do not occur during cold storage of corn oil eggs. This is supported by the observation that the putty-like yolks previously reported by Shenstone and Vickery(2) were produced only during cold storage of eggs from the *Sterculia foetida* oil lots. If eggs were stored at room temperature, this did not occur. Pink discoloration of eggs occurred only in eggs from Lot 4.

Summary. The yolks of eggs from hens having a one-egg clutch average took up significantly more water than those from hens having 2-4 egg clutch sizes. Yolks from hens having 2, 3, and 4 egg clutch sizes showed no significant differences in water uptake. Yolks from eggs stored overnight and from hens given *Sterculia foetida* oil were not significantly heavier and did not take up more water than eggs from hens given corn oil. However, when the eggs were stored one month the yolks from the hens fed *Sterculia foetida* oil increased 20% in weight and showed an 80% greater water uptake than those from the corn oil fed hens.

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Relation of Age to Radiomagnesium Exchange in Bone.* (26111)

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Evidence suggests that the reactivity of the skeleton, as measured by isotope exchange, declines with age(1). Studies in our laboratory have shown that radiosodium exchange in young bone exceeds that of old bone in both the rat and cat(2). A direct relationship between liberation of magnesium from skeleton and rate of bone growth has been demonstrated by Duckworth and Godden(3). The observed depletion of bone magnesium in calves fed a magnesium deficient diet(4), together with the finding of a normal bone content in lactating cows with hypomagnesemia(5), also suggests that bone magnesium is more labile in young animals.

This report concerns exchange of radiomagnesium in the bones of rats of various ages. Data are also included on age changes in bone content of stable magnesium.

Methods. Magnesium^{28} (T½ 21 h.) obtained from Brookhaven National Laboratories, was supplied as a solution of $MgCl_2$ in concentrated HCl. Sodium hydroxide was added to bring the pH to about 5. Due to the short half-life of the isotope it was necessary to inject more than trace amounts of stable magnesium. The amount actually injected was in the range of 0.1 to 0.16 meq. Mg/100 g body weight, and average dose of Mg^{28} was $4x10^5$ c/m/100 g body weight.

Groups of female rats aged 20, 30, 60, 180 days were injected with measured amounts of the isotope intraperitoneally and after 4 hours were exsanguinated by decapitation. In some of the 180-day and 30-day animals,

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TABLE I. Experimental Results.

Age, days	Wt, g	No. of animals	Equilibration time, hr	Bone water, %	Bone magnesium, meq/g wet wt	% Mg^{28} exchanged
20	44- 48	7	4	39.1 ± 1.7*	.18 ± .02*	31 ± 4.2*
30	60-100	12	4	24.2 ± 3.3	.25 ± .02	40 ± 8.3
		8	14	26.6 ± 1.8	.25 ± .02	36 ± 7.5
60	130-180	4	4	17.9 (17.7-18.4)†	.29 (.28-.30)†	6.2 (2.1-7.6)†
180	170-225	10	4	12.7 ± 1.1	.35 ± .05	4.1 ± 1.9
		6	14	13.9 ± 3.8	.30 ± .02	5.2 ± 1.2

* Stand. dev. † Range.

the experimental period was extended to 14 hours. All of the animals were fasted during the experiment, except the 30-day rats which were sacrificed after 14 hours.

After sacrifice, the hind limbs were removed and the long bones stripped of muscle and periosteum. The cancellous bone was removed and the marrow separated from the remaining cortical bone, which was placed at once in a tared vial. Radioactivity of bone and serum was determined in a scintillation counter, the usual corrections being made for decay and resolving time.

After drying to constant weight in a vacuum oven at 65°C, the samples were ashed in a muffle furnace at 525°C for 48 hours. The ash was dissolved in HCl, filtered, and made up to volume.

Serum magnesium was determined by the method of Orange and Rhein(6). Prior to anlaysis of the bone ash solutions, calcium and phosphorus were removed by passage through a cation exchange column consisting of a 0.5 x 37 cm column of the sulfonate polystyrene resin, Dowex-50, 50-100 mesh. An amount of bone ash solution corresponding to 50-80 mg of dry bone was added to the column and allowed to absorb on the resin.

