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Maintenance and Exchange of the Aromatic Amino Acid Pool in *Escherichia coli*

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The pool of phenylalanine, tyrosine, and tryptophan is formed in *Escherichia coli* K-12 by a general aromatic transport system [Michaelis constant (K_m) for each amino acid approximately 5×10^{-7} M] and three further transport systems each specific for a single aromatic amino acid (K_m for each amino acid approximately 2×10^{-6} M, reference 3). When the external concentration of a particular aromatic amino acid is saturating for both classes of transport system, the free amino acid pool is supplied with external amino acid by both systems. Blocking the general transport system reduces the pool size by 80 to 90% but does not interfere with the supply of the amino acid to protein synthesis. If, however, the external concentration is too low to saturate specific transport, blocking general transport inhibits the incorporation of external amino acid into protein by about 75%. It is concluded that the amino acids transported by either class of transport system can be used for protein synthesis. Dilution of the external amino acid or deprivation of energy causes efflux of the aromatic pool. These results and rapid exchange observed between pool amino acid and external amino acids indicate that the aromatic pool circulates rapidly between the inside and the outside of the cell. Evidence is presented that this exchange is mediated by the aromatic transport systems. Mutation of *aroP* (a gene specifying general aromatic transport) inhibits exit and exchange of the small pool generated by specific transport. These findings are discussed and a simple physiological model of aromatic pool formation, and exchange, is proposed.

Each of the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, is taken up in *Escherichia coli* K-12 and *Salmonella typhimurium* by two classes of transport systems (1, 3). Firstly, a general transport system, specified by the gene *aroP*, transports all three aromatic amino acids with Michaelis constants (K_m) for each about 5×10^{-7} M. High concentrations (of the order 10^{-4} M) of histidine, cysteine, methionine, leucine, aspartic acid, and alanine also have affinity for this system. The second class of transport system is much more specific for the aromatic amino acid in question. The K_m of each specific uptake is about 2×10^{-6} M.

Britten and McClure (2) have reviewed the pool formation and exchange properties of several amino acids (chiefly proline and valine) in *E. coli*. Little is known, however, of the fate of the aromatic amino acids taken up into the cell by the general and specific aromatic transport systems. The experiments described in the present paper demonstrate that the amino acid pools formed in *E. coli* K-12 by both classes of aromatic transport system are available for protein synthesis and that they circulate rapidly between the inside and outside of the cell through the transport systems. Thus, exchange between internal and external amino acid is rapid, and pool formation and maintenance is a dynamic, energy-requiring process, dependent upon continued active transport.

A brief summary of this work has appeared previously (4).

MATERIALS AND METHODS

Organisms. Wild-type *E. coli* K-12 (W1485) and KB3100, an *aroP*$^-$ derivative of W1485, have been described previously (3).

Media and growth conditions. Cells were grown in medium A of Davis and Mingioli (6) or L-broth of Luria and Burrous (11) at 37 C on gyratory shakers (New Brunswick Scientific Co., New Brunswick, N.J.; 200 rev/min).

Maintenance and preservation of stock cultures. Stocks were preserved on anhydrous silica gel. A modification of the method of Perkins (13) described for Neurospora was followed. A sample (1.0 ml) of overnight culture of *E. coli* in L-broth was mixed with an equal volume of sterile skim milk (10%, w/v); then 1-ml

samples of the mixture were uniformly distributed over chilled, anhydrous silica gel (12 to 20 mesh) in duplicate, plugged test tubes (13 by 100 mm). The tubes were half-filled with gel. Tests on 63 different stocks at times ranging from 10 to 36 months after preservation have indicated 100% viability. Stocks may be regenerated by inoculating 10 to 20 grains of silica gel into L-broth (10 ml) and shaking at 37 C. A similar technique has independently been developed by Grivell and Jackson (8) for *E. coli* and several other microorganisms. The method combines the virtues of a stable, dormant stock with ready availability and the convenience of re-use of the same stock (there being a large number of impregnated silica gel granules in each stock tube).

Chemicals. L-Tyrosine-*3,5-*^3H (44.6 Ci/mmole) and generally labeled ^{3}H-L-phenylalanine (6.3 Ci/mmole) were purchased from New England Nuclear Corporation, Boston, Mass. Generally labeled ^{3}H-L-tryptophan (3.4 Ci/mmole) was purchased from The Radiochemical Centre, Amersham, England. Other chemicals used in this work have been described previously (3).

Measurement of amino acid transport. The techniques for measuring amino acid transport in growing cells and under glucose-starved conditions have been described previously (3). Unless otherwise stated, the cell density in all assays was 0.067 mg dry weight/ml. Under the conditions used, the counting efficiency [in a LS250 spectrometer (Beckman Instruments, Inc., Fullerton, Calif.)] was approximately 85% for ^{14}C-labeled amino acids and 30% for ^{3}H.

Extraction of the trichloroacetic acid-soluble pool. This technique has been described previously (3).

P1-mediated transduction. This technique has been described previously (5).

RESULTS

Effect of external amino acid concentration on pool composition and protein synthesis. Figure 1 shows the time course of ^{14}C-tyrosine uptake by growing W1485 and KB3100 in medium A at 20 C. If the external tyrosine concentration is 10^{-5} M (this is sufficient to saturate both the general and tyrosine-specific transport systems), then the trichloroacetic acid-soluble pool of ^{14}C-tyrosine is derived from both systems in W1485 (Fig. 1a). It has previously been shown (3) that 92% of the ^{14}C label appearing in this pool (after 2 min of incubation at 37 C) is free tyrosine.

If general transport of ^{14}C-tyrosine is blocked by mutation of *aroP* (as in Fig. 1c) or by competition by a 100-fold excess of unlabeled tryptophan (Fig. 1a), then the ^{14}C-tyrosine pool size is reduced by about 80% (from 2.8 to 0.6 μmole/g dry weight of cells). This indicates that at least this proportion of the pool is generated by general transport in minimal medium. In the absence of general transport the tyrosine-specific transport system is unable to maintain this large pool.

Although the pool size of free ^{14}C-tyrosine is substantially reduced when general transport is blocked, the incorporation of exogenous tyrosine into cold trichloroacetic acid-insoluble material (predominantly protein) is not interrupted (Fig. 1a, c). Thus, when the external tyrosine concentration is 10^{-5} M, the general transport system is not needed to supply exogenous tyrosine for protein synthesis. This function is assured by the tyrosine-specific transport system. Since a significant concentration gradient is maintained by this system (about 20:1 over initial external tyrosine concentration), it is unlikely that diffusion into the cell plays a major role in supplying tyrosine for protein synthesis under these conditions.

If the external ^{14}C-tyrosine concentration is only 10^{-7} M so that the tyrosine-specific transport system with a K_m of about 2×10^{-6} M cannot efficiently operate, then almost the entire trichloroacetic acid-soluble ^{14}C-tyrosine pool in W1485 is derived from general transport (Fig. 1b). This renders incorporation of exogenous tyrosine into protein very sensitive to competition by exogenous tryptophan. Addition of 10^{-5} M tryptophan to the medium inhibits ^{14}C-tyrosine incorporation into protein by about 75% (measured at 4 min). The residual ^{14}C-tyrosine uptake in the presence of tryptophan can be accounted for by diffusion.

