methanol. After the product had reacted, the liquid was cooled and left in the ice-box for several hours. A thick precipitate appeared. This was filtered, washed with methanol, and dried. The white-yellowish product obtained had $[\alpha]_D^{20} = +22°$; it was redissolved in methanol at 50–60° C and put into the ice-box again for several hours. The precipitate formed was then quite white and had $[\alpha]_D^{20} = +31.2°$. After a third recrystallization from diluted methanol, 10.2 g of white leaflets was obtained, with the following constants: $[\alpha]_D^{20} = +35.9°$; m.p. = 110–120° (with decomposition); *N* calc.: 3.87 (as bitartrate), found 3.78.

The free base D isopropyl nor-adrenaline, prepared from the bitartrate, gave: m.p. = 164–165° (with decomp.); *N* calc.: 6.66 found 7.07; $[\alpha]_D^{20} = +48.8°$ (as chlorohydrate).

The methanolic solutions containing the levoisomer were dried, the residue dissolved in H_2O, and the free base liberated with NH_4OH. After standing, the crystals were filtered and washed to obtain a white product that showed $[\alpha]_D^{20} = -42°$. They were dissolved in diluted HCl and precipitated again with NH_4OH; 3.6 g of white microcrystalline powder was obtained with the following constants: m.p. = 162–164° (with decomp.); *N* calc.: 6.66 found 7.07; $[\alpha]_D^{20} = -50°$ (as chlorohydrate).

For the pharmacological assays the D isomer as bitartrate, the L isomer as chlorohydrate, and the racemate as sulfate were employed. Equimolecular solutions were prepared and injected intravenously into tracheotomized dogs under light chloralose anesthesia. The vagi were cut and the arterial blood pressure was recorded with mercury manometers.

No tachyphylactic phenomena have been observed; a slight degree of racemization occurs for the solutions of L isomer: 7–12% after 3–5 months. For this reason only fresh solutions were used for the comparison of potency.

The percentage decrease in the blood pressure as a function of the amount administered in micrograms of free base per kilogram body weight has been taken into account for this comparison; furthermore, the duration of the effects observed, i.e., the time required for the blood pressure to revert to its initial value, has also been considered for a better evaluation of potency. This time, in seconds, multiplied by the percentage variation in the blood pressure, yields a quantity *A* (= amount of action) whose log is linearly related to the log dose, as clearly shown by the following regressions:

L isomer: $y = 3.35187 + 0.46124x$; $r = +0.954$; $P < 0.01$
Racemate: $y = 3.26252 + 0.38248x$; $r = +0.952$; $P \simeq 0.01$
D isomer: $y = 0.79125 + 1.16049x$; $r = +0.999$; $P = 0.001$

where $y = \log A$ and $x = \log$ dose.

Deviation from parallelism (shown by the numerical value of the regression coefficient) prevents any comparison of potency, unless the "amount of action," for which the comparison is made, has not been previously fixed. Taking for this amount arbitrary values we obtain:

Potency ratio	log *A*		
	3.0	3.5	4.0
Levo/racemate	1.336	2.095	3.28
Levo/dextro	317.5	87.45	24.6

Since racemates usually are pharmacologically half as potent as one of the optically active isomers (the latter being almost inactive or displaying different properties), the figures of the second row in the table seem to be more reliable.

The optical and pharmacological behaviors of isopropyl nor-adrenaline, as regards the differences between racemate and L isomer, also run quite parallel to those of epinephrine and nor-adrenaline, although the former are pressure-decreasing and the latter are pressure-increasing compounds. The D isomer, on the contrary, appears to be about 90 times less potent than the L isomer, whereas the values which have been put forward for the L/D potency ratio (as regards the blood pressure effects) are 12–15 (*5*) and 15–30 (*6*) for epinephrine; 25–33 (*7*) and 40 (*8*) for nor-adrenaline. These differences probably may be accounted for by differences in the racemization rates of these compounds in body fluids.

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Manuscript received March 25, 1953.

Accumulation of Phenylalanine by a Phenylalanineless Mutant of *Escherichia coli*

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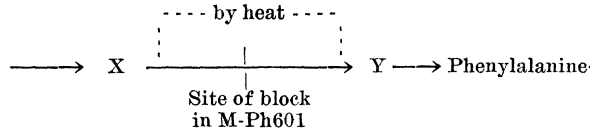
While studying aromatic biosynthesis with nutritionally deficient mutants of *Escherichia coli* (communior) isolated in our laboratories by the penicillin method of Davis (*1*), it was found that a mutant strain requiring phenylalanine for growth (M-Ph601) accumulates a considerable amount of that very substance, when cultivated in Davis' minimal medium (*2*) supplemented with a limited amount of the amino acid. It therefore resembles the similar mutant reported by Simmonds (*3*).

