metallic gold was bound by the alpha globulins. Although radiogold salts have been shown(3) to interact with the albumin fraction of plasma, Aurcoloid is relatively free of ionic gold.

The effect of gelatin in prolonging the retention of Aurcoloid in the circulation was apparently not mediated at the cellular level since colloidal gold was rapidly ingested by the RES regardless of whether the gold particles were coated with gelatin or plasma proteins(4). As Aurcoloid was eventually removed from the circulation by the RES, either the gold-gelatin-alpha globulin interaction was reversible or the total complex was phagocytised. Dissociation of the complex was not demonstrated in plasma electrophoretic patterns of animals injected with gelatin and Aurcoloid.

Summary. The relative mobilities of Aurcoloid and gelatin have been demonstrated *in vivo* and *in vitro* by paper electrophoretic methods. Addition of Aurcoloid and gelatin to plasma resulted in a radiogold-gelatin complex which migrated at the same rate as alpha globulins. Aurcoloid in plasma did not migrate. The formation of an Aurcoloid-gelatin complex was associated with a decreased rate of disappearance of radiogold from the circulation.

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Action of Parathyroid Extracts on Stable Bone Mineral Using Radiocalcium as Tracer.* (22229)

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When radiocalcium is injected into rats it becomes deposited in the skeletons of the animals in 2 forms which differ greatly in relative ease of mobilization and excretion. These forms of incorporation of radiocalcium and the parts of the skeletal bone mineral associated with each are referred to as the stable bone mineral and labile bone mineral(1,2). Fifty-two days after injection, radiocalcium is distributed almost exclusively in the stable fraction.

The present work describes the action of subcutaneously injected parathyroid extracts on radiocalcium (Ca^{45}) incorporated in the stable bone mineral.

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Procedure. Twenty-four adult-male rats of comparable age and weight (380-420 g) were given intraperitoneal injections of high specific activity radiocalcium. This was administered in physiological saline solution in 3 doses of 2 cc each, allowing 2 hour intervals between injections. The total injected dose amounted to approximately 9.9 x 10^5 counts per minute according to the methods of sample preparation and counting conditions employed. After 72 days the left foreleg of each rat was amputated at the shoulder. Within this time the radiocalcium had presumably been distributed preponderantly in the stable fraction of the skeleton(1,2). The humeri of the amputated legs were divested of adhering flesh and stored in a freezer to be compared with opposite pairs from the same animal obtained later at the time of sacrifice. One day after removal of the legs the rats were given a subcutaneous injection of 100 USP units of

Parathormone.‡ One group of rats received only a single injection and were sacrificed in pairs at intervals of 2, 4, 8, 14, and 24 hours after injection. The remaining rats received daily injections and were sacrificed in pairs at the end of 2, 3, 4, 5, 7, 9, and 13 days. The humeri of the right legs were obtained and then the ends of all humeri, including those from the amputated legs, were separated from the shafts by means of a rotating dental Carborundum disk, using recognizable anatomical landmarks. Epiphyseal and diaphyseal regions were then analyzed for Ca^{45} in order to detect any intra-skeletal shift of radiocalcium which might have occurred subsequent to the injection of Parathormone. Total calcium was also determined. Urine was collected daily from 6 of the rats housed in metabolism cages, and from 2 control rats. The control animals had undergone amputation but received isotonic saline injections instead of Parathormone. Radiocalcium was determined in the urine and humeri, using the method of sample preparation described by Armstrong and Schubert(3).

Results. The results of radioactivity measurements of the individual bone samples are recorded in Table I. Analysis for total calcium in these samples showed no differences either on a dry fat-extracted or ash basis. It is therefore convenient to express specific activity in terms of ash weight. It was found that no large replacement of radiocalcium in the stable bone mineral by non-radioactive calcium occurred, since the specific activities of experimental samples and the opposite leg control samples were practically identical in each case. The epiphyseal and diaphyseal regions of a given pair of bones were not significantly different with respect to specific activity either before or after Parathormone treatment, demonstrating that no major intrahumeral shift of radiocalcium occurred.

