

Table 1. Fixed ammonium nitrogen in shales and granite rocks.

Sample	Total N ($\mu\text{g/g}$)	Fixed $\text{NH}_4^+ - \text{N}$	
		($\mu\text{g/g}$)	(% of N)
<i>Shales</i>			
A (1445–1449 feet)	500	330	66.0
B (1976–2018 feet)	810	420	51.9
C (2318–2329 feet)	680	420	61.8
D (596– 600 feet)	610	410	67.2
<i>Granite rocks</i>			
A (highly weathered)	86	27	31.0
B (slightly weathered)	35	10	28.6
C (unweathered)	32	18	56.3
D (moderately weathered)	26	9	34.6
E (moderately weathered)	21	5	23.8
F (moderately weathered)	37	10	27.0

mens are given in Table 1. The nitrogen contents of the granite rocks were generally lower than the values reported by Rayleigh (5) for igneous rocks. The results show that all of the samples contained nitrogen, which was not removed by boiling 1N KOH but was released as ammonia by HF. As hydrofluoric acid dissolves silicate minerals, there can be little doubt that the nitrogen occurred as fixed ammonium. Indirect evidence for the presence of fixed ammonium in rocks is provided by the study of Rayleigh (5), who found that the nitrogen in igneous rocks was liberated as ammonia by heating with caustic soda, but not with caustic potash. It is now known that caustic soda expands the d (001) spacing of silicate minerals, thereby allowing the ammonium ions to escape; caustic potash contracts the d (001) spacing, thereby preventing their release (1).

The origin of the fixed ammonium in the granite rocks must be regarded as a subject for speculation. The silicate minerals in granite rocks have crystal lattices that are contracted and incapable of fixing ammonium. I am of the opinion that ammonium was present in the substrate at the time the minerals were synthesized and competed with potassium for the pockets formed by the hexagonal oxygen rings in the crystalline nuclei of the minerals.

The nature of the nitrogen in the granite rocks that was not recovered as fixed ammonium is not known. Ammonia was liberated from rocks A, B,

D, E, and F during the treatment with boiling 1N KOH. This nitrogen could have been in the form of exchangeable ammonium produced through the release of fixed ammonium from silicate minerals during weathering. The observation of Engols and Navarre (6) that substantial concentrations of ammonia nitrogen occur in unpolluted surface and ground waters of the uplands of northern Georgia as a result of the leaching of granite rock undergoing rapid weathering can be attributed to the liberation of ammonium ions from silicate minerals through weathering.

It is of interest to consider some possible implications of the results obtained from this study. First, they indicate that a vast reservoir of nitrogen exists in the terrestrial areas of the earth as ammonium ions held within the lattice structure of silicate minerals. The amount of combined nitrogen in the silicate phase of the primary lithosphere of the earth has been estimated to be about 50 times the amount present as gaseous nitrogen in the atmosphere (5, 7); the amount of nitrogen in sedimentary rocks has been estimated as about one-sixth that in the atmosphere (7, 8). If it is assumed that an average of one-third of the nitrogen in igneous rocks, and one-half of that in sediments, is fixed ammonium, the amount of nitrogen present as fixed ammonium in the earth's crust would be almost 20 times that present as elemental nitrogen in the atmosphere.

Second, the results provide a clue to the origin of nitrogen in the atmosphere. Scientists generally believe that the nitrogen content of the atmosphere increased during geologic times, presumably through de-gassing of the earth (7, 9). It has been known for a long time that nitrogen is given off during the heating of igneous rocks. This has led to the conclusion that the nitrogen in volcanic gases is primary and thus is a new addition to the atmosphere. Scott *et al.* (10) found that fixed ammonium ions in some clay minerals were released at temperatures as low as 400°C; this is well below the temperatures reached in volcanoes.

Third, the results afford an explanation of the chemical nature of the nitrogen in stony meteorites. Biddhue (11) found that the fusion of meteorite powder with caustic soda and mercuric nitrate resulted in the production of ammonia. This ammonia was believed not to be entirely in the free state. Nitrogen is liberated from stony meteorites during entry into the earth's atmosphere, and I postulate that some of it originates as ammonium ions held within the lattice structure of silicate minerals.

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Changes in Soluble Citrate of Pigeon Bone during Egg Laying

Abstract. Accompanying the structural changes in medullary bone during the egg-laying cycle there are local increases in the concentration of soluble citrate. Since citrate can form both soluble and insoluble compounds with calcium, it is suggested that this anion may have a regulatory function in bone apposition and resorption.

Bone is a highly organized aggregate composed mainly of a protein matrix, apatite, water, and electrolytes. Like other tissues, it responds readily to changes in the composition of the surrounding body fluids and to the metabolic activities of the neighboring cells. Notable examples of this lability are the metaphyseal dissolution which follows the administration of parathyroid extract and the changes in avian medullary bone during egg laying.

We have suggested that resorption of bone could be initiated by the accumulation of intermediate metabolites formed by the contiguous connective tissue cells (1, 2). In support of this theory it was shown that, after the administration of parathyroid extract in the rabbit, dehydrogenase activity of bone slices was diminished and respiration was impaired (2). In this circumstance, the anions of di- and tricarboxylic acids could accumulate at physiologic pH, dissolving calcium from the apatite crystal and competing for calcium with the fixed colloidal anions of the bone matrix (3). The formation of these soluble calcium complexes would thus contribute to the dissolution of the structure.

