total erythroid tissue to EP dose is greater ($p<0.01$) in the 48-hour response. Total uptake saturates above 8 units in the "early" response but not in the "late." Marrow uptake drops at higher doses (16 units) in the "early" response but does not do so in the "late." The curve for uptake by circulating erythrocytes shows only one slope in the "early" but two distinct slopes in the "late" response: one from 1–4 units the other much steeper from 4–16 units. The results are discussed in relation to the possibility of different mechanisms being involved in the two responses. The "early" one reflecting the effect of EP on proliferation of recognizable erythroid precursors, the late differentiation of "stem cells."

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Bone Salt Mobilization Effected by Ascorbic Acid* (32880)

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It is accepted that ascorbic acid plays a role in normal bone growth (1). The extent of the physiological function of vitamin C in bone has not been clarified; however, its requirement is usually considered to be limited to the part it plays in matrix formation. In this regard, an influence on both collagen formation (2) and alkaline phosphatase activity (3) has been demonstrated. These (2,3) are only selected references since both effects have been shown by a number of investigators and have been reviewed elsewhere (4). The possibility that ascorbic acid influences bone calcification directly has been a point of research interest, but no conclusive evidence has been presented illustrating such a role. Similarly, there is no unequivocal demonstration linking ascorbic acid to bone catabolism. Recently some work has been reported which suggests that ascorbic acid may play a role in bone destruction (5,6). Those reports suggested that ascorbate enhanced the *in vitro* release of both calcium and phosphate (5) and resulted in bone ash reduction in rachitic chicks given this compound (6). The latter results imply that bone demineralization may have been increased by the ascorbic acid, although it could be that the reduced ash value was effected by a stimulation of matrix formation.

In the present case, results are presented which suggest that exogenous ascorbic acid can cause the mobilization of skeletal ^{45}Ca. Since the isotope was given several days prior to the vitamin, it may be assumed that much of the ^{45}Ca was mainly located in mature crystals when the ascorbate was administered. If so, it follows that the increase in blood ^{45}Ca following ascorbic acid administration resulted because bone salts in the so-called nonexchangeable fraction (7) were mobilized.

Materials and Methods. Three separate experiments were conducted to test the idea that ascorbic acid could influence bone salt mobilization. Procedures similar in these studies are as follows. Male leghorn chicks were placed on a diet used previously (8) at 1 day of age and given this feed until the experiment was completed. When 8 days of age, each chick was given ^{45}Ca intramuscularly. The isotope, administered in solution with normal saline, was given at a level of 20 μC/100 gm of body weight in the first study and 10 μC/100 gm in the last two cases. When 14 days old, the animals were injected intraperitoneally with

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TABLE I. Ascorbate and Blood ^{45}Ca Activity.

Time of measurement (hours postinjection)	No. of animals	Blood ^{45}Ca activity (cpm/ml)		
		Control	Exptl.	Exptl./control
0	8	480 ± 18[a]	—	—
1	6	504 ± 43	587 ± 94	1.16
2	6	484 ± 18	555 ± 58	1.15
4	6	457 ± 44	576 ± 26	1.26[b]
8	6	538 ± 21	758 ± 55	1.41[c]
24	6	521 ± 35	407 ± 17	0.78[c]

[a] Mean ± the standard error of the mean.

[b,c] Significantly different from the control group at the 5 and 1% confidence levels, respectively.

TABLE II. Exogenous Ascorbic Acid and Skeletal ^{45}Ca Mobilization.

Time of measurement (hours postinjection)	No. of animals	Blood ^{45}Ca (cpm/ml)		
		Control	Exptl.	Exptl./control
4	8	296 ± 22[a]	442 ± 35	1.49[c]
8	8	325 ± 7	436 ± 33	1.34[c]
24	8	336 ± 32	252 ± 40	0.75
6	12[b]	282 ± 16	363 ± 20	1.29[c]

[a,c] See Table I.

