CITRIC ACID AND CALCIUM METABOLISM

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Recently there has been increasing realisation of the important part played by citric acid in the body's metabolism. Thus administration of pyruvic acid, the intermediate metabolite common to the utilisation of carbohydrate, protein and perhaps fat, causes increased urinary excretion of citrate. Many tissues can form citrate by condensation of pyruvate and oxalacetate followed by reduction, and Krebs (1937) postulated the existence of a tissue respiration cycle in which pyruvate and oxalacetate yielded a succession of six carbon acids, among which was citric acid, until by loss of hydrogen and carbon dioxide oxalacetate was regenerated ready to continue the cycle with another molecule of pyruvate (Fig. 1).

Since Dickens (1941) showed that the skeleton contains 90 per cent or more of the body's citric acid and Class and Smith (1943) demonstrated its presence in various body concretions, evidence has been accumulating that this substance plays an important part in the metabolism of bone. Schersten (1931) found that the citrate content of the whole blood of man varied from 1·5 to 4·0 milligrams/100 millilitres; whereas, according to Pucher, Sherman and Vickery (1936), that of the dog is slightly lower, from 0·9 to 1·9 milligrams/100 millilitres. Several factors influence the blood citrate level. Boothby and Adams (1932) have shown that it decreases with advancing age and Agrell (1946) has demonstrated a rise after muscular work. The blood level was found by Smith and Orten (1938) to be dependent on the dietary citric acid and is increased by oral administration of citrate or citrate precursors such as lactate, malate, fumarate or succinate. According to Ostberg (1931) values for the normal daily citrate excretion of human adults vary from 0·2 to 1·0 grammes and lower results are found in dogs. The citric acid is evidently the product of endogenous metabolism since Sherman, Mendel and Smith (1936) have found that it continues to appear in the urine during starvation. During the menstrual cycle variations occur in urinary citrate, and decreases are observed after oestrogen administration according to Shorr, Bernheim and Taussky (1942).

The metabolism of calcium has long been thought to be associated with that of citric acid. Thus citrate has been shown by Pincus, Peterson and Kramer (1926) to form a soluble complex with calcium and it has been assumed that such a soluble complex exists in blood and accounts for the non-ionisable part of the diffusible fraction of serum calcium. Gomori and Gulyas (1944) showed that citrate injections in dogs made serum calcium more ultrafilterable so that a rapid urinary excretion of calcium took place. The state of calcium in the blood stream appears thus to influence its urinary excretion, and if the diffusible fraction of serum calcium is increased then the glomerular filtration rate may also be increased. Chang and Freeman (1950) injected neutralised citric acid into dogs and similarly found not only increased plasma and urine citrate but also marked urine calcium increases. On the other hand, calcium chloride injections produced no change in plasma citrate but reduced urine citrate.

Alwall (1944) showed that parathormone produced not only a raised serum calcium but also a parallel rise in serum citrate. Increases in both urine calcium and citrate have been found by Shorr, Almy, Sloan, Taussky and Toscani (1942) after parathormone injections in a hypoparathyroid patient, and similar results were produced in a hyperparathyroid patient with calcium injections. In Gomori's experiments above, in which dogs were injected with
citrate, microscopic examination showed hyperaemia and oedema of the bone marrow, transformation of osteoblasts into spindle cells, increase in numbers of osteoclasts far beyond normal, fragmentation of trabeculae and areas of early fibrosis—a picture seen after toxic doses of parathormone.

Vitamin D is known to raise serum calcium levels when administered in small doses to rachitic animals and in much larger doses to normals. When such excessive doses are given to normal or to parathyroidectomised dogs Freeman and Chang (1950) found not only that the serum calcium is raised but also the serum citrate. They suggested that excess vitamin D increases the liberation of calcium and citrate from the bone reservoir. Shohl and Butler (1939) showed that citrate had an action similar to that of vitamin D in rickets. This has been explained by Glanzmann, Meier and Walthard (1946) as follows: In the absence of vitamin D most of the calcium which may have dissolved in the acid medium of the stomach is reprecipitated as insoluble calcium phosphate at the more alkaline reaction of the intestine and thus eliminated in the faeces. With citrate in the diet, soluble unionised calcium citrate does not reprecipitate to form insoluble calcium phosphate (or insoluble calcium phytate if phytic acid from cereals is present). On a milk diet, for example, citrate in a like manner may prevent the formation of insoluble calcium soaps with the milk fatty acids. The citrate
part of the calcium citrate circulating in the blood may be oxidized away at ossifying zones leaving calcium free for bone salt formation.

Kuyper's (1938) experiments indicate that the precipitate of calcium, phosphate and citrate formed in vitro in the presence of suitable concentrations of these substances is actually a complex of these three such as may exist in bone. Since Sobel, Rockenmacher and Kramer (1945) found that changes in the calcium and phosphate contents of the diet produce parallel changes in their blood levels and in turn in their contents of bone, one is tempted to speculate whether the same close correlation would not be found in the case of citrate.

The kidneys and parathyroid glands have long been recognised as intimately related to the metabolism of calcium and phosphorus. Thus patients with advanced renal insufficiency frequently have hyperplasia of the parathyroid glands. According to Albright and Reifenstein (1948) the kidney insufficiency produces phosphate retention with a compensatory low serum calcium level as adjustment to the high serum phosphate, and parathyroid hyperplasia to meet this tendency. A theory of parathormone action (Ellsworth 1932) regards a decreased tubular reabsorption of phosphate as the first step. Thus phosphaturia leads to hypophosphataemia and a reciprocal rise in serum calcium. The rising serum phosphate levels after vitamin D administration are attributable only in part to increased phosphate absorption and mainly to the decreased parathyroid activity resulting from the raised serum calcium. It therefore seems that vitamin D has an effect similar to that of parathormone in increasing urinary phosphate excretion and that this effect of vitamin D is separate and distinct from its effect in increasing calcium absorption from the intestine. The kidney is also concerned in citrate metabolism. Thus experiments of Martensson (1940) showed that the serum of nephrectomised rabbits had four times as much citric acid as normals and that renal parenchyma oxidized citric acid rapidly. Similar observations have been made by Freeman and Chang (1950) in dogs and it has been found also that citrate injections produced much greater increases in plasma levels in nephrectomised animals compared with normals whether the normals had their ureters ligated or not. The kidney thus appears to be the main site of removal of circulating citrate. Support for this view comes from the work of Buffa and Peters (1949) who used fluoracetate in vivo to jam the citric acid cycle in rats so that citrate accumulated. Although increases in citrate were recorded in most tissues after fluoracetate poisoning, the kidney showed by far the largest, reaching seventy times its normal citrate content.

Whereas the metabolic relationships of calcium and phosphorus are determined by their physical properties and indestructible nature so that retentions of these substances are easily followed from the differences between their intakes and excretions, it must be remembered that the prominent position in intermediary metabolism occupied by citric acid makes its concentration in a particular organ or fluid independent of intake. Thus a rise in blood citrate does not necessarily result from either increased food citrate or from mobilisation from the bone reservoir, but may merely indicate an interruption of the citric acid cycle.

Precisely how all these observations fit into calcification mechanisms is not clear at present, but the function of citrate in bone certainly requires increased attention.

REFERENCES

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