated	11.6 ± .96	8.3 ± .80	23	12

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† Oak Ridge Institute of Nuclear Studies Research Participant from Rice Institute, Houston, Texas.

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TABLE II. Optical Densities of Bone Autoradiograms from Controls and Sheep Treated with Parathyroid Extract.

	Optical density (mean and S.D.) Plate	Funnel	No. of samples Plate	Funnel	No. of animals	Level of sig. for mean diff.	% reduction* Plate	Funnel
P^{32}								
Control	1.250 ± .045	.848 ± .025	25	15	5			
Treated	1.064 ± .032	.745 ± .025	40	24	8	.01	62.0	34.5
Ca^{45}								
Control	.729 ± .053	.636 ± .048	10	10	2			
Treated	.754 ± .062	.631 ± .037	10	10	2	None	0	0

* A difference of .3 in optical density represents an increase or decrease of 100%.

extract (Table I). This is the reverse of what normally happens in rats, dogs, and man. The average serum phosphate for the control sheep used in these experiments was 6.1 mg % as compared with 8.3 mg % for the treated animals. There was no significant difference between the serum calcium levels of the control and experimental.

Effect on isotope excretion by the kidney. The renal excretion values for P^{32} and Ca^{45} in these experiments are incomplete. However, the results of these preliminary data are too important to exclude from this report. Based on 4 treated and 2 control animals given P^{32}, the total urinary excretion of the isotope by the treated sheep averaged only slightly over 2 times that of the control. This is a markedly small increase as compared to the 30-fold increase seen in the rat(4). Based on one experimental and one control animal, there was no significant difference in the renal excretion of Ca^{45}, however, previous studies in other species have never shown marked changes in calcium excretion following parathyroid extract administration.

Effect on removal of P^{32} and Ca^{45} from bone. As a result of parathyroid administration, a marked removal of P^{32} occurred from the primary spongiosa directly beneath the epiphyseal plate of the long bone and also from the endochondral bone of the funnel as shown in Fig. 1. It is of particular interest that radioactive phosphorus once deposited in the endochondral bone of the funnel is normally the last to be removed in the process of growth(6). Fig. 1 also demonstrates that there was no significant removal of Ca^{45} as a result of the same experimental treatment which caused such a marked P^{32} removal. The autoradiographic data for all the animals are summarized in Table II, which presents the mean densitometer readings for the autoradiograms. It is clear that the densitometric estimates support the visual observations as illustrated in Fig. 1.

Discussion. The most conspicuous difference in the effect of parathyroid extract on calcium and phosphate metabolism in the sheep as compared with other mammals was the failure of the extract to produce a large increase in phosphate excretion and to cause a decrease in phosphate blood level. The blood, urine and bone data obtained can be interpreted to give the following picture of parathyroid action: In the sheep, the parathyroid extract had a direct action on bone causing a release of phosphate, but there was little or no effect upon the kidney. This caused the blood phosphate to be increased, which may in turn have resulted in the slightly increased urinary excretion of phosphate which was observed. It is clear that in this case the removal from the bone was not a secondary effect caused by deficit of phosphate in the blood arising from the increased urinary excretion which does occur in rats and dogs.

There seems little question, both from the results with rats(4) and the data reported here, that more radiophosphate was removed from bone than was radiocalcium. In the light of present knowledge of bone formation and destruction it is difficult to conceive of a large effect on bone phosphate without a similar effect on bone calcium. It should first be noted that recently deposited Ca^{45} and P^{32} is probably located in the bone crystal in sites accessible for exchange or metabolic activity so that the removal of radioactivity

