ADAPTIVE ENZYMATIC PATTERNS IN THE BACTERIAL OXIDATION OF TRYPTOPHAN

R. Y. STANIER AND MARTHA TSUCHIDA

Department of Bacteriology, University of California, Berkeley, California

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It has been shown recently that analysis of adaptive enzymatic patterns ("simultaneous adaptation") can be used to advantage in the unraveling of microbial metabolic reaction chains (Stanier, 1947). In essence, such analyses represent an extension and refinement of the customary kinetic approach to the problems of intermediary metabolism, made possible by the substrate-activated nature of many microbial enzymes. Simultaneous adaptation has been used to establish the nonparticipation of the tricarboxylic acid cycle in the oxidation of acetate by *Azotobacter agilis* (Karlsson and Barker, 1948), to study the mechanisms for the bacterial oxidation of aromatic compounds (Stanier, 1947, 1948), to examine the feasibility of suggested intermediates in nitrogen fixation (Burris and Wilson, 1946), and to investigate the utilization of uronic acids by *Escherichia coli* (Cohen, 1949).

The principle of simultaneous adaptation can be applied effectively only when it is possible to formulate a priori the various pathways that might be followed in an adaptively controlled metabolic process and to obtain a reasonable number of postulated intermediates for testing each formulation. These requirements at present limit severely the applicability of the method. After considering the various dissimilatory processes for which the above-mentioned limitations might not prove too restrictive, we concluded that the oxidation of tryptophan offered most promise. A good deal is now known about the degradation and synthesis of this amino acid, and many of the postulated intermediates in its metabolism were available to us. A study of the bacterial oxidation of tryptophan was, accordingly, undertaken.

MATERIALS AND METHODS

Through the kindness of Dr. S. H. Hutner we obtained several strains of bacteria, originally isolated by appropriate enrichment procedures, which were known to be capable of using the oxidation of tryptophan as the sole source of energy for aerobic growth. As a result of preliminary experiments on the adaptation to and oxidation of tryptophan by these organisms, one strain (Hutner, str. 7) was selected for detailed study and has been used throughout the experiments reported below. The characteristics of this organism, which will be designated here as *Pseudomonas* sp., will be described in a forthcoming publication by Dr. Hutner.

Stock cultures were maintained on yeast agar slants. In order to produce suspensions of cells with the desired enzymatic patterns for manometric work, the organism was grown on synthetic or semisynthetic media containing a single

compound as the source of carbon and energy. All these media had the following basal composition: NH_4NO_3 0.1 g, K_2HPO_4 0.1 g, $MgSO_4$ 0.05 g, agar 1.5 g, and distilled water 100 ml. The specific compound that served as a source of carbon and energy was added at a final concentration of 0.2 to 0.5 per cent, and the pH was adjusted to 7.0 to 7.2. So-called "unadapted" cells (i.e., cells not adapted to any compounds related to tryptophan) were produced by using asparagine as the carbon source. The organism grows sparsely on a strictly synthetic medium, and at times a small amount of yeast extract (0.01 per cent) was added to the basal medium in order to improve development. This addition has no effect on the adaptive patterns with which we were concerned, since the amount of tryptophan thereby introduced into the medium is very small. Even growth on a medium containing 0.5 per cent yeast extract results in only a partial activation of the tryptophan-oxidizing enzyme system. All cultures were incubated at 30 C.

Our supplies of certain compounds to which we wished to adapt *Pseudomonas* sp. were extremely limited. In such cases, specific adaptation was achieved by exposing suspensions of initially "unadapted" (asparagine-grown) cells to a small amount of the compound in question, rather than by growing them in its presence. As a rule, the activation of these resting cell suspensions was conducted in Warburg vessels with double side arms, one of which contained the activating substance. After the addition of this substance, the course of adaptation could be gauged from the oxygen uptake, and at the point when the activating substance had been completely metabolized, as judged by a return to the autorespiratory rate of oxygen consumption, a second compound could be added from the other side arm.