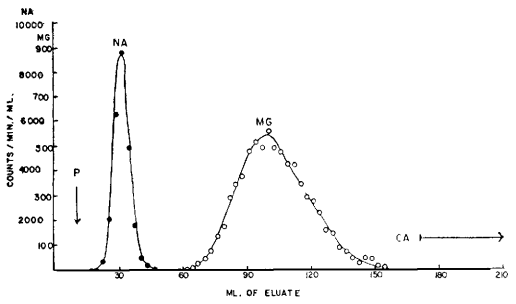

FIG. 1. Elution pattern of bone ash solution to which Na^{22} and Mg^{28} had been added.

This was then eluted with 0.7 N HCl at a flow rate of about 0.7 cc/min. The initial 25 cc was discarded as it contained the phosphorus, and the following 125-150 cc were collected, and analyzed for Mg by the method of Orange and Rhein. The column was regenerated with 100 cc of 2 N HCl, and washed with distilled water.

Fig. 1 demonstrates separation of P, Na, Mg, and Ca in various portions of the eluate, utilizing the isotopes of Na and Mg. In a series of 12 runs, an average of 95% (s.e. 1.6) of the added Mg^{28} was recovered. The solutions used in these trials also contained 0.004-0.016 meq. Mg and about 0.5 meq. Ca, to simulate the proportion of these 2 ions as they occur in bone. It was found that addition of larger amounts of either Mg or Ca caused a shift in the elution pattern.

Results are summarized in Table I. It is apparent that the exchange of Mg^{28} is much (5-10 times) greater in the 20- and 30-day-old animals than in the 60- and 180-day-old groups. Per cent exchange is calculated as the ratio specific activity† bone/specific activity serum (x100). Prolonging equilibration period to 14 hours did not increase the exchange.

The Table also illustrates the progressive decline in bone water content and the progressive increase in stable magnesium content as the animals become older.

Discussion. The exchange of Mg^{28}, expressed as bone: serum specific activity, is much more rapid in the younger than in the older animals. This finding is in keeping with the age differences which we have previously reported for radio-sodium exchange

† Counts/min./meq Mg.

in bone(7), though the latter isotope exchanges more rapidly at all ages than does Mg^{28}. Langemann(8) has reported that Mg^{28} accumulates in the bones of young rats about twice as fast as in the adult, when results were expressed as per cent of injected dose per gram of bone. This author also found that dietary Ca and Mg influenced uptake of Mg^{28} in bone. In this connection it should be noted that the 30-day-old animals in our experiments were fasted for 12 hours prior to injection of Mg^{28}, whereas the 20-day-old animals were allowed food during this period. The lowered uptake of Mg^{28} in the latter group may have been due in part to this necessary circumstance.

Changes in bone water content are in keeping with those previously described in our laboratory(2). The increase in stable magnesium content is analagous to that previously noted for Na and Ca. It is apparent that experiments involving bone composition should be designed with this age factor in mind.

It is of interest that for about 10-15 minutes following injection many of our animals exhibited decreased activity and responsiveness, and stretching of the hind limbs. In a preliminary study of blood levels it was found that serum magnesium concentration rose following injection of the isotope solution to a value 50-100% above normal within 20-30 minutes, subsequently falling to normal in 4 hours. Simultaneous measurements of serum Mg^{28} confirmed the observation of Rogers and Mahan(9) that the level drops rapidly during the first 4 hours, followed by a much slower decline.

Neuman has shown that the magnesium of bone is a surface-limited ion(10). The greater degree of exchange exhibited by the bone of the young animal may be related to the smaller crystal size(11), or to the greater water content, implying a greater degree of accessibility to circulating body fluids(12).

The chromatographic method for separating Mg from Ca and P proved to be satisfactory. The method described here is essentially the same (though a small column was used) as that previously reported from our laboratory(13). It is essential that these interfering ions be removed, at least in bone, prior to determination of Mg by the method of Orange and Rhein. We found that failure to do so gave values which were about double those found by the chromatographic method for which it is known that recovery of added Mg^{28} is satisfactory. Others have also reported the use of Dowex columns for separation of Mg and Ca(14,15).

Summary. In the rat, the exchange of Mg^{28} in cortical bone occurs much more rapidly in young animals than in old. The stable magnesium content of bone increases with age, varies inversely with bone water content. A method for separating Mg from Ca and P by cation exchange chromatography is described.

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