phosphorus balance, but fed a diet containing a Ca/P ratio of 4, excreted more Sr^{89} than did their controls (Table I). These animals did, however, have an increased urinary calcium. The animals which were in negative calcium balance had a depressed urinary calcium and excreted no more Sr^{89} than the controls. Thus, it would appear that alterations in the dietary Ca/P ratio such that they result in an increased urinary calcium will cause an acceleration of the release of skeletally bound radioactive strontium.

The effectiveness of magnesium ions in increasing the elimination of skeletally bound isotope is also dependent on its ability to increase urinary calcium.

If the diet contains calcium, increased magnesium intake decreases fecal calcium, *i.e.*, increases absorption from the gut and makes the calcium balance more positive. In these experiments, the magnesium supplemented rats consumed more calcium than their controls and it is conceivable that increase in the calcium balance may in part be derived from the increased calcium intake. However, despite the increased calcium intake by the magnesium supplemented rats, fecal calcium was always less. Moreover, in a series of experiments in which magnesium supplemented rats were pair-fed with their controls the effect of magnesium was to decrease consistently fecal calcium and to increase the balance regardless of the dietary Ca/P ratio, provided the diet contained more than traces of calcium (to be published). The decreased fecal Sr^{89} observed previously(1) and in these experiments must be the result of increased absorption of this ion which resulted from the excess magnesium intake. The rate of renal elimination must be greater than that of absorption to account for the increased overall elimination (Table I).

Summary. The removal of skeletally bound Sr^{89} is accelerated by diets whose Ca/P ratios are 4 or greater. Also, supplemental magnesium increases the removal of isotope if the dietary Ca/P ratio is 2 or less. Large dietary Ca/P ratios and supplemental magnesium produce hypercalciuria which is essential for increased Sr^{89} elimination. Increased intake of magnesium ions routinely decreases urinary phosphorus and fecal calcium causing their balance to become more positive.

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The Activating Effect of Lead on the Precipitation of Calcium Phosphate.* (29997)

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For many years one of the most puzzling features of calcification has been how hydroxyapatite crystals can form *in vivo* at a

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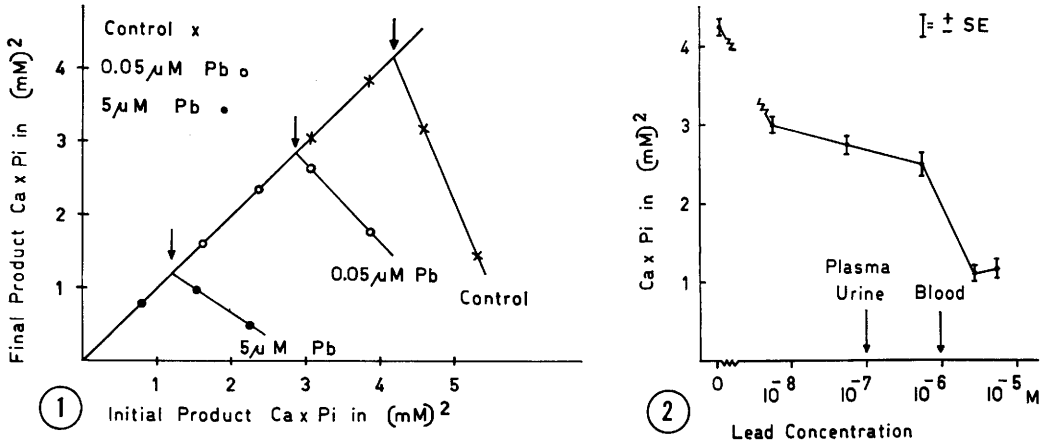

FIG. 1. Typical experiment showing influence of lead on minimum concentration Ca × P in $(mM)^2$ (represented by inflection points) required for formation of calcium phosphate crystals *in vitro*. Ionic strength = 0.16; pH = 7.4; temperature = 37°C.

FIG. 2. Influence of lead on minimum concentration Ca × P in $(mM)^2$ required for crystal formation *in vitro* at $\mu = 0.16$, pH = 7.4 and 37°C.

calcium phosphate concentration much below that required for crystal formation *in vitro* (1). The demonstration that collagen(1,2,3, 4) and elastin(5) can induce calcium phosphate precipitation *in vitro* at relatively low Ca × P products seemed to clarify this. In recent years, interest has therefore been centered around the activating role of the matrix in calcification and little attention has been devoted to the possibility that inorganic substances might have a similar effect and therefore also take part in the control of mineralization.