All experiments were performed with the Warburg respirometer, at a temperature of 30 C in an atmosphere of air. Cell suspensions were prepared by harvesting the growth from plate cultures 18 to 24 hours old, washing once, and resuspending in M/60 phosphate buffer of pH 7.0. Carbon dioxide determinations were made by the "direct" method. Ammonia determinations were made on the supernatants from the cells used in Warburg experiments, Nessler's reagent being employed.

RESULTS

General nature of the attack of L-*tryptophan.* The oxidation of L-tryptophan by *Pseudomonas* sp. is a strictly adaptive process. When cells grown on a medium containing asparagine as the sole source of carbon are tested manometrically for their ability to attack L-tryptophan, no oxygen consumption in excess of autorespiration occurs for 60 minutes following substrate addition, after which there is a typical exponential increase in the rate of oxygen uptake until a steady maximum rate is reached (figure 1). Cells grown with L-tryptophan as a carbon source show an immediate oxygen uptake when tested in a similar manner, and the rate remains steady to the point of substrate exhaustion. Cells grown on yeast extract attack L-tryptophan without a latent period, but the initial rate of oxidation is low and increases several times in the course of 2 to 3 hours (figure 1).

Presumably the amount of tryptophan in yeast extract is sufficient to cause partial adaptation.

When oxidizing limiting amounts of L-tryptophan, adapted cells consume approximately 145 microliters (6.5 micromoles) of oxygen per micromole of substrate, after which there is a return to a rate of oxygen uptake little, if any, greater than the autorespiratory one. The oxidation is accompanied by substantial carbon dioxide production, for which data are presented in table 1.

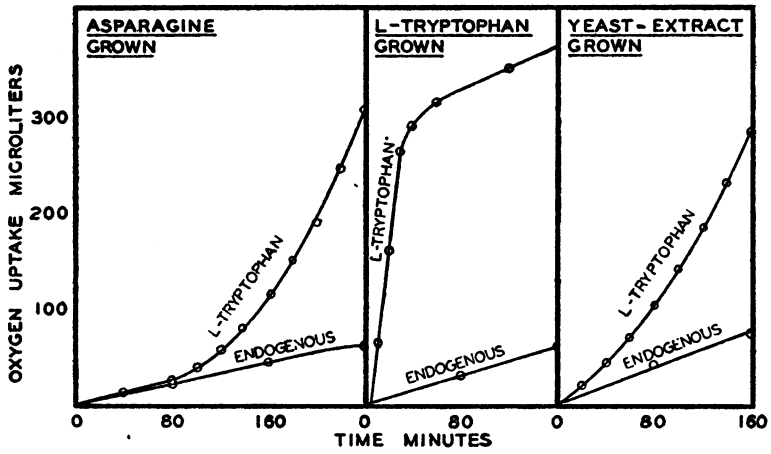


Figure 1. Effect of conditions of cultivation on the rate of oxidation of L-tryptophan (2 micromoles) by *Pseudomonas* sp.

TABLE 1

Oxidation of 3 micromoles of L-tryptophan by adapted cells of Pseudomonas sp.

TIME AFTER SUBSTRATE ADDITION	OXYGEN UPTAKE	CO_2 PRODUCTION	R.Q.
min	*microliters*	*microliters*	
15	109	101	0.93
30	252	246	0.98
60 (oxidation complete)	435	417	0.96

Oxygen uptake, microliters per micromole: 145.
CO_2 output, microliters per micromole: 139.
Average R.Q., microliters per micromole: 0.96.