There was, however, a greatly increased movement of radiocalcium out of the skeleton as a result of stable bone mineral resorption associated with the large dosages of Parathormone. Fig. 1 shows the effect of Parathormone treatment on the daily renal excretion of radiocalcium released from stable bone mineral. In order to improve counting conditions, the daily urine volumes of 3 rats were pooled, by housing the 3 together in the same metabolism cage. Group A in Fig. 1 refers to a group of 3 rats receiving daily Parathormone injections, the results being expressed as percent of the injected dose excreted per rat per day. Group B refers to a parallel, entirely similar group of 3 rats receiving Parathormone daily, the urine volumes from which were also pooled for counting. The control value is based on the urinary analysis of 2 rats, with amputated legs, receiving subcutaneous saline injections instead of Parathormone. Prior to treatment of the experimental rats with Parathormone and in the control rats, the urinary excretion

TABLE I. Comparison of Ca^{45} in Epiphyseal and Diaphyseal Regions of Rat Humeri before and after Parathormone Injections, 23 Rats.

	Specific activity (% of injected dose/g of bone ash)			
	Epiphysis		Diaphysis	
Treatment	Control leg	Exp. leg	Control leg	Exp. leg
PTH,* single inj.				
2 hr	.66	.65	.71	.73
	.48	.45	.58	.59
4	.69	.66	.69	.70
	.57	.58	.62	.66
8	.61	.69	.69	.71
	.65	.64	.70	.69
14	.66	.64	.56	.62
	.62	.63	.69	.69
24	.56	.61	.65	.65
	.57	.53	.60	.59
PTH,* daily inj.				
2 days	.59	.56	.59	.60
	.63	.60	.65	.51
3	.61	.59	.65	.63
	.48	.48	.52	.51
4	.60	.57	.60	.43
	.67	.64	.66	.64
5	.57	.56	.61	.60
	.57	.59	.59	.60
7	.55	.54	.59	.55
	.55	.58	.57	.59
9	.65	.64	.64	.67
	.54	.59	.58	.59
13	.67	.62	.70	.71

* PTH = 100 USP units of Lilly Parathormone, inj. subcut.

‡ A gift of Eli Lilly Co., Indianapolis, Ind.

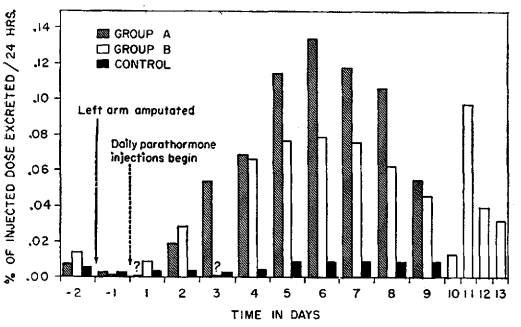

FIG. 1.

of Ca^{45} was between .009% and .013% of the injected dose per day. After a single Parathormone injection, radiocalcium in the urine was increased only slightly, but continued to increase during the first week of injections to a level of 0.08-0.14% (A). With repeated injections after the 6th day, however, the percentage of the injected dose in the urine began to decrease§ and after 13 days was down to .04%. In general this decline reflects the loss of Parathormone potency usually observed after repeated injections of bovine parathyroid extracts in normal rats(4).

Discussion. No decreases in the total calcium content or specific activity of radiocalcium in the experimental legs as compared to their previously amputated counterparts were observed. This might merely indicate that Parathormone injections mediate a complete withdrawal of the materials comprising bone, not acting upon calcium independently. It is possible, however, that the amount of material mobilized from the humerus, compared to the large amount present, is so small as to be undetectable by direct analysis of the bone. It is also possible, though unlikely, that radiocalcium appearing in the urine is derived to a lesser degree from the humerus than from other bones of the skeleton.

Unfortunately separation of the epiphyseal from the diaphyseal regions of the humeri, for the purpose of detecting intra-osteal shifts of Ca^{45}, resulted in variable losses of material. An accurate comparison between bone weights of amputated legs and experimental legs was thus prevented, but it would seem likely that the small amount of mineral mobilized from the vast skeletal reserves would not be detectable even to this method. The urinary excretion of radiocalcium appears to be the most sensitive indicator of a Parathormone effect on Ca^{45} deposited in the stable bone mineral.