At 10^{-7} M little active uptake of exogenous tyrosine is evident in the *aroP*$^-$, general transport-negative mutant KB3100 (Fig. 1d). The very small ^{14}C-tyrosine pool formed appears equivalent to the external tyrosine concentration and may, therefore, be largely accounted for by diffusion with only a low level of specific transport activity apparent. At 10^{-5} M almost all ^{14}C-tyrosine uptake in this mutant occurs via the tyrosine-specific transport system and is relatively insensitive to tryptophan (Fig. 1c).

These results demonstrate that the ^{14}C-tyrosine transported by both general and specific classes of transport system is available for protein synthesis.

Effect of dilution on pool maintenance. If cells with an established tyrosine pool are diluted so that the external tyrosine concentration falls below that which can be efficiently transported into the cell, there is a net loss of the pool. In Fig. 2a is shown the trichloroacetic acid-soluble pool formed in wild-type cells from ^{14}C-tyrosine (5×10^{-6} M, initial external concentration) by both the general and tyrosine-specific transport systems. When these cells are diluted 1:500, pool efflux occurs with a half-time of ca. 5 min. When the dilution factor is only 1:50, the half-time of efflux is 3 min.

The half-times of efflux quoted here and below are intended merely as physiological parameters for describing the phenomena observed in different strains under particular experimental con-

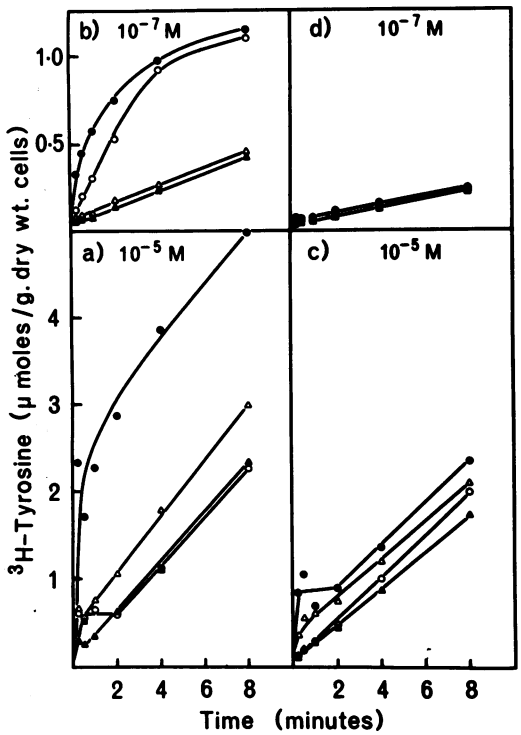

FIG. 1. *^{3}H-tyrosine (50 mCi/mmole) uptake in medium A at 20 C. Sample volume, 1.0 ml; total assay volume, 12.5 ml. (a) W1485, initial ^{3}H-tyrosine concentration, 10^{-5} M; (b) W1485, initial ^{3}H-tyrosine concentration, 10^{-7} M; (c) KB3100, initial ^{3}H-tyrosine concentration, 10^{-5} M; (d) KB3100, initial ^{3}H-tyrosine concentration, 10^{-7} M. Uptake into: whole cells (●); trichloroacetic acid-insoluble material (○); whole cells in presence of unlabeled tryptophan, 10^{-3} M (a and c); 10^{-5} M (b and d) (△); trichloroacetic acid-insoluble material in presence of unlabeled tryptophan (▲).*

ditions. It is not intended that the rates of efflux reported should be regarded as basic properties of the mechanism of pool maintenance. The rate of efflux no doubt would be affected by several factors, including the activity of the transport systems and the internal and external concentrations of the particular amino acid.

In Fig. 2b the uptake of ^{14}C-tyrosine by the general transport system is blocked by tryptophan (10^{-3} M) so that the pool is generated by tyrosine-specific transport alone. Efflux occurs on dilution (1:500 and 1:50) with a half-time of ca. 1 min. Similarly, in Fig. 2c, uptake in the *aroP*$^-$ mutant KB3100 is entirely via the tyrosine-specific system. In this strain, however, the pool loss on dilution is slower (half-times of loss on 1:500 and 1:50 dilution are 4 and 5 min, respectively). This slow loss is largely the result of removal of pool ^{14}C-tyrosine into protein rather than efflux (*unpublished results*). Thus, the *aroP*$^-$ allele affects not only general aromatic transport but also affects exit of the pool formed by tyrosine-specific transport.

In Fig. 2a efflux is incomplete after a 1:500

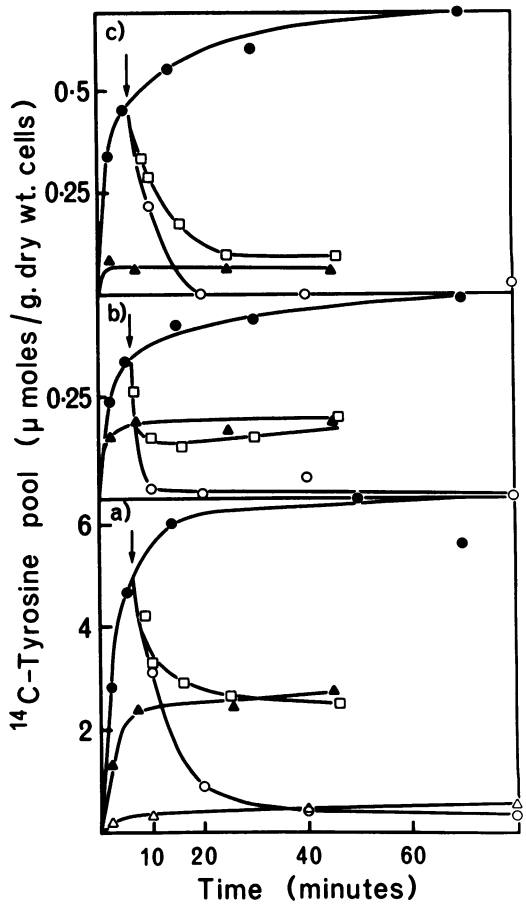

FIG. 2. *Effect of dilution on maintenance of the ^{14}C-tyrosine pool in glucose-starved W1485 and KB3100 at 20 C. Undiluted control: initial ^{14}C-tyrosine concentration, 5×10^{-6} M (20 mCi/mmole); cell density, 0.067 mg dry weight/ml; assay volume, 20 ml. Sample volume: 1.0 ml (control), 25 ml (1:50 dilution), 250 ml (1:500 dilution). The diluted samples were filtered through larger membrane filters (47-mm diameter) than the undiluted control samples (25-mm diameter). This minimized the time differences in filtration of samples so that these did not significantly influence the results. At 6 min (arrow), 8-ml volumes were removed from the control flask and diluted with medium A minus glucose. Pool formation and maintenance in: (a) W1485, (b) W1485 in presence of unlabeled tryptophan (10^{-3} M), (c) KB3100. Trichloroacetic acid-soluble ^{14}C-tyrosine in: undiluted control cells (●), cells diluted 1:500 at 6 min (○), cells diluted 1:50 at 6 min (□). Pool formation was also followed in: cells diluted 1:500 at 0 min (△) and cells diluted 1:50 at 0 min (▲).*