The total carbon dioxide production is 139 microliters (approximately 6 micromoles) per micromole of tryptophan oxidized, and the R. Q. remains constant (within the limits of experimental error) throughout the oxidation. As shown by the data in table 2, ammonia is also liberated as a result of the oxidation, about 1.3 micromoles being produced per micromole of tryptophan decomposed. These facts demonstrate that the oxidation is a far-reaching one. On the other hand, the figures for oxygen uptake and ammonia and carbon dioxide production are considerably less than those required for a complete oxidation of the tryptophan molecule according to the equation:

$$C_{11}H_{12}O_2N_2 + 11.5\ O_2 \rightarrow 11CO_2 + 3H_2O + 2NH_3.$$

This discrepancy results, at least in part, from the occurrence of substantial oxidative assimilation. In the presence of 2,4-dinitrophenol (DNP) at concentrations between M/4,000 and M/8,000, the oxygen uptake per mole of tryptophan decomposed is substantially increased (see, e.g., figures 3 and 4), although even under these circumstances the theoretical uptake for complete oxidation has never been observed. The highest oxygen uptakes obtained in the presence of DNP have been approximately 80 per cent of the theoretical figure. The possibility thus exists that a small part of the tryptophan molecule is converted to organic end products not further attackable by the organism. We have not attempted to search for these hypothetical end products. Assuming that the discrepancies from the values for complete oxidation result exclusively from oxidative assimilation, the following equation best fits the data.

$$C_{11}H_{12}O_2N_2 + 6.5O_2 + 2H_2O \rightarrow 6CO_2 + 5(CH_2O) + 2NH_3,$$

giving a theoretical R.Q. of 0.93 (found 0.96), a theoretical oxygen uptake of 145 microliters per micromole (found 143), and a theoretical carbon dioxide

TABLE 2

The production of ammonia from L-tryptophan by adapted cells of Pseudomonas sp.

TRYPTOPHAN OXIDIZED	NH_3-N FORMED	NH_3 PRODUCED	NH_3 PRODUCED
micromoles	*micrograms*	*micromoles**	*micromoles per micromole of tryptophan oxidized*
0.0	10.0		
1.0	27.0	1.21	1.2
1.3	31.5	1.55	1.2
2.67	61.5	3.70	1.4
4.0	77.0	4.80	1.2

* Corrected for blank value.

production of 134 microliters per micromole (found 139). The figures for ammonia production suggest that both nitrogen atoms are liberated as ammonia, part of the ammonia produced (about 35 per cent) then being assimilated and utilized by the cells.

Stereoisomerism and adaptive response. Figure 2 shows the oxygen uptake with different amounts of L- and of DL-tryptophan by cells grown in the presence of the L-isomer. The curves for the racemic mixture are those characteristic of a two-step oxidation with an intervening adaptive lag. The initial oxidation of DL-tryptophan proceeds at the characteristic rate for the oxidation of the pure L-isomer and ceases abruptly when the total oxygen uptake is exactly half that with an equivalent molarity of the pure L-isomer; this portion of the curve clearly represents the oxidation of the L-moiety. Following reversion to the autorespiratory rate, there is a slow and continuous increase in the rate of oxygen uptake until the total amount is double that at the initial break, when a second and permanent reversion to the autorespiratory rate occurs. We interpret the secondary rise in oxygen consumption as reflecting a much slower oxidation of

the D-moiety by means of a second, initially unadapted, enzyme system. Since the final total oxygen uptake per mole is identical for DL-tryptophan and for the L-isomer, it can also be inferred that the two enzyme systems oxidize tryptophan to the same extent.

In order to demonstrate more conclusively the initially unadapted condition of the D-tryptophan-oxidizing enzyme system in cells grown on L-tryptophan, use was made of the fact, originally discovered by Monod (1944), that DNP will completely block adaptation at molarities that do not affect dissimilatory mechanisms. The oxidation of L-, D-, and DL-tryptophan by cells adapted to the L-isomer was studied in the presence and absence of DNP (figure 3). In ac-