During our work on the isolation of physiological substances which might regulate calcification, we noticed that extremely low concentrations of lead could activate calcium phosphate precipitation *in vitro*. In view of its possible biological significance we have investigated this effect in more detail.

Methods. The test system was essentially the same as that used previously for assessing the nucleating activity of collagen(2,4). It consists of determining the minimum ion product [Ca] × [P] necessary to form calcium phosphate crystals *in vitro* under physiological conditions of pH (7.4), ionic strength (0.16) and temperature (37°C). A series of solutions was made up containing KCl for maintenance of ionic strength, barbital as buffer, a constant calcium concentration of 1.3 mM and progressively increasing phosphate concentrations ranging from 0.5 mM to 4 mM. Lead was added as lead acetate to a final concentration between 5×10^{-9} M and 5×10^{-5} M. The solutions were incubated for 3 days at 37°C, then filtered through a membrane filter to remove any crystals which had formed. By plotting the arithmetical [Ca] × [P] product in the filtrate after incubation against the product before incubation it was possible to determine graphically the minimum [Ca] × [P] product necessary for crystal formation (Fig. 1).

Results. A typical experiment is shown in Fig. 1. Whereas a [Ca] × [P] product of 4.2 $(mM)^2$ is normally necessary for crystal formation, the presence of lead at concentrations of 0.05 μM and 5 μM decreases this value to 2.85 $(mM)^2$ and 1.21 $(mM)^2$ respectively. The various experiments are summarized in Fig. 2, which shows that lead at concentrations as low as 5×10^{-9} M facilitates calcium phosphate precipitation.

Discussion. These results show that lead has an activating effect on calcium phosphate crystal formation, not only at the high concentrations present in lead intoxication but also at the lower levels normally present in biological fluids such as blood(6) plasma(7) and urine(8). The mechanism of this activation is unknown; for its elucidation the properties of the lead apatites would first need to

be thoroughly investigated. It is of interest to note that the [Ca] × [P] products at which crystals are formed in the presence of lead are as low as those obtained formerly with the most active nucleating collagens(2, 4). It is thus tempting to speculate that the local concentration of lead might play some role in the regulation of calcification. This is supported by the fact that lead does induce ectopic calcification. This has been demonstrated by Selye, who placed this element in his category of "direct calcifiers"(9,10,11).

Summary. Physiological concentrations of lead have been shown to decrease the minimum product [Ca] × [P] necessary to form calcium phosphate crystals *in vitro*. The possible importance of this activating property in biological calcification is discussed.

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Histamine Metabolism in the Pregnant and Non-Pregnant Hamster. (29998)

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It has been shown in this laboratory that the foetuses of all the mammals investigated form histamine, *i.e.*, are endowed with histamine forming capacity (HFC), which in some species attains very high levels(1). Part of this histamine of foetal origin is excreted in the urine as the free amine. Even maternal tissues may exhibit greatly elevated HFC during pregnancy, for instance the mouse kidney. In the present investigation a hitherto unknown site of singularly high HFC in pregnancy has been disclosed.

Methods. The experiments were done on the female golden hamster, *Cricetus auratus*; only 2 animals (No. 2 and 4 in Table V) were of an inbred strain of white hamsters.

Free non-isotopic histamine was determined in 24-hour urine samples collected with the animal in a metabolic cage. The animal was fed a paste of ground pellets mixed with water, 10-12 g of this diet daily, which contained only about 1 μg histamine/g, and water was allowed *ad libitum*. Hydrochloric acid, sufficient to give the urine a pH value below 2, was added to the collecting vessels to prevent formation of histamine by bacteria during the collecting periods. The histamine content of the urine samples was assayed on the guinea pig ileum by the method originally devised by Guggenheim and Löffler(2). The figures for excretion of free non-isotopic histamine are given as μg base/24 hours. To ascertain the identity of the substance with histamine, a histamine antagonist was used according to Reuse's procedure(3).

The histidine decarboxylase activity *in vivo* was determined by injecting ^{14}C-histidine and measuring urinary excretion of radioactive histamine and methylhistamine, a catabolite of endogenously formed histamine. ^{14}C-L-Histidine, 100 μg, labelled in the 2-position of the imidazole ring, was injected subcutaneously, and the urine sam-