dilution (to 10^{-8} M external tyrosine). Most of the remaining pool, however, can be accounted for by continuing general transport activity. The rate of uptake is the limiting factor which determines the extent of efflux under these conditions. This is shown if only a 1:50 dilution (to 10^{-7} M external tyrosine) is made. Then the residual pool after efflux is quite large (2.5 μmoles/g dry weight of cells) in wild-type cells where general transport is active (Fig. 2a), whereas in Fig. 2b and c where general transport is blocked the residual pool after 1:50 dilution is less than 10% of this size, reflecting the lower affinity of the specific transport system for tyrosine. When the dilution factor is 500, no detectable pool remains in these cells (Fig. 2b and c) nor is there any detectable pool forming activity. Closely analogous results to those described in this section for tyrosine were obtained for exit of the phenylalanine pool upon dilution in W1485 and KB3100 (*unpublished results*).

It is concluded from these observations, firstly, that the aromatic pool is labile and rapidly lost from the cell when the external amino acid concentration falls below that required for efficient active uptake. Pool maintenance, therefore, requires continuous active transport. Secondly, it is concluded that the *aroP*$^-$ mutant KB3100 is defective, not only in general aromatic transport, but also in exit of the pool formed by the tyrosine- and phenylalanine-specific transport systems. This conclusion implies that exit does not occur simply by passive diffusion but is mediated by a system specified, at least in part, by the *aroP* gene.

Energy requirements for pool formation and maintenance. A further experiment (*see* Fig. 3) which illustrates the dynamic nature of the aromatic pool shows that energy is required both for formation and maintenance of the pool. In Fig. 3a, ^{14}C-tyrosine pool formation by the general and tyrosine-specific transport systems was allowed to proceed in wild-type W1485 for 4 min at 37 C, and then energy production was blocked with a combination of azide and iodoacetamide [after Kay and Gronlund (9)]. This caused almost complete efflux of tyrosine with an apparent half-time for exit of 5 min. Similar results were observed under the influence of 2,4 dinitrophenol (10^{-3} M). A preformed ^{14}C-phenylalanine pool was similarly lost when 2,4 dinitrophenol (10^{-3} M) was added to the medium. In Fig. 3a, ^{14}C-tyrosine pool formation (measured at 2 min) was 75% inhibited by azide and iodoacetamide. Inhibition rose to 95% at 10 min. These metabolic inhibitors had similar effects on ^{14}C-tyrosine pool formation and maintenance in wild-type cells where general transport of tyrosine was blocked by excess tryptophan, thus leaving only tyrosine-specific transport active (Fig. 3b). Under these conditions, however, the half-time of pool exit was much shorter (2 min). Pool formation by the tyrosine-specific transport system was similarly affected in the *aroP*$^-$ mutant KB3100 (Fig. 3c), but the pool exhibited a significantly longer half-time of efflux (7 min) under the influence of azide and iodoacetamide.

It is concluded that both aromatic pool formation and maintenance require energy. The possibility cannot be excluded, however, that since the measurement of pool formation in these experiments required pool maintenance, the effect of the inhibitors on transport is a consequence of their effect on pool maintenance. It is also concluded that mutation of the gene specifying general aromatic transport (*aroP*) slows the exit of the tyrosine pool formed by the tyrosine-specific transport system.

Exchange of the aromatic pool with external amino acids in wild-type (W1485). The preformed, saturated, aromatic pool exchanges rapidly with external aromatic amino acids. The half-time for exchange is 20 sec at 37 C. This is shown in Fig. 4a where addition of unlabeled tryptophan (2×10^{-4} M) displaces the pool formed from 10^{-5} M ^{14}C-tyrosine in wild-type cells in medium A *minus* glucose. Similar results (*unpublished*) were obtained in medium A except that protein synthesis is more rapid. Unlabeled tyrosine similarly displaces the preformed ^{14}C-tyrosine pool (*not shown*). Under the conditions shown in Fig. 4a, about 80% of the total aromatic pool is generated by general transport and 20% by specific transport (3). Figure 4b shows displacement of a preformed ^{14}C-phenylalanine pool by unlabeled tryptophan (unlabeled phenylalanine caused a similar pool displacement), whereas Fig. 4c shows displacement of a preformed ^{14}C-tryptophan pool by tyrosine.

That the displacements observed are caused by actual exchange of the aromatic pool is indicated by double-labeling experiments as shown in Fig. 5. In Fig. 5a the ^{14}C-tyrosine pool is formed and exits when a 20-fold concentration of exogenous ^{3}H-tyrosine or ^{3}H-tryptophan is added. The simultaneous entry of the latter amino acid is readily followed by measuring the uptake of ^{3}H counts by the cells. When ^{3}H-tyrosine is added, exchange is not strictly mole for mole as might have been expected. In fact, ca. 1.3 moles of ^{3}H-tyrosine enter for every mole of ^{14}C-tyrosine that exits. This may be explained by the high concentration of ^{3}H-tyrosine (10^{-4} M) compared with ^{14}C-tyrosine (5×10^{-6} M). The former concentration is saturating for both general and specific transport of tyrosine, thus ensuring a greater up-

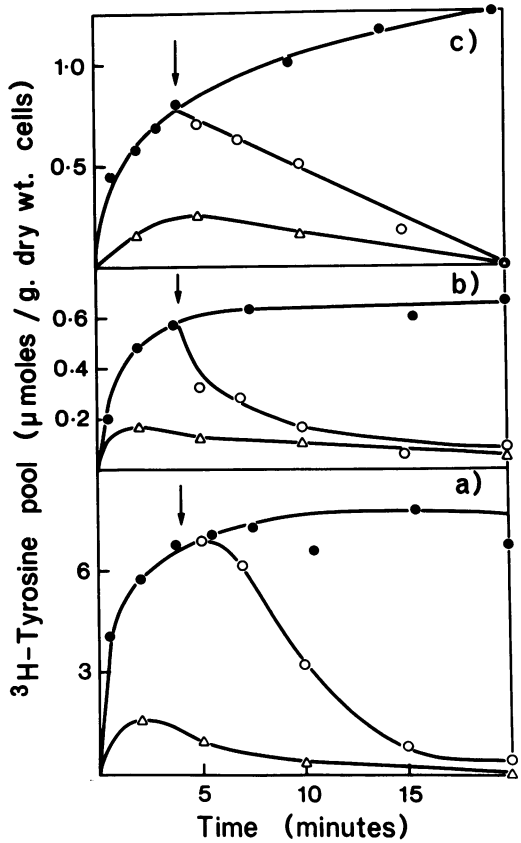

FIG. 3. *Effect of sodium azide (3×10^{-2} M) plus iodoacetamide (10^{-3} M) on ^{3}H-tyrosine pool formation and maintenance in glucose-starved W1485 and KB3100 at 37 C. Initial ^{3}H-tyrosine concentration, 5×10^{-6} M, 100 mCi/mmole; sample volume, 2 ml; total volume (control), 60 ml. (a) W1485, (b) W1485 in presence of unlabeled tryptophan (10^{-3} M), (c) KB3100. Trichloroacetic acid-soluble ^{3}H-tyrosine in: control (●), cells transferred from control at 4 min (arrow) to a flask containing azide plus iodoacetamide (○), cells incubated with azide plus iodoacetamide from 0 min (△).*