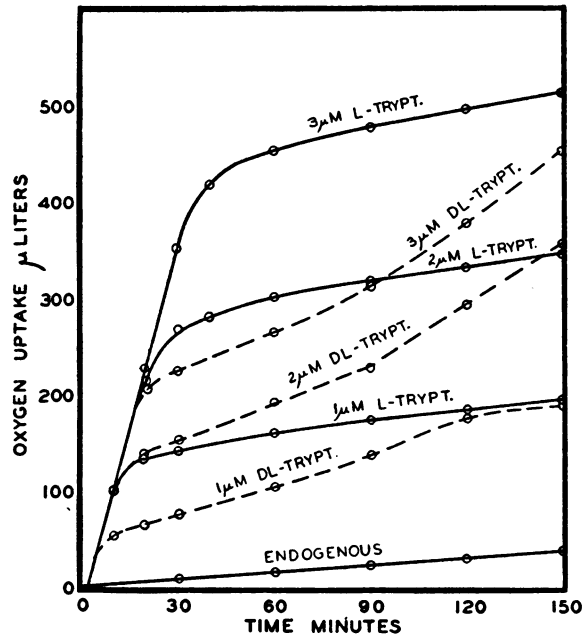


Figure 2. The oxidation of 1, 2, and 3 micromoles of L- and of DL-tryptophan by *Pseudomonas* sp. adapted to L-tryptophan.

cordance with expectation, treatment of such cells with M/4,000 DNP completely prevents attack on the D-isomer: the oxygen uptake with 2 micromoles of the racemic mixture is the same as with one micromole of the pure L-isomer, whereas with the pure D-isomer it remains permanently at the autorespiratory level. The same cell suspension without DNP oxidizes D-tryptophan after a typical adaptive lag and shows a secondary oxygen uptake with the racemic mixture of the type already described above.

If a similar experiment is performed with cells that have been grown on DL-tryptophan, entirely different results are obtained (figure 4). In such cells, both enzyme systems have been activated, and DNP is consequently unable to prevent oxidation of the D-isomer. Both in the presence and absence of DNP the L-isomer is oxidized somewhat more rapidly than the D-isomer, but the

difference is insufficient to cause more than a slight inflection in the later part of the curves for the oxidation of the racemic mixture. With all three substrates, the only effect of DNP is to increase somewhat the total oxygen uptake, a consequence of the blockage of assimilation.

In view of the striking specificity with which L-tryptophan activates and is acted upon by the L-tryptophan-oxidizing enzyme system, we anticipated that a relationship of similar specificity would obtain between the D-isomer and the enzyme system acting upon it. This proved not to be the case. Cells that have been adapted to the D-isomer alone are always simultaneously adapted to the L-isomer and actually oxidize the latter somewhat more rapidly than the former.

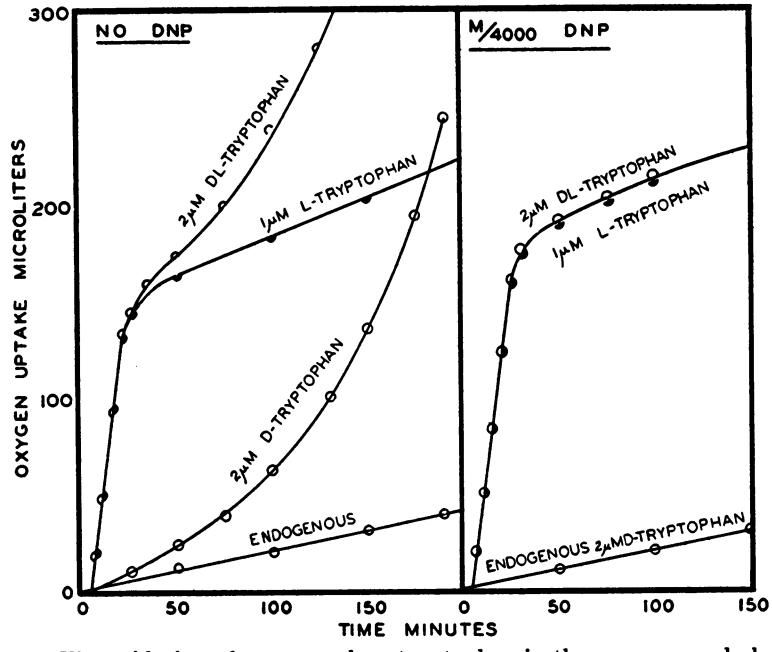


Figure 3. The oxidation of L-, D-, and DL-tryptophan in the presence and absence of DNP of *Pseudomonas* sp. adapted to L-tryptophan.