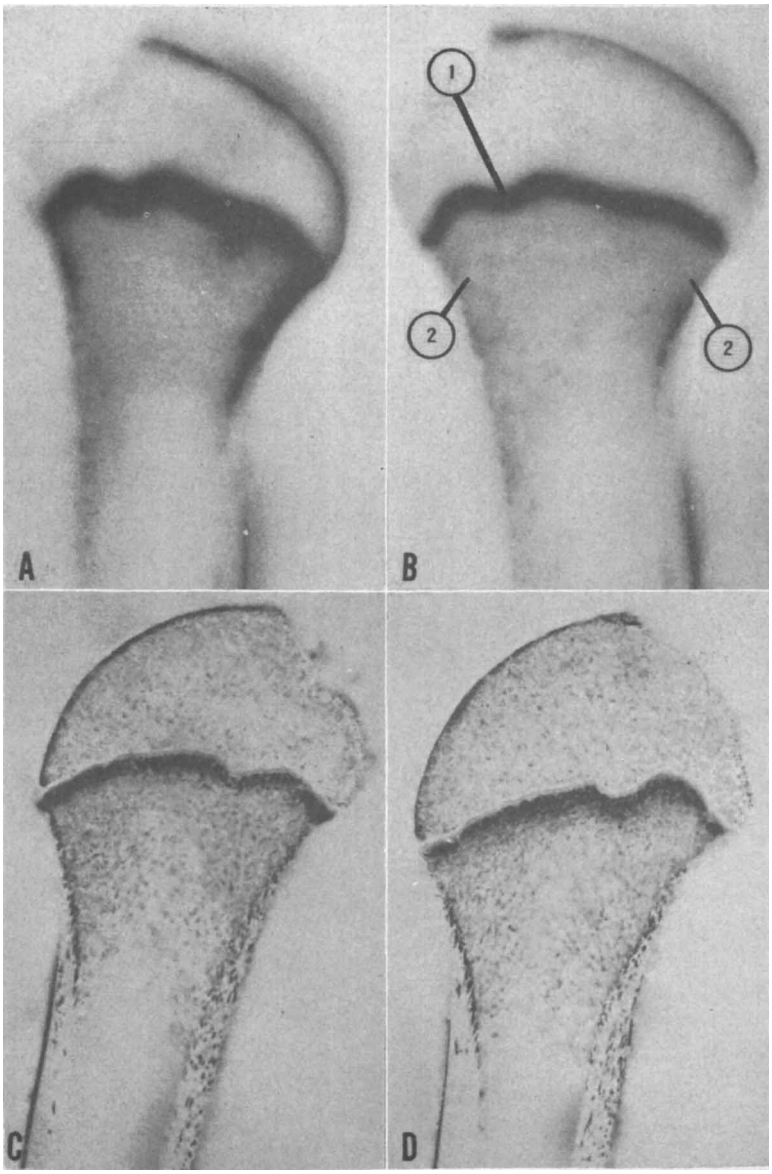

FIG. 1. Autoradiograms showing the localization of P^{32} and Ca^{45} in distal ends of sheep femurs. A. P^{32} in control sheep. B. P^{32} in sheep given 30 i.u./kg parathyroid extract; note removal from primary spongiosa (1) and from funnel (2). C. Ca^{45} in control sheep. D. Ca^{45} in sheep given 30 i.u./kg parathyroid extract; note the lack of removal from primary spongiosa and funnel.

does not represent stoichiometric removal of bone phosphorus and calcium. It may well be that the 2 elements at least in the active sites, are more independent of each other than has been generally realized and that parathyroid action is primarily concerned with phosphate ion and only indirectly with calcium ions.

Another possible explanation is that the parathyroid action causes the bone calcium and phosphorus to be placed in a state where ions of both elements are readily exchangeable with other body stores. Since there is a large pool of phosphate and very little calcium outside of bone, such a mixing of bone elements with the body stores would cause a large de-

crease in P^{32} and a very small decrease in Ca^{45} specific activities.

Summary. The effect of parathyroid extract administration upon the removal of radiocalcium and radiophosphorus from the long bones of sheep and its subsequent excretion in the urine was studied. Over a 5-day period of treatment, radiophosphorus but not radiocalcium was removed from the bone. The parathyroid extract produced only a slight rise in the urinary excretion which may be accounted for by the elevated serum phosphorus. Daily doses of 1000 i.u. of parathyroid extract over a 7-day period resulted in elevation of the serum phosphorus levels but not of serum calcium. It is concluded that in the sheep, parathyroid extract acted directly upon the bone and that any effect upon the kidney was minor in the dosage range and time periods employed; also, it appeared that phosphorus metabolism was much more influenced than was calcium metabolism.

1. Fleischmann, W., *Comparative Physiology of the Thyroid and Parathyroid Gland,* Thomas Press, Springfield, Ill., 1951.

2. Tweedy, W. R., Chilcote, M. E., and Patras, M. C., *J. Biol. Chem.,* 1947, v168, 597.

3. Talmage, R. V., Kraintz, F. W., and Kraintz, L., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 553.

4. Talmage, R. V., Lotz, W. E., and Comar, C. L., *ibid.,* 1953, v84, 578.

5. Lotz, W. E., Gallimore, J. C., and Boyd, G. A., *Nucleonics,* 1952, v10, 28.

6. Comar, C. L., Lotz, W. E., and Boyd, G. A., *Am. J. Anat.,* 1952, v90, 113.

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Prolonged Administration of Chloramphenicol in Monkeys. (20859)

S. SASLAW, T. D. STEVENSON, AND C. A. DOAN.

From the Department of Medicine, College of Medicine, Ohio State University, Columbus.

The relationship of chloramphenicol to blood dyscrasias in certain susceptible persons has been reviewed recently(1). To date no standard method is available to predict by experimental methods the potential hemocytotoxic effects in humans of new drugs. In earlier studies from this laboratory, the monkey has proved to be a valuable animal for hematologic study(2-5). It is the purpose of this paper to present the results of prolonged chloromycetin administration to monkeys.

Methods and materials. A total of 15 young adult *Macaca mulatta* were used in these preliminary studies. One group of 6 animals was administered 750 mg of chloromycetin daily except Sundays for 15 months from July, 1952 through October, 1953. The contents of 3 250 mg capsules were suspended in water, drawn up into a 50 ml syringe and introduced by stomach tube. Any residual material was rinsed through with water. Since these animals weighed between 5 to 6 lb at the beginning, the dosage approximated 250-350 mg/kilo. Two monkeys received a similar daily dose on alternate weeks for 15 months. Two had daily doses of 5 g and another 2 received 470 mg chloromycetin palmitate twice daily. Another pair of monkeys were started on 750 mg daily after receiving 200 r of total body irradiation. Three monkeys, after 2 months on vit. B-free synthetic diet, were given 750 mg chloromycetin daily along with a normal diet. Five to 7 complete blood counts were performed over a 2-week period before therapy, for base-line purposes. In addition, a pre-treatment bone marrow aspiration was also done. Serial blood counts (total 90 to 100) were then done daily during the first month and then bi-weekly thereafter. Bone marrow aspirations (total 6 to 7) were performed at approximately 2 to 3 month intervals. Chemical(6) and microbiologic(7) determinations of chloromycetin were done at weekly intervals to insure absorption of the drug. Weights were checked weekly.

Results. As can be seen in Fig. 1, 6 monkeys (A,B,C,D,E,F) given 750 mg daily re-