take than the latter concentration which is not completely saturating for tyrosine-specific transport (K_m, ca. 2×10^{-6} M). When the external amino acid is ^{3}H-tryptophan, only 1 mole of ^{3}H-tryptophan enters for every 2 moles of ^{14}C-tyrosine that exit. This may be explained either by the fact that the pool formed by wild-type cells from exogenous tryptophan is only about half the size of the pool formed from tyrosine (*see* Fig. 4a and c) or by a slightly lower affinity of the transport systems for tryptophan compared with tyrosine. Figure 5b shows the exchange in wild-type cells between a preformed ^{14}C-phenylalanine pool and external ^{3}H-tryptophan. It can be seen again that exchange is not mole for mole (more tryptophan enters than phenylalanine exits). This may be explained by the higher tryptophan concentration and by the fact that a larger pool is formed from tryptophan under these conditions than from phenylalanine (*see* Fig. 4b and c) or by a slightly

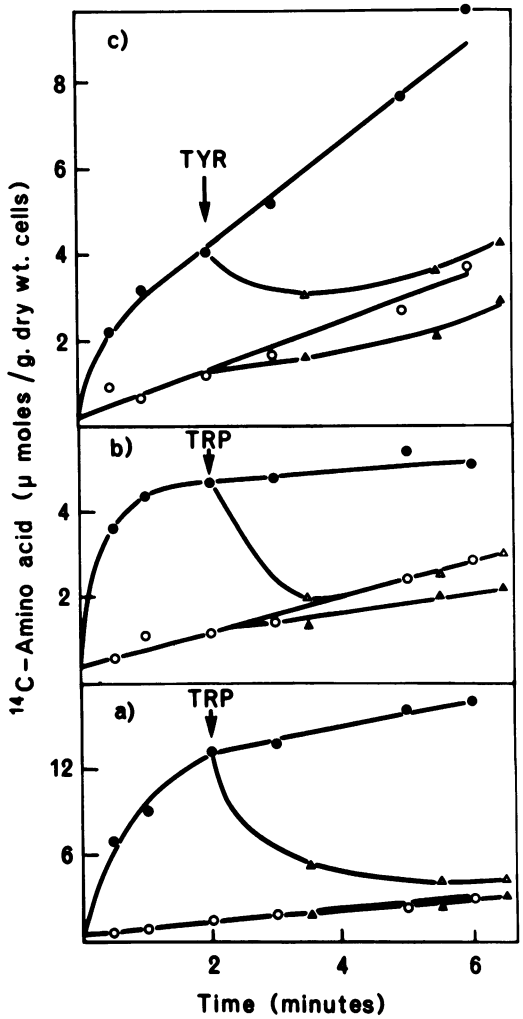

FIG. 4. *Displacement by unlabeled external aromatic amino acids (2×10^{-4} M) of the preformed ^{14}C-aromatic amino acid pool in glucose-starved W1485 at 37 C. Initial ^{14}C-amino acid concentration, 10^{-5} M, 5 mCi/mmole. Sample volume, 1.0 ml; total volume, 20 ml. Cells were transferred at 2 min (arrow) from the control to a flask containing unlabeled amino acid. Displacement of: (a) ^{14}C-tyrosine by unlabeled tryptophan (TRP), (b) ^{14}C-phenylalanine by unlabeled tryptophan, (c) ^{14}C-tryptophan by unlabeled tyrosine (TYR). ^{14}C-amino acid in: whole cells, control (●), trichloroacetic acid-insoluble material (○), whole cells after exposure to unlabeled amino acid (△), trichloroacetic acid-insoluble material after exposure to unlabeled amino acid (▲).*

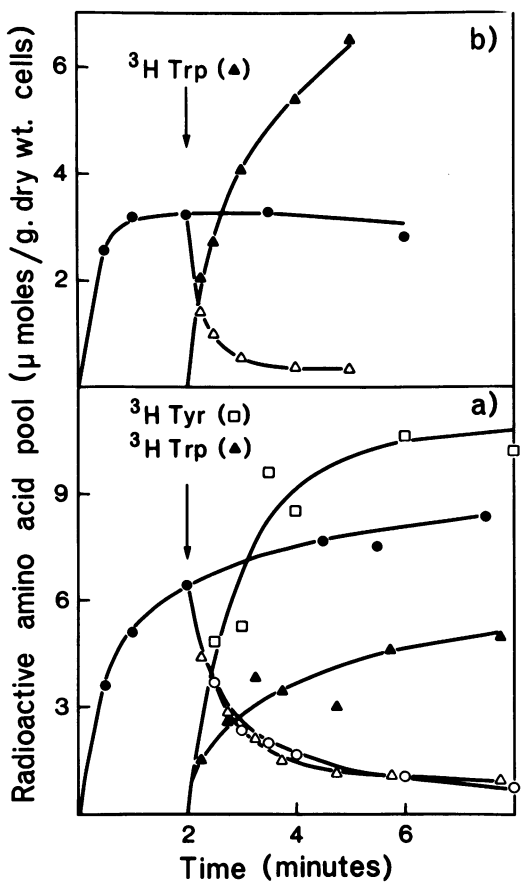

FIG. 5. *Exchange at 37 C in glucose-starved W1485 of: (a) ^{14}C-tyrosine pool (initial ^{14}C-tyrosine concentration, 5 × 10^{-6} M, 20 mCi/mmole) with external ^{3}H-tyrosine and ^{3}H-tryptophan (both 10^{-4} M, 25 mCi/mmole) and (b) a ^{14}C-phenylalanine pool (initial ^{14}C-phenylalanine concentration, 10^{-5} M, 20 mCi/mmole) with external ^{3}H-tryptophan (2 × 10^{-4} M, 25 mCi/mmole). Sample volume, 2 ml; total volume per assay, 90 ml (a) and 40 ml (b). Cells were transferred at 2 min (arrow) from the control to flasks containing ^{3}H-amino acid. Trichloroacetic acid-soluble ^{14}C-amino acid in: control (●), cells exposed to ^{3}H-tyrosine (○), cells exposed to ^{3}H-tryptophan (△). Trichloroacetic acid-soluble ^{3}H-tyrosine (□), ^{3}H-tryptophan (▲).*

lower affinity of the transport systems for phenylalanine compared with tryptophan.

