# Reports

## Phenylalanine and Tyrosine Metabolism in E. coli Strain K-12

A previous report (*1*) from this laboratory described the inhibition by β-hydroxyphenyl-DL-alanine (phenylserine) of the growth of *Escherichia coli* strain K-12 and two of its mutants, a phenylalanine auxotroph and a tyrosine auxotroph. Although, under certain conditions, L-tyrosine and L-phenylalanine each acted as a competitive antagonist of phenylserine for strain K-12, the experimental data were in accord with the view that phenylserine specifically inhibits the utilization of phenylalanine but not of tyrosine, and that, in the presence of the antimetabolite, exogenous tyrosine may serve as a precursor of the phenylalanine required to overcome the inhibition. The latter hypothesis has now been tested directly by growing strain K-12 in the presence of both phenylserine and L-tyrosine-$C^{14}$, and by isolating phenylalanine and tyrosine from the bacterial protein (*2*).

Strain K-12 was grown in 6 lit of a synthetic basal medium (*1*, *3*) containing, per liter, 3.75 g of glucose, 5.5 mmole of phenylserine, and 0.14 mmole of L-tyrosine, plus sufficient uniformly labeled L-tyrosine-$C^{14}$ (*4*) to provide 2054 count/min μmole of L-tyrosine in the medium. The culture was incubated under forced aeration for 25 hours at 35°C. Preliminary experiments had shown that, under such highly aerobic conditions, 5.5 m*M* phenylserine completely inhibited bacterial growth in the basal medium for 25 hours or longer and that the addition of at least 0.14 m*M* L-tyrosine was required to overcome the inhibition.

The cells were harvested by centrifugation and were washed first with 200 ml of cold 0.9 percent saline containing 0.5 mmole of unlabeled L-tyrosine and then once with 0.9 percent saline, twice with water, and three times each with absolute ethanol and with ether. The resultant bacterial powder (in the amount of 1.6 g) was hydrolyzed in 25 ml of 6*N* hydrochloric acid in a stream of $N_2$ for 25 hours, and the hydrolyzate was concentrated in a vacuum to remove excess acid. The residue was suspended in 120 ml of water and filtered, and the filtrate was shaken with about 3 g of acetic acid-washed Darco-G 60 to adsorb the aromatic amino acids (*5*). After the Darco had been washed well with water, it was shaken with two 50-ml portions of 95 percent ethanol to elute the adsorbed amino acids (phenylalanine, tyrosine, and some arginine).

The ethanol solution was concentrated to dryness, the residue was taken up in 1 ml of 4*N* hydrochloric acid, and the amino acid mixture was subjected to column chromatography on Dowex 50 resin (*6*). The column was eluted with 4*N* hydrochloric acid, and tyrosine and phenylalanine were isolated from the appropriate fractions.

The tyrosine was recrystallized three times from water to constant radioactivity; the yield was 10.3 mg. The phenylalanine was freed of impurities derived from the resin by paper chromatography on washed Whatman No. 54 paper, first with butanol-pyridine-water (2/1/1) as the solvent and then with butanol-acetic acid-water (4/1/1) as the solvent. Finally, the material was dissolved in hot 95 percent ethanol, the solution was filtered, and after removal of most of the solvent, and chilling, 9.3 mg of phenylalanine was obtained. The purity of the isolated compounds was checked by paper chromatography prior to $C^{14}$ analysis in a gas-flow counter in the Geiger region.

Several samples of bacterial tyrosine were counted (sample weights 0.071 to 0.289 mg) and had a specific radioactivity equivalent to 2334 count/min μmole of amino acid. The bacterial phenylalanine contained a barely detectable amount of $C^{14}$; the specific radioactivity of the sample counted (1.113 mg) corresponded to about 0.19 count/min μmole of phenylalanine.

The work of Davis (*7*) and his associates has established that, in *E. coli*, phenylalanine and tyrosine are formed by independent pathways from a common precursor, prephenic acid. In the experiment described in preceding paragraphs, none of the prephenic acid formed by bacteria grown in the presence of phenylserine and L-tyrosine-$C^{14}$ was used for the synthesis of bacterial tyrosine, since the specific radioactivity of the isolated amino acid (2334 count/min) was equivalent to that of the tyrosine supplied in the medium (2054 count/min). The absence of significant $C^{14}$ from the bacterial phenylalanine indicates that all the bacterial phenylalanine must have been synthesized from prephenic acid derived from glucose and shows clearly that no phenylalanine was formed from exogenous tyrosine. It may be concluded, therefore, that the conversion of prephenic acid to tyrosine is essentially irreversible, even under conditions where the bacteria require large amounts of phenylalanine. In this connection, it is of interest that the conversion of prephenic acid to phenylalanine also is irreversible, since studies with auxotrophic mutants of strain K-12 indicate that these bacteria do not make tyrosine from phenylalanine (*8*).

The ability of exogenous tyrosine to overcome the inhibitory action of relatively low concentrations of phenylserine, therefore, may be ascribed to the "sparing" action of exogenous tyrosine on bacterial prephenic acid. This sparing action would permit the biosynthesis of the extra phenylalanine required by the bacteria for growth in the presence of phenylserine. The amount of extra phenylalanine formed should be proportional to the amount of prephenic acid spared, and this, in turn, should depend on the concentration of exogenous tyrosine. The observed effect, therefore, will be a competitive antagonism between tyrosine and phenylserine.