The adaptive response to D-tryptophan is thus effectively identical with the response to DL-tryptophan; the curves in figure 4 could represent both equally well.

It might be argued that the results obtained with the D-isomer were caused by the use of a preparation contaminated with a small amount of L-tryptophan. However, the data in figure 3 provide strong evidence against this possibility. If our D-tryptophan had contained any of the L-isomer, there should have been an appreciable, rapid oxygen uptake by cells adapted to the L-isomer in its presence; of this there is not the slightest indication. As a check on the possibility that an excess of D-isomer might inhibit the oxidation of a small amount of L-tryptophan by cells adapted to it, we studied the oxidation of a fixed amount of the L-isomer by L-tryptophan-adapted cells in the presence of various known

quantities of the D-isomer. Neither the rate nor the total quantity of oxygen consumed during the initial rapid oxidation of the L-isomer was affected, even by a tenfold excess of D-tryptophan. We feel that this possible source of experimental error is consequently eliminated.

Several interpretations of the dissimilar responses by *Pseudomonas* sp. to the isomers of tryptophan are possible, and we have been unable so far to obtain conclusive evidence as to which is correct. Considered from the standpoint of

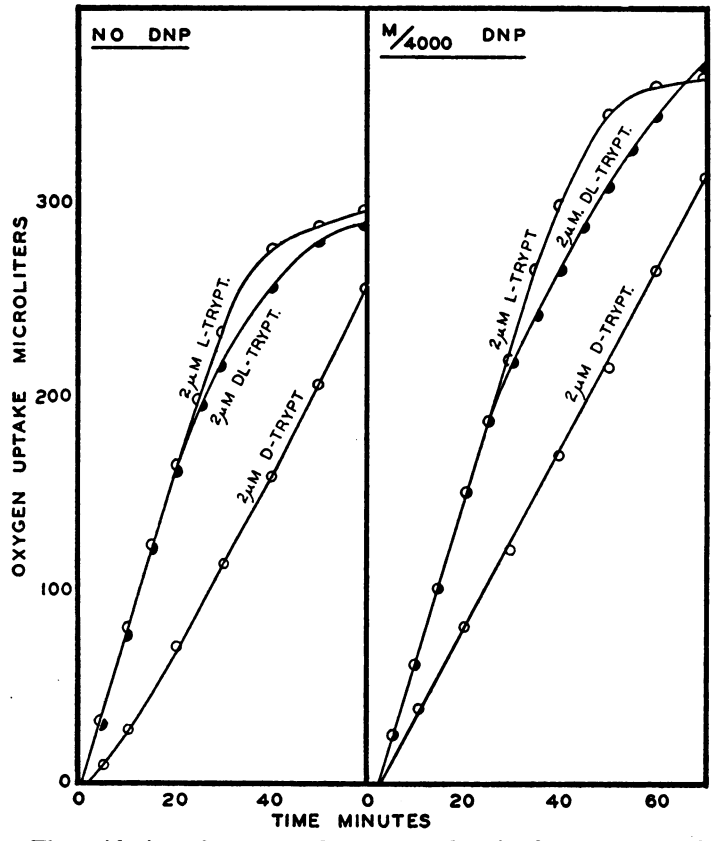


Figure 4. The oxidation of L-, D-, and DL-tryptophan in the presence and absence of DNP by *Pseudomonas* sp. adapted to both isomers by growth on DL-tryptophan.