In addition to the aromatic amino acids, certain other amino acids and their analogues displace (presumably by exchange) the preformed ^{14}C-tyrosine pool. These are listed in Table 1. Aspartic acid, serine, lysine, glycine, asparagine, arginine, proline, cystine, glutamic acid, isoleucine, glutamine, and thiolhistidine caused less than 20% loss of the tyrosine pool. Those amino acids and analogues which displace the tyrosine pool have previously been shown to compete with tyrosine for uptake by the general aromatic transport system (3). Valine is an exception to this rule, however. On reexamination of its effect on general transport of ^{14}C-tyrosine, it was found to be a marginal inhibitor (20%). It is presumed that all of the amino acids and analogues listed in Table 1 are transported by the general transport system, but this has been directly shown only for phenylalanine, tyrosine, and tryptophan (3).

It is concluded from these experiments that the

TABLE 1. *Displacement of a preexisting tyrosine pool by external amino acids and analogues in wild-type E. coli (W1485)*[a]

Additions[b]	% ^{3}H-tyrosine pool displaced[c]
None	0 (12.0)
Tyrosine	75
Phenylalanine	70
Tryptophan	73
Histidine	46
Cysteine	35
Valine	34
Leucine	26
Methionine	20
(DL) PFP	84
TA	81
(DL) PAP	78
(DL) 4MT	82
(DL) 5MT	73
(DL) 6MT	78
E-Cys	48
Me-His	71
A-Leu	40

[a] The time course of formation of the trichloroacetic acid-soluble ^{3}H-tyrosine pool was followed by sampling 2-ml volumes at 3-min intervals from a control flask containing glucose-starved cells (total volume, 80 ml) at 37 C (*see* Materials and Methods). Initial ^{3}H-tyrosine concentration was 5 × 10^{-6} M (50 mCi/mmole).

[b] Unless otherwise indicated all amino acids and analogues were the L-isomers. The concentration of all additions was 10^{-3} M (with respect to L-isomer). PFP, *p*-fluorophenylalanine; TA, β-2-thienylalanine; MT, methyltryptophan; E-Cys, *S*-ethylcysteine; Me-His, 1-methyl-histidine; A-Leu, 4-azaleucine.

[c] Commencing at 5 min after pool formation had begun, 5-ml volumes of cells were transferred at 3-min intervals from the control flask into flasks containing each of the additions indicated. These were shaken for 2 min at 37 C, and then 2-ml samples were taken and the trichloroacetic acid-soluble ^{3}H-tyrosine pool measured as described in Materials and Methods. These assays were compared with the corresponding assays from the unsupplemented control which was continuously sampled. Thus, the displacements shown are those occurring over a 2-min time range. The number in parentheses indicates the mean pool size in micromoles per gram dry weight of cells in the unsupplemented control.

pool exchange process in wild-type cells and the general transport system have similar substrate specificities.

Exchange of the pool formed by specific aromatic transport. A further link between the active transport systems and pool exchange is indicated by the altered properties of pool exchange in the general transport-negative mutant KB3100. Firstly, the specificity of exchange is altered. It can be seen in Fig. 6a that the preformed ^{14}C-tyrosine pool does not exchange with external tryptophan. In these cells the small tyrosine pool is generated by the tyrosine-specific transport system alone, and it will only exchange with external tyrosine (*see* Fig. 7b). A similar specificity of exchange is seen in wild-type cells when general transport of tyrosine is blocked with tryptophan. This is shown in Fig. 7a where the ^{14}C-tyrosine pool formed by tyrosine-specific transport is exchanged with external ^{3}H-tyrosine. The exchange is not strictly mole for mole. In fact, in

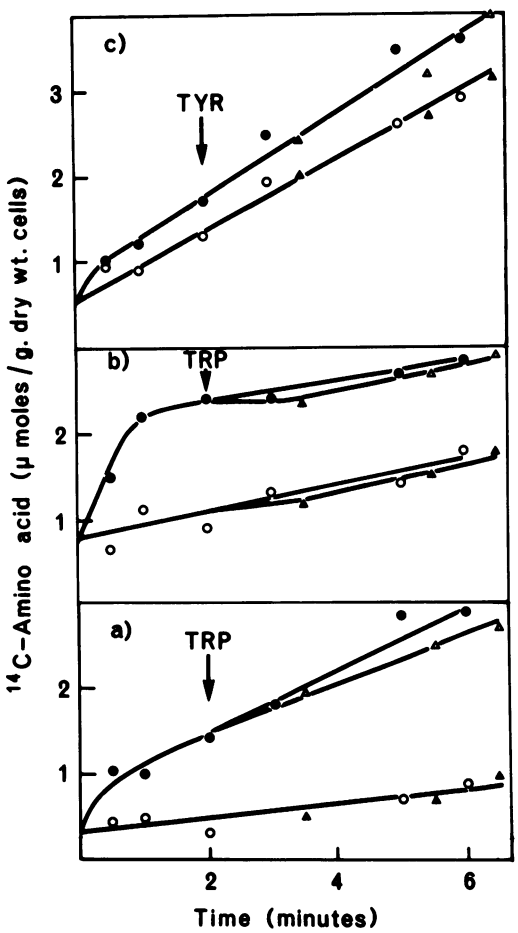

FIG. 6. *Effect of unlabeled, external aromatic amino acids (2 × 10^{-4} M) on the ^{14}C-aromatic amino acid pool in glucose-starved KB3100 at 37 C. Effect of: (a) unlabeled, external tryptophan (TRP) on a ^{14}C-tyrosine pool, (b) unlabeled, external tryptophan on a ^{14}C-phenylalanine pool, (c) unlabeled, external tyrosine (TYR) on a ^{14}C-tryptophan pool. ^{14}C-amino acid in: whole cells, control (●), trichloroacetic acid-insoluble material (○), whole cells after exposure to unlabeled amino acid (△), trichloroacetic acid-insoluble material after exposure to unlabeled amino acid (▲). Other experimental details as in Fig. 4.*