We agree with others that, of the tricarboxylic acid anions, special attention should be accorded to citrate (4-7). There are several reasons for this emphasis. (i) The dissociation constant of calcium citrate is very low, and hence

the solution of insoluble calcium salts is favored in the presence of this anion (8). From experiments on the equilibration of apatite systems and of powdered bone with buffered citrate, it is evident that soluble (and insoluble) complexes are formed with bone mineral (5, 6, 9). (ii) Citrate occupies a position of central importance in the scheme of cellular metabolism, and bone contains the enzyme systems necessary for the synthesis and utilization of this compound (2, 4). (iii) Citrate occurs in relatively high concentration in the extracellular matrix of bone (10). (iv) An elevation of serum citrate levels often accompanies bone resorption (11, 12).

In testing our theory, we studied soluble citrate concentrations in medullary bone of the pigeon during the egg-laying cycle (13). At such times, rapid transformations occur in the tibia and femur (14). For approximately 1 week preceding calcification of the egg, a period of bone apposition is dominant. Then during calcification of the egg (a clutch of two eggs is laid within 40 hours) resorption predominates, and the serum citrate level is elevated (12). In the third stage, this cyclic activity is discontinued and bone apposition and resorption are minimal. The soluble citrate concentrations were determined during these three stages.

Previous efforts to demonstrate local changes in citrate concentration in bone have been clouded by the inclusion of a large quantity of citrate from the insoluble extracellular phase (15). We have attempted to overcome this difficulty by a procedure which largely sequesters cells and soluble extracellular material. Hence, our results measure intracellular citrate plus any liberated soluble extracellular fraction.

Thirty pairs of mated pigeons were used for these experiments. The birds were examined daily until their egg-laying cycle was determined. They were then sacrificed during the resting, apposition, or resorption period. The exact period was confirmed by gross examination of the ovaries and ovulatory tract and by gross and histologic examination of the long bones. Material for citrate determination was obtained from the tibia and femur. For this procedure the bones were excised and split longitudinally, and the cells in the medullary portion were aspirated through a fine-bore (1 mm) glass tube into a chilled flask containing a small amount of distilled water. A piece of 100-mesh wire screen, moistened with water, was interposed between the bone and the tip of the tube to prevent aspiration of bone particles. The surface of the bone was continually shaved with a razor blade to expose fresh areas of cells. The aspirated material was homogenized and then lyophilized.

Table 1. Soluble citrate aspirated from pigeon medullary bone.

No. of birds	Stage	Citric acid* (μg/g)	P
8	Resting	167 ± 82	
7	Apposition	846 ± 203	
15	Resorption	1361 ± 304	< .001

* Mean values and standard deviations expressed as micrograms per gram of lyophilized material.

Fifty-milligram portions of the dried material were analyzed for citric acid by L'Heureux and Roth's modification of the method of Natelson, Pincus, and Natavoy (11).

The results, expressed as micrograms of citric acid per gram of dry aspirated material, are given in Table 1. During the resting phase the citrate level was 167 μg/g. This level increased about five times during the stage of bone apposition. The highest concentration, representing an eightfold increase over the resting level, occurred in the resorptive stage.

These values may be compared with the citrate concentration in the serum (approximately 5.7 mg/ml) observed during the resting stage (12). To express the data in terms of tissue water, we will assume that the extracted cellular material has a water content of 80 percent. Then the calculation yields the following values: resting stage, 4.2 mg/100 ml; appositional stage, 21.2 mg/100 ml; and resorptive stage, 34.0 mg/100 ml. Thus, the concentration of citrate from the bone sites during the cycle exceeds that in serum from resting birds by approximately 4 to 6 times. We have no information about the rate of formation and utilization of citrate by the bone; nevertheless, this high local citrate concentration alone would be expected to have an important effect on the state of the bone salts and bone matrix.

In vitro experiments with calcium phosphate, apatite, and bone powder, it has been shown that citrate can react with calcium to form both soluble and insoluble compounds (6, 9). When a small amount of citrate is present, it is coprecipitated with calcium phosphate (or apatite). On the other hand, larger concentrations lead to the formation of soluble calcium citrate and to solubilization of the altered apatite. Although the nature and relationships of many of the components and phases of bone are still ill defined, we assume that in bone, citrate is also distributed between an insoluble phase in the calcified matrix and a soluble phase, as a calcium complex. This concept, in conjunction with our present and earlier experimental observations, leads to the following hypothesis concerning the role of citrate in bone

apposition and resorption. During bone formation, the citrate released through cellular metabolism is largely deposited in the insoluble solid phase. As citrate production continues and the concentration increases, the soluble calcium citrate complex is formed, removing calcium from the ionic, crystalline, and protein bound fractions. Accompanying this realignment in the phase relations there is a disaggregation of the structure of bone. As a corollary of this view, the role of citrate in cell metabolism and its equally significant part in bone apposition and resorption illustrates the unity of structure and function.

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Display of Moving Parts of a Scene

Abstract. Methods for emphasizing the moving parts of a scene. By photographic or electronic means, a past image can be subtracted from the present one to emphasize the moving parts of a motion picture scene. Rhythms and patterns of motion become more noticeable, and changes in velocity can give an impression of accelerations and the force pattern.

In a motion picture, it is possible to emphasize those parts of a scene that move by suitable use of photographic or electronic methods. In essence, the method compares the image at one instant with that at a slightly later in-