[b] The 6-hour assessment was made on a different date from the 4-, 8-, and 24-hour assessments and constitutes a separate experiment.

either physiological saline solution (pss) or this material containing ascorbic acid (reagent grade L-ascorbic acid). The ascorbate crystals were dissolved in the saline solution just prior to administration and given at a level of 10 mg/100 gm of body weight. Comparable volumes of solution (0.5 ml/100 gm of body wt.) were given in each case.

In the first study (Table I) blood samples were collected by cardiac puncture from 6 animals at 1, 2, 4, 8, and 24 hours following the injection. Additionally, blood samples from 8 animals which received no injection were used for zero time observations. Each collection was made on a separate group of animals. Eight animals per group were used in the second study (Table II) with blood collections made at 4, 8, and 24 hours postinjection. The third study (Table II) involved 12 animals per treatment with only 1 observation made at 6 hours.

The whole blood samples were first dried overnight at approximately 105°C and then ashed at 600°C. The ash was taken up in 1.0 *N* HCl and 0.1-ml samples plated on stainless steel planchets. The plating pipette was rinsed once with distilled water, and the plated sample distributed uniformly over the planchet surface with ethanol. The samples were dried and ^{45}Ca activity determined with a Beckman gas flow detector (Low Beta II model no. 160006). Since the sample was small, containing little ash, no corrections for self-absorption were needed.

Results and Discussion. Blood ^{45}Ca activity was elevated following the injection of ascorbic acid (Tables I and II). This increase became statistically significant after 4 hours and continued to be so up to 8 hours following the administration of vitamin C. No assessments were made between 8 and 24 hours; thus, it is not known whether the 8-hour observation was the time of greatest response. However, it was apparent that the effect did not persist beyond 24 hours since the blood ^{45}Ca activity at that time was approximately 25% lower than control animals (Tables I and II). From this it appeared that the response to the

exogenous ascorbic acid was a two-phase situation. First, a mobilization of previously deposited skeletal ^{45}Ca occurred, followed by a greater than control movement of ^{45}Ca from the blood. Of interest are the possible physiological implications that can be attributed to these observations.

Mobilization of bone salts is usually regarded as being a parathyroid hormone function. Secretion of this hormone occurs when fluid ionic calcium level is decreased. Thus, if ascorbic acid effected a decrease in ionic calcium, it would obviously stimulate parathyroid gland activity. In that case, the results reported here would be attributed to an indirect effect by ascorbic acid; that is, a decrease in fluid ionic calcium level which stimulated the release of parathyroid hormone. The current experiments were not designed to answer this question. However, earlier results suggested that a stimulation of parathyroid gland activity did not occur following the injection of ascorbic acid (unpublished). In that particular study it was noted that the excretion of ^{32}P was slightly lower than controls in chicks given ascorbic acid at levels similar to those employed in the current work. The ^{32}P had been given 5 days before the ascorbic acid, and the measurement of excreta ^{32}P was from 0–48-hours postinjection of the ascorbate.

The elevation of blood ^{45}Ca observed in the present studies could seemingly have resulted by means other than active bone resorption. The possibility of physical dissolution was considered although such an effect seemed remote. To test this possibility chicks of similar breed, age, and treatment as previously used were given 0, 5, 10, and 20 mg of ascorbic acid/100 gm of body weight. Blood pH values were determined at 20 and 60 min, following injection with a blood–gas analyzer (Instrumentation Lab., Inc., Model 102). It was noted that blood pH values did not deviate from normal in any of the ascorbic acid-injected chicks.