[1] The authors wish to thank Prof. Y. Kuwajima for his continuous encouragements for this study.

Following confirmation of the discovery that a substance that accumulates in the filtrates is responsible for the growth of the mutant, it was still uncertain whether that substance is phenylalanine or its precursor. Decisive evidence that the substance involved is actually phenylalanine was, however, obtained by paper chromatographic analysis of the filtrates together with four other unidentified amino acids (by ninhydrin test). The paper chromatogram showed the presence of phenylalanine. Furthermore, it was confirmed that M-Ph601 responds only to the eluate from the paper bearing the phenylalanine spot.

The conclusion that the phenylalanineless mutant accumulates phenylalanine itself may seem rather strange, since, according to current theory, the mutant exacting for a substance has a genetic block at a certain reaction in the biosynthesis of that substance. We could, however, find a possible explanation for this phenomenon by the following observations. M-Ph601 grown on the minimal medium-agar containing phenylalanine (10 μg/ml) was harvested and thoroughly washed with physiological saline. The washed cells were then suspended in the minimal medium (10^9 cells/ml) and shaken at 37° C. After 1 hr of shaking, the suspension was filtered with a Seitz filter. This filtrate (reinforced with the minimal medium) failed to support the growth of M-Ph601. But the same filtrate, if autoclaved (120° C, 10 min), acquired the ability to support the growth of the mutant.[2] It is obvious that this emergence of the growth-supporting activity is not due to the destruction of an inhibitor by heat, since M-Ph601 was found to be able to grow vigorously in the nonautoclaved Seitz filtrate if phenylalanine was added. Typical results of these shaking experiments are presented in Table 1.

These findings strongly suggest the following possibility. Shaking of the cells of the mutant with the minimal medium results in the accumulation of compound X, which is so unstable that it is readily convertible to compound Y, the product of the blocked reaction, by heat or other factors. Thus the filtrate, if not autoclaved, contains only compound X, substrate of the blocked reaction, and therefore is inactive. After autoclaving, however, compound Y is produced and thus the filtrate acquires the activity. Compound Y may not be phenylalanine itself but is considered to be its precursor, since paper chromatography of the autoclaved filtrate gave no spot corresponding to phenylalanine. These relations can be readily understood from the following scheme:



From these shaking experiments it may be possible to explain the results of the culture experiments as follows. In the culture experiments, too, the accumulation of compound X does take place as the first sequence. But, due to its instability, it is gradually converted to compound Y during the prolonged incubation. Phenylalanine thus produced may then be excreted into medium for reasons unknown as yet.

TABLE 1

RESPONSE OF M-PH601 TO THE SHAKING FILTRATES*

Filtrate shaken with		Treatment	Growth
1	M-Ph601	Autoclaved	0.31
1a	M-Ph601	Not autoclaved	0.05
1b	M-Ph601	Not autoclaved plus phenylalanine†	0.14
2	Wild type	Autoclaved	0.04
2a	Wild type	Not autoclaved	0.05

* All media were used after reinforcement with 1.5 volumes of minimal medium. Growth was measured after 24 hr of cultivation and expressed in the optical density units at 460 mμ.

† Phenylalanine (3 μg/ml) is added.

[2] With the cells of M-Ph601 harvested from the peptone-bouillon media, no such phenomena were observed.

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Manuscript received February 13, 1953.

Autocatalytic Growth of a Mutant Due to Accumulation of an Unstable Phenylalanine Precursor[1]

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Simmonds (*1*) observed that a phenylalanine auxotroph of *E. coli* accumulates a growth factor for itself. In the course of searching for intermediates between shikimic acid (*2*) and phenylalanine we encountered the same phenomenon and concluded, on the basis of paper chromatography with several solvents (using the ninhydrin reaction and bioautography for detection), that at least part of the accumulated active material is phenylalanine. This accumulation was surprising, since mutation-induced growth requirements are generally attributed to loss of activity of a single enzyme, and hence would not be expected to be accompanied by excessive synthesis and excretion of a product of the blocked reaction.

The phenylalanine accumulation became understandable when it was found (*3*) to be preceded by accumulation of an unstable substance, provisionally called prephenylalanine (PPA). The same explanation has been independently arrived at by Katagiri and Sato (*4*), whose manuscript I was privileged to

[1] Aided by a grant from the Rockefeller Foundation.