adaptive response, the data could indicate that D-tryptophan activates nonspecifically the enzyme system attacking the L-isomer, as well as activating specifically the separate enzyme system attacking the D-isomer. Considered from the standpoint of *enzyme action*, however, the experiments are interpretable as indicating that the L-tryptophan-oxidizing enzyme system is specific in its action on the L-isomer, but the D-tryptophan-oxidizing enzyme system can act on both. Unless this dual action of the latter enzyme system were of a very special kind, though, exposure to L-tryptophan should cause simultaneous adaptation to the D-isomer, since it would provide a substrate activation equivalent to that

provided by the D-isomer for an enzyme system capable of action indifferently on both. Since this does not occur, an explanation in terms of enzyme action can be envisaged only if one assumes a *sequential relationship* between the D- and L-isomers; if the first step in the attack on the D-isomer involved the action of a racemase that transformed it to the L-isomer, the latter would in fact constitute an intermediate in the decomposition of the former, and the dissimilar adaptive responses would be explicable. Such an interpretation is compatible with the finding that any treatment that activates the D-tryptophan-oxidizing enzyme system causes an even greater rate of attack on the L-isomer, but at the same time this very fact precludes the possibility of demonstrating a racemizing action on D-tryptophan by living cells. Other observations concerning steric specificity, which are reported in the next section, tend to complicate further any attempt to interpret the foregoing results in terms of a racemizing enzyme. At present, therefore, the explanation of these findings remains obscure.

Evidence concerning the path of tryptophan oxidation. On the basis of what is at present known about the comparative biochemistry of tryptophan, a number of possible pathways can be postulated *a priori* for its oxidative degradation. These include:

(1) Reversal of the synthetic mechanism (Tatum and Bonner, 1944; Tatum, Bonner, and Beadle, 1944), involving a primary cleavage to indole and serine and a subsequent oxidation of the two fragments, anthranilic acid occurring as an intermediate in the further breakdown of indole.

(2) Rupture of the tryptophan molecule with the primary production of indole, ammonia, and pyruvic acid, the indole and pyruvic acid undergoing subsequent oxidation. The initial rupture is the mechanism for indole formation by *Escherichia coli*, as shown by Wood, Gunsalus, and Umbreit (1947).

(3) Primary attack on the aliphatic side chain, by oxidative deamination to indolepyruvic acid and subsequent oxidation through indoleacetic acid.

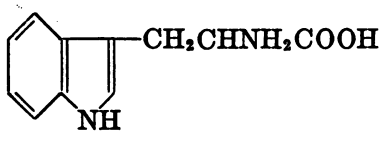
(4) Primary attack on the indole nucleus, with elimination of the second carbon atom in the five-membered ring, leading to the formation of kynurenin and thence, by oxidative deamination and ring closure, to kynurenic acid. This is the mechanism proposed by Kotake (1931, 1933) for tryptophan oxidation by the rabbit and by *Bacillus subtilis*.

In the light of the postulates concerning simultaneous adaptation (Stanier, 1947), one can predict specific adaptive patterns corresponding to each of these possible pathways in cells adapted to tryptophan. On the basis of (1), the cells should be simultaneously adapted to indole, serine, and anthranilic acid. On the basis of (2), they should be simultaneously adapted to indole and pyruvic acid, and possibly also to anthranilic acid. On the basis of (3), they should be simultaneously adapted to indolepyruvic and indoleacetic acids. On the basis of (4), they should be simultaneously adapted to kynurenin and kynurenic acid. The experiments performed to check these predictions have given extremely clear-cut results, which make it evident that none of the first three proposed pathways is operative and suggest strongly that the oxidation actually proceeds through the compounds in the fourth pathway.

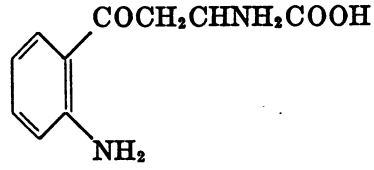
Figure 5 presents the data on some of the intermediates that would occur in the first three pathways. The oxidation of indole and serine, either singly or in combination, is clearly far too slow in comparison to that of tryptophan for these compounds to act as intermediates. The same is true for indolepyruvic and indoleacetic acids. Data on anthranilic acid are given in figure 10, and show that it, too, cannot be considered a feasible intermediate.