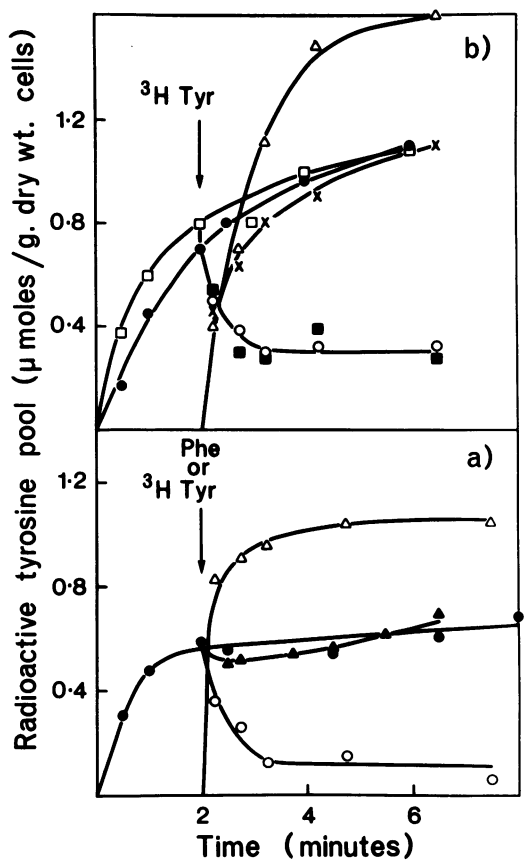

FIG. 7. *Exchange of the ^{14}C-tyrosine pool at 37 C in glucose-starved cells. Initial ^{14}C-tyrosine concentration, 5 × 10^{-6} M, 40 mCi/mmole. ^{3}H-tyrosine concentration, 5 × 10^{-5} M, 40 mCi/mmole. (a) W1485 in presence of unlabeled tryptophan (10^{-3} M). Trichloroacetic acid-soluble ^{14}C-tyrosine in: control (●), cells exposed to ^{3}H-tyrosine at 2 min (arrow) (○), cells exposed to unlabeled phenylalanine (5 × 10^{-5} M) at 2 min (▲). Trichloroacetic acid-soluble ^{3}H-tyrosine (△). (b) KB3100 in presence and absence of unlabeled tryptophan (10^{-3} M). Trichloroacetic acid-soluble ^{14}C-tyrosine in: control (●), control in presence of unlabeled tryptophan (□), cells exposed to ^{3}H-tyrosine at 2 min (arrow) (○), cells exposed to ^{3}H-tyrosine in presence of unlabeled tryptophan (■). Trichloroacetic acid-soluble ^{3}H-tyrosine taken up in absence of unlabeled tyrptophan (△) and in presence of unlabeled tryptophan (×). Experimental procedure as in Fig. 5.*

W1485 (Fig. 7a) 2 moles of ^{3}H-tyrosine enter for every mole of ^{14}C-tyrosine that leaves. In KB3100, 4 moles of ^{3}H-tyrosine enter for each mole of ^{14}C-tyrosine that leaves (Fig. 7b). One probable explanation of this nonequivalence is that ^{3}H-tyrosine is added at a higher and more saturating concentration (5×10^{-5} M) for tyrosine-specific transport than ^{14}C-tyrosine (5×10^{-6} M). In the case of KB3100, another factor is residual general transport of ^{3}H-tyrosine (since the concentration is so high). If this uptake is blocked with tryptophan (10^{-3} M), the ratio of ^{3}H-tyrosine entry to ^{14}C-tyrosine exit becomes 2:1 (cf. W1485). The specificity of this exchange in W1485 is indicated in Fig. 7a by the failure of the tyrosine pool to be displaced by unlabeled, external phenylalanine (contrast the specificity of tyrosine pool exchange in W1485 shown in Table 1). Similarly, Fig. 6b and c indicate that in KB3100 there is no displacement of the ^{14}C-phenylalanine pool by external tryptophan and that there is no displacement of the ^{14}C-tryptophan pool by external tyrosine (contrast W1485 in Fig. 4b and c).

The second major difference in the exchange properties of KB3100 and wild-type W1485 is shown in Fig. 8. In wild-type cells in which general transport is blocked, the small tyrosine pool formed by tyrosine-specific transport is displaced rapidly by unlabeled external tyrosine at various concentrations until equilibrium is reached (Fig. 8a, b, and c). In KB3100, however, in order to demonstrate displacement of the pool formed by tyrosine-specific transport, it is necessary to strictly control the external tyrosine concentration. In contrast to W1485, displacement in KB3100 by unlabeled tyrosine at external concentrations of 2×10^{-5} M, 5×10^{-5} M, and 2×10^{-4} M is only partial (Fig. 8a, b, and c). Similar results have been obtained with respect to the phenylalanine pool formed in KB3100 (*see* Fig. 9). Here the velocities of phenylalanine pool formation and displacement were observed to be slower in KB3100 than in W1485. The presence of excess unlabeled tryptophan in the KB3100 experiments (to simulate the wild-type conditions in Fig. 8 and 9) did not alter the displacement characteristics of KB3100. The tryptophan pool formed by tryptophan-specific transport is too small to reliably perform similar experiments.

One possible explanation of the behavior of KB3100 is that it is a double mutant with a genetic defect in general transport (*aroP*$^{-}$) and in another element common to all three specific aromatic transport systems. This is unlikely since the transport and exchange defects of KB3100 are both cotransduced (frequency 30%) with the *leu* locus. Testing of transport and exchange properties (with respect to tyrosine) in four *leu*$^{+}$ *aroP*$^{-}$ transductants from the cross P1 (KB3100) $\times$ W1485 *leu*$^{-}$ (*leu*$^{+}$ selected, *aroP*$^{-}$ unselected) has yielded identical results to those obtained with the mutant KB3100. Unless the hypothetical locus affecting specific transport is very closely linked to *aroP*$^{-}$, it seems unlikely that KB3100 is a double mutant.

K_m values of the three specific aromatic transport systems in KB3100 and W1485 were calculated from the Lineweaver-Burk plots shown in Fig. 10. Both strains have similar K_m values (all are in the range $2\text{–}3 \times 10^{-6}$ M). Thus, although general aromatic transport is almost completely absent, the specific transport systems in KB3100 are not altered in their affinities for the aromatic amino acids. The slopes of the plots for KB3100, however, are greater than those for specific transport in W1485. This indicates that the initial velocities and maximal velocities for these transport systems are lower in KB3100 than in W1485. The reason for this alteration is unknown.

It is concluded from the observations on pool exchange through both the general and specific aromatic transport systems that, in wild-type cells, the aromatic pool circulates rapidly between the inside and outside of the cell. The transport and exchange properties of KB3100 suggest either that an intact general transport system is required for exchange of the pool formed by the specific aromatic transport systems (*see* below) or that the general and specific transport systems share a macromolecular element specified by the gene, *aroP*. The finding that the K_m values of the specific transport systems in KB3100 are unaltered, whereas general transport is almost completely absent, favors the former hypothesis. On the other hand, the altered velocities of specific transport in KB3100 favor the latter hypothesis.

DISCUSSION

The lability of the pool when active transport is interrupted by dilution of the external amino acid or by energy poisons indicates that the pool is dynamic and dependent on continued active transport. The rapid exchange between the aromatic pool and external amino acids indicates that the pool is rapidly circulating between the inside and outside of the cell. A simplified working model for aromatic pool formation and exchange which unites the data so far obtained is shown in Fig. 11 for the case of labeled tyrosine (Tyr*). This model is adapted from a general model for active transport described by Pardee (12). G represents a hypothetical, macromolecular element of the general aromatic transport system and is specified, at least in part, by *aroP*.