The increased quantity of radiocalcium appearing in the urine shortly after initiation of daily injections of Parathormone indicates that these extracts influence the stable bone mineral to yield calcium to the body fluids. During 13 days of Parathormone treatment in rats, at least 60 times as much Ca^{45} was lost by renal excretion as in control animals.

The present experiments have an interesting correlation to a theory of parathyroid action recently formulated by McLean, who describes a dual mechanism for controlling the level of calcium in the plasma(5). One part, independent of the parathyroids, acts by simple chemical equilibrium with the labile fraction of the bone mineral to maintain a constant but low plasma calcium level of approximately 3.5 meq/l. The second part of the dual mechanism is required to raise the plasma calcium to the normal level of 5 meq/l and is mediated by the parathyroid glands. McLean postulates that the parathyroid hormone releases calcium from the stable crystals of hydroxyapatite, as well as from the labile fractions of bone mineral. The present work provides experimental evidence that the stable bone mineral does indeed participate in releasing calcium to the body fluids as a result of injecting parathyroid extracts. This mechanism makes an otherwise inaccessible source of calcium available for homeostatic regulation.

Summary. The effect of Parathormone injections causing mobilization of radiocalcium from incarceration in stable bone mineral of rat, has been studied by observing specific activities of bones and amounts of radiocalcium appearing in urine prior to and following subcutaneous injections of bovine parathyroid extracts. From the greatly increased renal excretion of Ca^{45} following injection of

§ The unusually high value for Group B on day 11 was probably due to inadequate washing of cage and hence incomplete collection of urine on day 10.

parathyroid extracts it can be reasonably concluded that these extracts are able to produce mobilization of stable bone mineral from the rat skeleton.

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Incorporation of C^{14}-Acetate into Intestinal Fatty Acids of Rats with Cannulated Bile Ducts.* (22230)

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Incorporation of C^{14} from acetate into intestinal fatty acids has been demonstrated in experiments of short duration by various investigators(1-3). Evidence is available which indicates that the labeled intestinal fatty acids are synthesized independently of the liver fatty acids(2) and probably by the intestine itself(3,4). However, the possibility of transport of significant amounts of highly radioactive fatty acids from liver to intestine via the bile has not been investigated. This pathway was suggested to us by the findings of Gould *et al.*(5), that significant amounts of synthesized liver cholesterol are transported via the bile. The experiments described in this paper were designed to ascertain the role of bile in the acquisition of labeled long chain fatty acids by the intestine after administration of radioactive acetate.

Methods and materials. Adult male Sprague-Dawley rats weighing 200 to 300 g were used, and animals of the same weight were paired in each experiment. The bile duct of one rat was cannulated with polyethylene-50 tubing just distal to its bifurcation, and the cannula led to the outside of the body through an incision immediately below the right costal margin. A sham operation was performed in the control animal. Infection was not a problem and recovery usually occurred in 7 to 9 days. The sham-operated animal was pair-fed with the cannulated rat. During the first 2 days post-operatively there was low food consumption which led to a 5-10% decline in body weight. Subsequently, food consumption returned to normal and body weight remained at a constant level or showed a slight gain. When the rats had recovered, they were injected intraperitoneally with a tracer dose of radioactive acetate (1 ml of solution containing 3 mg of $CH_3C^{14}OO$ Na and 10 μc of activity) and placed in a cage designed to hold animals immobile(6). Water was given *ad libitum* but food was withheld after C^{14} administration. Three pairs of animals were allowed to metabolize the radioactive acetate for 5 hours; 2 pairs were killed 30 minutes after injection. Samples of bile were collected in the 5-hour experiments at 30-minute intervals for the first hour and at hourly intervals thereafter. A 0.025 ml aliquot of each bile sample was dried on a planchet and counted as such to determine the relative activity of the whole bile. In the 30-minute experiments 0.025 ml samples were taken at 5-, 15-, and 25-minute intervals. No corrections for self-absorption were applied to these measurements. The liver, intestine, bile and combined contents of cecum and intestine were taken for chemical and

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