The foregoing data may also be explained by an alternative hypothesis, analogous to that of Cohen and Rickenberg (*9*) for the competitive antagonism between other bacterial amino acids and their antimetabolites. According to this view, exogenous tyrosine would exert its antagonism to phenylserine by preventing the uptake of the latter from the medium by the bacterial cells. However, phenylserine does not compete with phenylalanine for entrance into *E. coli* cells (*9*), and it is unlikely that it competes with tyrosine. Furthermore, the inhibition of the growth of strain K-12 and, especially, of its phenylalanine auxotroph by phenylserine can be enhanced by the addition of tyrosine to the medium (*1*). Since, in the absence of phenylserine, tyrosine is not inhibitory, the synergistic effect of the tyrosine-

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phenylserine mixtures implies the entrance of both of the amino acids into the bacterial cells rather than the selective exclusion of phenylserine by tyrosine.

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**References and Notes**

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## Bony Mechanism of Automatic Flexion and Extension in the Pigeon's Wing

In 1875 Garrod (*1*) noted that Bergmann, and later Strauss-Durckheim, had earlier described a mechanism for automatic flexion and extension in birds' wings. Garrod also credited Alix for an observation of the same thing, reported in 1874 in Paris. Coues (*2*) further described the device. The function was suggested from gross examination of dissections, and no experimental evidence was offered. Since the date of these papers, no further research on this apparatus has been published.

Briefly, the theory of operation was that the radius and ulna move in a fashion similar to a pair of "drawing parallels." The proximal end of the radius butts against the humerus, while the distal end is in contact with the fused carpals (scapholunar), which in turn are in contact with the anterior (digit II side) surface of the carpometacarpal articulation (Fig. 1). Humeroulnar flexion forces the radius distally, and the radius forces the manus into a flexed position,

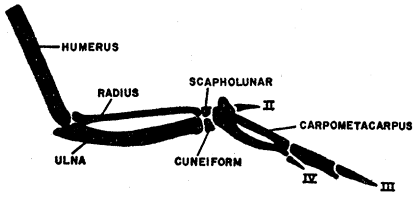


Fig. 1. Diagram of bones in pigeon's wing.

using the distal end of the ulna and the fused posterior carpal bones (cuneiform) as the fulcrum. Extension at the humeroulnar joint, through ligamentous attachments between the various bones, pulls the radius proximally and drags the manus into an extended position (with the same distal fulcrum as in flexion).

Experiments were devised to test these views of bone movement in the bird's wing. In two domestic pigeons, *Columba livia,* all flexors of the manus were cut; the birds were under general anesthesia, and it was a simple matter to cut all flexor tendons at the "wrist." After recovery from the operation, these birds flew in near-normal manner, but when they were perched the manus seemed to droop (extend) slightly. After 1 month, flight was so nearly normal that it was difficult to distinguish experimental birds from others in the room.

Cutting of the extensor tendons to the manus caused some further loss of delicate control, but the birds soon were able to fly. Eventually, these birds that had no muscular control of the hand flew easily, but they were always distinguishable in their awkwardness.

In two other pigeons, surgery was used to shorten the radius and prevent its simultaneous contact with the humerus and scapholunar. The left and right forewings were entered dorsally and a 5-millimeter section of the radius was sawed from the middle of the length of this bone. After insertion of a bone pin in the marrow cavity, the cut ends of the radius were pulled together with metal sutures. After recovery, the birds could fly, and wing-action was nearly the same as before the operation.

The flexor tendons for the hand were then cut. The birds could not fly, and the manus remained in an extended position at all times. Further surgery, to cut the extensor tendons, caused the hand to dangle, without visible control.

An attempt was subsequently made in each wing to remove the bone pin and insert a longer one to bring the radius back to its normal length. This was successful only on one side of one of the two birds; in the three other sites of bone shortening there had been too great a deposit of calcium on the outside of the bone to permit further surgery. The manus of the one wing in which the length of the radius had been restored was flexed and extended as the bird jumped from perch to perch.

At autopsy it was found that the radius had been shortened only 3 millimeters in one instance and 4 millimeters in the other two; normal length in the radii, as measured from x-ray photographs, was 48 millimeters.

It thus seems evident that flexion and extension of the manus can be accomplished without contraction of muscles extending to the manus. Flexion and extension at the humeroulnar joint, through action of the muscles of the upper arm, causes the same actions at the wrist.

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14 June 1957

## Liquid Scintillation Counting of $C^{14}$- and $H^3$-Labeled Amino Acids and Proteins

The method for tritiation of organic compounds described by Wilzbach (*1*) makes it possible to prepare with relative ease a wide variety of labeled compounds of high specific radioactivity. The applicability of the method in the labeling of intact protein molecules has now been demonstrated (*2*). Although the labeling procedure leads to degradation of a portion of each sample, a good yield of native protein of high specific radioactivity is obtained, as is evidenced by chromatographic homogeneity and retention of full enzymatic activity.

Effective use of these compounds in biological studies depends on the availability of a simple and sensitive system for radioassay. This is provided by the liquid scintillation counting technique. But, because the phosphors used in this system are nonpolar and are generally incorporated into an organic solvent (*3*), significant quantities of highly polar substances such as amino acids and proteins cannot be dissolved in the phosphor solution in unmodified form. A method for suspending such material in scintillating gels has been described for use in counting $C^{14}$-labeled compounds (*4*), but this is not applicable to the assay of tritium.

Passman *et al.* (*5*) have reported the use of a methanolic solution of the hydroxide form of a quaternary amine, Hyamine (*6*), to form a complex with $C^{14}O_2$ which is soluble in organic solvents, thus making it possible to dissolve up to 5 mmole of $C^{14}O_2$ in 35 ml of phosphor-containing toluene. As is described in the next paragraph, this same quaternary amine hydroxide (and several other quaternary amine hydroxides —for example, choline and tetraethyl ammonium hydroxide) can also complex amino acids and intact proteins. The phosphor dissolved in toluene can then be added to the methanolic solution of protein-amine complex, providing a clear