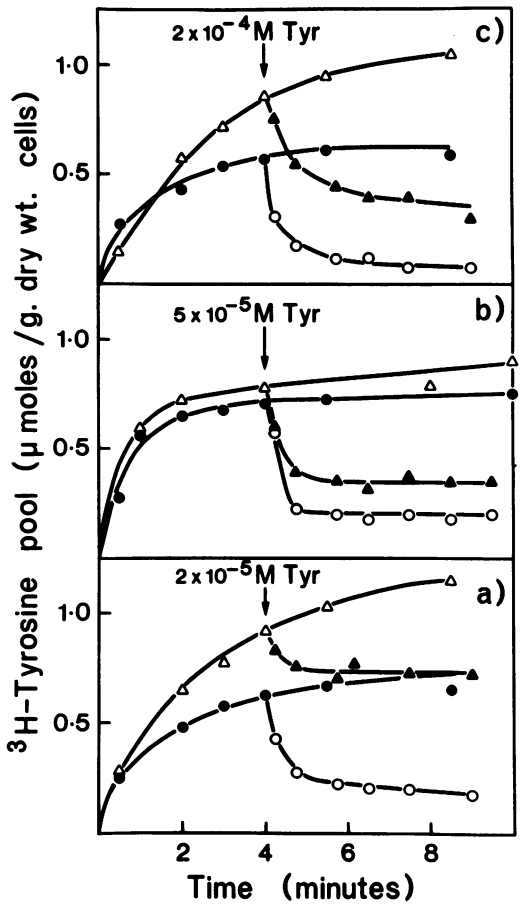

FIG. 8. *Displacement by external tyrosine of the ³H-tyrosine pool formed by the tyrosine-specific transport system in glucose-starved W1485 and KB3100 at 37 C. Initial ³H-tyrosine concentration, 5 × 10⁻⁶ M, 50 mCi/mmole. Unlabeled tryptophan (10⁻³ M) was present in the assays of W1485 to block ³H-tyrosine pool formation by the general aromatic transport system. Sample volume, 2 ml; total assay volume, 50 ml. Cells were transferred from the control to a flask containing unlabeled tyrosine at 4 min (arrow). Displacement by: (a) 2 × 10⁻⁵ M tyrosine, (b) 5 × 10⁻⁵ M tyrosine, (c) 2 × 10⁻⁴ M tyrosine. Trichloroacetic acid-soluble ³H-tyrosine in: W1485 control (●), W1485 exposed to unlabeled, external tyrosine at 4 min (○), KB3100 control (△), KB3100 exposed to unlabeled, external tyrosine at 4 min (▲).*

It has high affinity for phenylalanine (Phe), tyrosine, and tryptophan (Trp), and lower affinity for cysteine, histidine, leucine, methionine, valine, alanine, and aspartic acid (*see* reference 3). S represents a hypothetical, macromolecular element of the tyrosine-specific transport system. It has high affinity for tyrosine and lower affinity for phenylalanine (*see* reference 3). The relationship of G and S to each other and to the cell membrane is not known. Which reactions in the transport sequence require energy (~P) and which are therefore sensitive to azide and iodoacetamide are also unknown. By analogy with other active transport systems, the initial binding reaction is depicted as non-energy requiring (12). The return of G and S to their original state on the membrane exterior is not shown.

If the loss of tyrosine from the pool by passive diffusion is relatively slow, then the binding, translocation, and release of tyrosine inside the cell

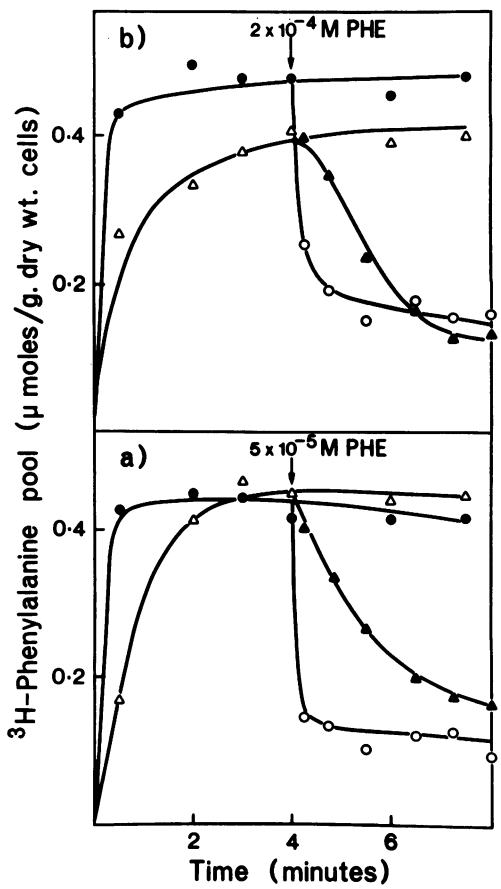

FIG. 9. *Displacement by external phenylalanine of the ³H-phenylalanine pool formed by the phenylalanine-specific transport system in glucose-starved W1485 and KB3100 at 37 C. Initial ³H-phenylalanine concentration, 5 × 10⁻⁶ M, 100 mCi/mmole. Unlabeled tryptophan (5 × 10⁻⁴ M) was present in the assays of W1485 to block ³H-phenylalanine pool formation by the general aromatic transport system. Displacement by: (a) 5 × 10⁻⁵ M phenylalanine, (b) 2 × 10⁻⁴ M phenylalanine. Trichloroacetic acid-soluble ³H-phenylalanine in: W1485 control (●), W1485 exposed to unlabeled, external phenylalanine at 4 min (○), KB3100 control (△), KB3100 exposed to unlabeled, external phenylalanine at 4 min (▲). Experimental procedure as in Fig. 8.*

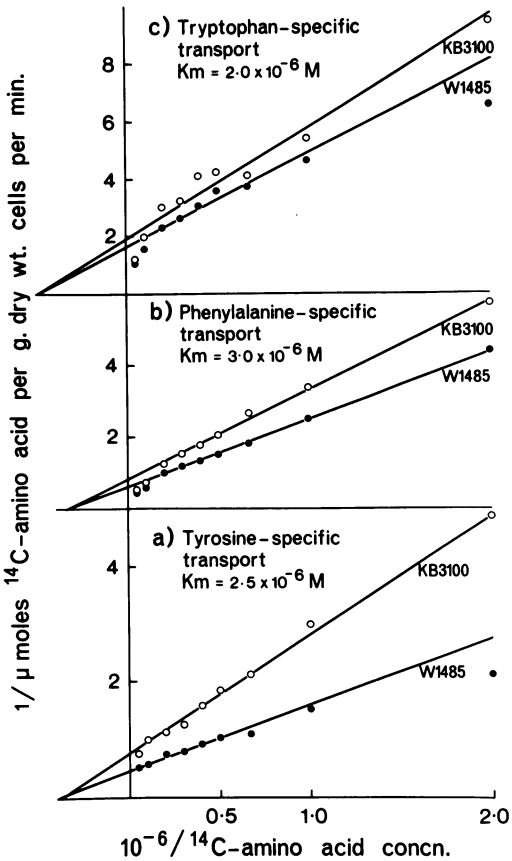

FIG. 10. *Lineweaver-Burk plots of specific aromatic transport in W1485 (●) and KB3100 (○). Uptake of: (a) ¹⁴C-tyrosine in the presence of 5 × 10⁻⁴ M tryptophan, (b) ¹⁴C-phenylalanine in the presence of 5 × 10⁻⁴ M tryptophan, (c) ¹⁴C-tryptophan in the presence of 5 × 10⁻⁴ M phenylalanine. Assay procedure: 2-ml volumes of glucose-starved cells were added to flasks containing ¹⁴C-amino acid, glucose (0.2% final concentration) and other additions where indicated. After shaking for 30 sec at 37 C, the contents of each flask were filtered, washed, and counted as described in Materials and Methods. Specific activity of ¹⁴C-amino acid, 50 mCi/mmole.*

must all be reversible in order to explain the rapid exchange of pool tyrosine with external amino acid.