On the other hand, kynurenin and kynurenic acid fulfill the postulated requirement for intermediates in tryptophan oxidation, since tryptophan-adapted cells are always simultaneously adapted to them and oxidize them at a rate comparable to the rate of tryptophan oxidation (figure 6). As shown by experiments with asparagine-grown cells, the oxidation of kynurenin and kynurenic acid is strictly adaptive, both substances being attacked in such experiments only after adaptive lags of the same order as the lag with tryptophan.

There are other experimentally verifiable consequences of the assumption that kynurenin and kynurenic acid participate in the reaction chain leading from tryptophan. In the first place, a fixed and calculable relationship should exist between the total oxygen uptakes with equimolar amounts of the three substrates, the figures for kynurenin and kynurenic acid being proportionately less than for tryptophan, in view of their more oxidized states. From the gross equations:

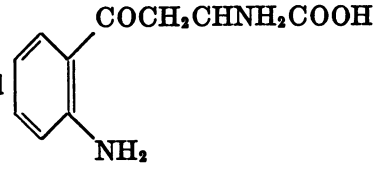


$$+ 1.5\ O_2 \rightarrow$$

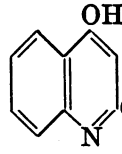


$$+ CO_2 \ldots\ldots 1$$

and



$$+ 0.5\ O_2 \rightarrow$$



$$+ NH_3 \ldots\ldots 2$$

it is evident that the oxygen uptakes with kynurenin and kynurenic acid should be less by 1.5 and 2.0 moles per mole, respectively, than the oxygen uptake with tryptophan, assuming, of course, that the carbon atom eliminated from the indole nucleus appears as carbon dioxide, rather than undergoing assimilation.

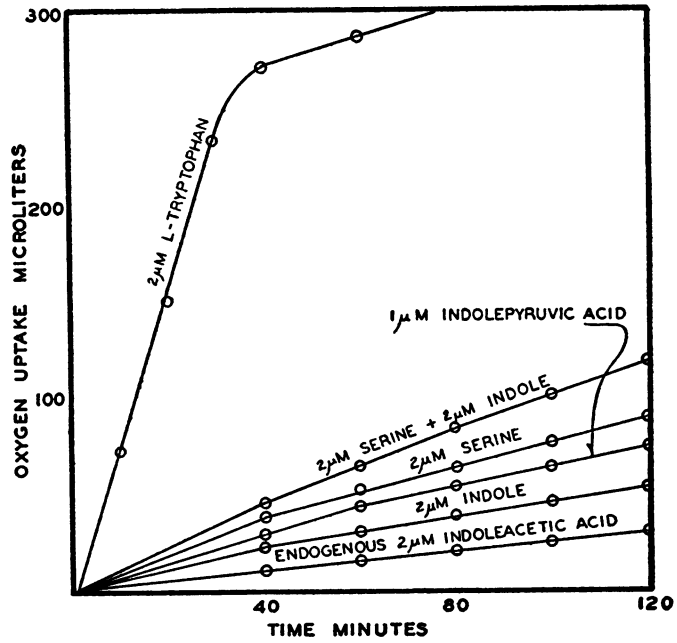


Figure 5. The oxidation of L-tryptophan and of various possible intermediates in its dissimilation by *Pseudomonas* sp. adapted to L-tryptophan.