Next, the possibility of calcium chelation by the exogenous ascorbic acid was considered despite earlier observations which suggested that vitamin C had only a minor influence (10). Twenty-four chicks, similar in all respects to earlier studies, were given either pss or this material containing ascorbic acid (10 mg/100 gm of body wt.). Blood samples were collected via cardiac puncture from 4 animals of each experimental group at 2, 6, and 24 hours after injection. The blood samples were placed immediately in 4% TCA and the plasma ascorbic acid level determined (9). Changes in blood ascorbic acid levels of +45, +7, and —2% were noted at 2, 6, and 24 hours, respectively in the ascorbic acid-injected chicks. Thus, a marked increase in plasma ascorbic acid preceded the alteration in blood ^{45}Ca by 2 hours. The initial elevation in blood ascorbic acid could have caused a calcium-chelating effect which may have led to increased parathyroid gland secretion. The observation that this particular species responds to exogenous parathyroid hormone within 3–4 hours (11) supports such a suggestion, timewise. Despite this possibility, the fact remains that ascorbic acid has only a minor calcium-chelating capacity (10), and we have noted that ^{32}P excretion was not increased in chicks given ascorbic acid (unpublished, previously cited).

The second response to the injected ascorbate, a 25% reduction in blood ^{45}Ca activity, was observed at 24-hours postinjection. This effect could seemingly have resulted from an increased excretion of calcium or a return to a predominately deposition phase following the initial bone salt mobilization. The latter suggestion seems more probable. It is well accepted that ascorbic acid influences bone matrix formation (2–4) which presumably would enhance the movement of calcium from the blood to the bone.

Others (12) have presented indirect results indicating a skeletal requirement of ascorbic acid for bone mineral deposition. Many (4) have found evidence linking ascorbic acid to a function in bone matrix formation. It is believed that this work is the first illustration of a possible bone salt mobilizing influence. Whether this effect is direct, thus a true resorptive function, or was caused indirectly, remains to be clarified. If the effect were direct, it would imply that ascorbic acid had a more extensive role in skeletal physiology than appreciated heretofore.

Summary. The influence of exogenous ascorbic acid on the movement of ^{45}Ca from the

bone to the blood was measured in the young chick. The isotope was given 6 days prior to the vitamin and was presumably located mainly in mature crystals when the ascorbic acid was given. Blood levels of ^{45}Ca were significantly elevated from 4 to 8 hours following administration of ascorbic acid. Twenty-four hours after this injection blood ^{45}Ca was approximately 25% less than controls. These results suggest that ascorbic acid, as used in these studies, had a bone salt mobilizing influence. Whether this effect was direct or indirect remains to be elucidated. If the mobilizing effect were direct, it would indicate that ascorbic acid has a more extensive role in skeletal physiology than previously thought.

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Qualitative Studies of the Protein in Fetal Lamb Lung Fluid* (32881)

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During fetal life the trachea, bronchi, and alveoli are filled with fluid which is produced by the lungs(1). It is believed that the physicochemical properties of this fluid are important during the onset of respirations at the time of delivery, particularly in regards to the presence or absence of substances influencing the surface tension at the air liquid interface.

Adams *et al.* (2,3) have described the fetal lung fluid and the presence of surface active material in the fluid of lambs near term before the first breath. A direct relationship between the presence at postmortem of hyaline membrane disease and decreased surface activity of lung extracts also exists both in man(4) and animal (5). Abrams(6) has isolated a surface active lipoprotein from homogenates of mammalian lungs with an α globulin mobility in agar gel electrophoresis.

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Although fibrinogen is not present normally in fetal lung fluid(1), it is one of the main constituents of the hyaline membrane(7). It seems plausible that under conditions of fetal distress, changes in the composition of the lung fluid may occur favoring the formation of the hyaline membrane. The purpose of these studies was to determine the protein fractions normally present in the lung fluid of fetal lambs before the onset of respiration.

Materials and Methods. Thirteen lambs, at different gestational ages, ranging in weight from 1.9 to 4.6 kg were delivered by cesarean section. Special precautions were taken to maintain the placental circulation intact(2).

Pregnant ewes were anesthetized with nembutal, and 100% oxygen was given by a respiratory pump through a tracheostomy. The rate and volume of the pump was adjusted in order to maintain a normal maternal arterial pH and pCO_2.

After shaving the abdomen, with the ewe