It is not intended that the model should predict the actual number of transfers of tyrosine between the initial binding reaction and the release into the intracellular pool. The passage of the transport macromolecules, G and S, in unaltered form through the membrane is shown thus for simplicity only. Presumably, modification of either tyrosine or the transport macromolecule must occur to account for the accumulation of tyrosine inside the cell (otherwise, the forward and reverse reactions would proceed at the same rate and there would be no net accumulation of tyrosine).

In this model both general and specific transport are shown as freely reversible. One possible explanation of the behavior of the general transport negative (*aroP*⁻) mutant KB3100, however, is that the specific aromatic transport systems are not as readily reversible as the general aromatic transport system and that exit of the aromatic pool (whether generated by general or specific transport) is normally mediated in the wild-type by one or more elements of the general transport system. This would adequately explain the significantly slower pool efflux seen in KB3100 after dilution of external amino acid or energy deprivation (Fig. 2c and 3c). It would also explain the defective exchange of the tyrosine and phenylalanine pools formed by the tyrosine-specific and phenylalanine-specific transport systems in KB3100 (Fig. 8 and 9).

The energy requirements for the exchange of the aromatic pool are largely unknown and an energy requirement for efflux has not been established. The data as presented in the model indicate that the entry of external amino acid in the exchange process occurs via the active (i.e., energy-requiring) transport systems. On the other hand, the efflux observed on energy deprivation (Fig. 3) suggests that the exit reaction is non-en-

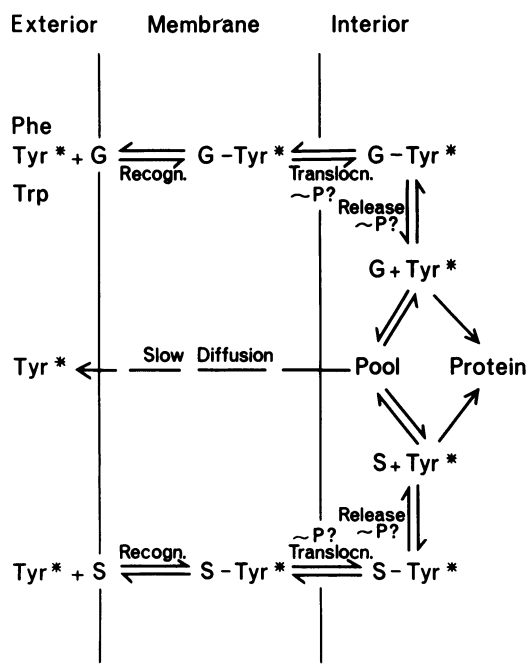

FIG. 11. *A physiological model for formation and exchange of the aromatic pool in E. coli K-12.*

ergy requiring. The altered exit properties of KB3100 (Fig. 2c and 3c) also suggest strongly that exit is a mediated process, involving the *aroP* gene product.

An earlier report by Kessel and Lubin (10) also implicates active transport in the exchange process. These authors described a mutant of *E. coli* W which was defective in active proline uptake (at 37 C) and which lacked the ability to carry out rapid exchange at 0 C between pool proline and external proline. These authors suggested a close relationship between the 0 C exchange and active uptake at 37 C. They proposed that the proline transport system in wild-type cells is not only responsible for active uptake at 37 C but also mediates the exchange of proline between the pool and the outside of the cell at 0 C.

At this stage it is not known whether the general and specific aromatic transport systems supply a single, homogeneous pool or whether pool compartmentation exists with general transport supplying one pool and the three specific aromatic transport systems supplying another (or one each). This point is at present under investigation. If the pool is homogeneous, then, when pool exchange of one particular aromatic amino acid for a second amino acid occurs via the general transport system, the specific transport system for the first amino acid must be able to maintain the observed, small, net pool in dynamic balance with the total pool which is turning over. This would be so since the entire pool of any one aromatic amino acid (whether generated by general or specific transport) would undergo continuous exchange through the general transport system.

The experiments on aromatic pool maintenance described here indicate that the pool is rapidly lost under conditions of external amino acid dilution or energy deprivation. These results contrast with those reported for the proline pool in *E. coli* by Britten and McClure (2). The differences may be explained either by basic differences in pool maintenance for different amino acids or as follows. Firstly, the dilution of proline in the experiment by Britten and McClure was not as great as the dilution of tyrosine in the 1:500 experiment reported in Fig. 2. In the former experiment considerable proline uptake may have remained (*see* Results section on comparison of the 1:50 and 1:500 experiments, Fig. 2). The Britten and McClure experiments on energy deprivation were based on glucose depletion, and these conditions are not paralleled in the experiments reported here so that a direct comparison cannot be made. It is possible, however, that the energy deprivation in the former experiments was insufficient to prevent continued proline uptake and hence pool maintenance (*see* reference 3).

In its general conclusion the present paper differs from Britten and McClure in that it proposes a labile, rapidly circulating aromatic amino acid pool, whereas the latter authors conclude that the proline pool is subject to only a small circulating flow between the cell interior and the medium. Britten and McClure, however, point out that in contrast to their conclusions based on pool maintenance experiments there is a rapid exchange observed between pool proline and external proline, and this indicates a large circulation of the pool.

The results reported here on pool exchange and pool maintenance in the presence of energy poisons are generally in agreement with those reported by Kay and Gronlund (9) for valine and proline in *Pseudomonas aeruginosa*. There is no evidence as yet for aromatic amino acid pool compartmentation of the kind reported for proline by these authors in *P. aeruginosa* and by Britten and McClure in *E. coli* (2).

DeBusk and DeBusk (7) reported that in *Neurospora crassa* the phenylalanine pool size is reduced by preincubation with tyrosine, tryptophan, and a number of other neutral amino acids. These same amino acids compete with phenylalanine for active transport. In view of the findings reported in the present paper, it may be speculated that the exclusion of phenylalanine from the pool in *N. crassa* could result from circulation of the pool between the interior and exterior of the cell where phenylalanine reentry through a general transport system is inhibited by competing amino acids in an analogous fashion to that proposed for *E. coli*.

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