

Gale E F. The bacterial amino acid decarboxylases. *Advan. Enzymol.* 6:1-32, 1946.
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Certain bacteria grown in an acid medium containing amino acids produce enzymes each of which is specific for the decarboxylation of an amino acid to the corresponding amine. Some of these enzymes were purified and their kinetics studied. The decarboxylases for lysine, arginine, ornithine, and tyrosine possessed a new coenzyme, codecarboxylase, replaceable by pyridoxal phosphate. [The *SCI*® indicates that this paper has been cited in over 265 publications.]

A New Range of Enzymes

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After graduating in biochemistry in 1936 from Cambridge University I was offered the chance to work with Dr. Marjory Stephenson on the breakdown of amino acids by bacteria. For the next three years I was mainly concerned with deaminase systems, the conditions under which they were formed, and the properties of the enzymes concerned. The early literature on "putrefaction" included many references to the production of amines by bacteria, but this did not occur in our work with washed suspensions of a variety of bacteria. Scrutiny of the literature showed that amines seemed to be produced when growth occurred in complex media containing fermentable carbohydrate leading to acid conditions—when we had found that deaminase production was inhibited. Following up these early clues, I found that bacteria grown in the presence of free amino acids under acid conditions did indeed have the ability to produce a new range of enzymes, each of which was specific for the decarboxylation of an amino acid. The paper cited summarises the work of the next seven years.

The discovery of these enzymes made available a quick and accurate method for the estimation of the

substrate amino acids in the free state in biological preparations. Professor Chibnall suggested that we use them for analysing protein hydrolysates,¹ and we also found we had a tool that made possible the long series of studies on amino acid transport and metabolism in bacteria and the effects thereon of antibiotics.² Purification of the enzymes revealed that some of them possessed a new coenzyme, which we called codecarboxylase. At that time the only known coenzymes were coenzyme 1 and 2, riboflavin and cocarboxylase.

We tried to purify codecarboxylase by the methods then available but unfortunately we had neither chromatographic or isotopic techniques but had to rely on chemical precipitations, extractions, and so on. We purified the codecarboxylase of yeast extract some 15,000 times, but even so it was probably not more than 3-5 percent pure. Its UV spectrum suggested a purine component, but we had a shock when we sent it away for microanalysis to a laboratory specialising therein and were informed that it was essentially free from phosphorus! This taught me not to rely on other people for analytical work! At this stage W.D. Bellamy and T.C. Gunsalus³ made splendid use of their "deficient culture" technique and showed that the tyrosine decarboxylase activity of *Streptococcus faecalis* was dependent on the presence of pyridoxin in the medium—the first hint of the function of pyridoxin. This soon led to the finding that the active substance was a derivative of pyridoxin—pyridoxal—and, in 1945, J. Baddiley and I carried out a phosphorylation of pyridoxal and found that the product activated our tyrosine apo-enzyme preparation. The decarboxylases were thus the enzymes that first demonstrated the existence of pyridoxal phosphate as a prosthetic group, while later work showed its importance in many other forms of amino acid metabolism.

One problem that exercised us was the function of these decarboxylases. It did not seem that the products—the amines—were essential as such. The enzymes we studied were formed only when bacteria grew in acid conditions (pH 3-5)—the lower the acidity the higher the activity. We proposed that the enzymes formed neutralisation mechanisms enabling the organism to maintain a neutral internal environment in an acid environment.⁴ Later studies have, of course, shown that some of the products (e.g., γ -aminobutyric acid) have important roles in mammalian biochemistry but are formed by enzymes that do not require such acid environments for activity.

1. Gale E F. The use of specific decarboxylase preparations in the estimation of amino acids and in protein analysis. *Biochemical J.* 39:46-52, 1945. (Cited 85 times.)
2. ———. The Heter Lectures: the nitrogen metabolism of Gram-positive bacteria. *Bull. Johns Hopkins Hosp.* 83:119-75, 1948. (Cited 65 times.)
3. Bellamy W D & Gunsalus T C. Tyrosine decarboxylation by streptococci: growth requirements for active cell production. *J. Bacteriology* 48:191-9, 1944. (Cited 15 times since 1945.)
4. Gale E F. Factors influencing the enzymic activities of bacteria. *Bacterial. Rev.* 7:139-73, 1943. (Cited 100 times since 1945.)