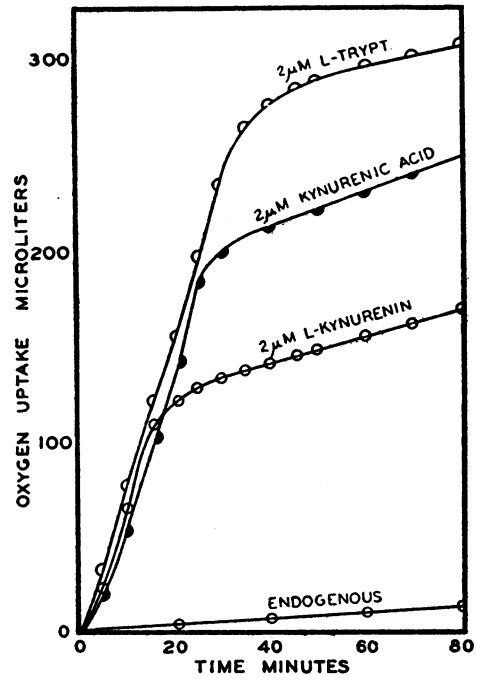


Figure 6. The oxidation of equimolar amounts (2 micromoles) of L-tryptophan, L-kynurenin, and kynurenic acid by *Pseudomonas* sp. adapted to L-tryptophan.

With kynurenic acid, the predicted oxygen uptake is always obtained, within the limits of experimental error (see, e.g., figure 6). Furthermore, the R.Q. is very close to the theoretical R.Q. derivable from the figures given previously for the oxygen consumption and carbon dioxide output with tryptophan. The data on the oxidation of L-tryptophan indicate an oxygen uptake of 6.5 moles per mole and a carbon dioxide production of 6.0 moles per mole, giving an R.Q. of slightly less than unity (0.93). Since the oxidation of tryptophan to kynurenic acid would result in the release of only one molecule of carbon dioxide, the R.Q. for the oxidation of kynurenic acid should, in consequence, be slightly greater than unity (5.0/4.5, or 1.11). In an actual experiment in which the R.Q. values were determined at three points during the oxidation of kynurenic acid, the figures obtained were 1.09, 1.18, and 1.14, which is in satisfactory agreement with expectation.

With kynurenin, however, the total oxygen uptake per mole is always far below the predicted level, being actually much lower (as shown in figure 6) than that with kynurenic acid. Our early experiments were performed with a sample of the natural L-isomer of kynurenin, and we assumed at first that the low total oxygen uptakes reflected gross impurity of the material. Subsequently, however, the experiments were repeated with synthetic DL-kynurenin, and oxygen uptakes of the same magnitude were obtained (allowing for the effects of stereoisomerism to be discussed below). It is consequently improbable that the anomalously low total oxygen uptakes with kynurenin result from the use of impure material, and we assume that the phenomenon is a real one. On the face of it, two explanations are possible. Kynurenin may not be involved at all as an intermediate in tryptophan oxidation, being broken down by an entirely different mechanism that results in a far lower total oxygen uptake per mole. But in this case, it is very difficult to understand how exposure to tryptophan can result in simultaneous adaptation to kynurenin (particularly in view of the carrying over of steric specificity in the adaptive response, described below), and how kynurenic acid, which meets so admirably all the requirements for an intermediate, can be fitted into a scheme for tryptophan degradation that does not involve also kynurenin. These difficulties can be circumvented by assuming that kynurenin is in fact an intermediate, but that there are two pathways for its metabolism, one (via kynurenic acid) resulting in a high oxygen uptake and the other unknown pathway resulting in a very low oxygen uptake. Furthermore, it is necessary to assume that when kynurenin is present in trace amounts (e.g., as an intermediate during the oxidation of tryptophan) it is nearly all decomposed via kynurenic acid, the other mechanism becoming substantially operative only when relatively large amounts of kynurenin are available.

If kynurenin is an intermediate in the oxidation of tryptophan, this particular sequence in the reaction chain would not involve the loss of asymmetry, since the side chain of the molecule that contains the asymmetric carbon atom is untouched. Hence one might predict that the stereoisomeric effects previously noted with tryptophan would carry over to kynurenin. Since both

L- and DL-kynurenin were available, this inference could be checked experimentally. As shown in figure 7, the attack on the stereoisomers of kynurenin is markedly affected by the specific adaptive patterns pre-established with respect to tryptophan. Cells that are adapted to both isomers of tryptophan are simultaneously adapted to both isomers of kynurenin, although (just as in the case of tryptophan) the oxidation of the D-isomer is somewhat slower than the oxidation of the L-isomer. On the other hand, cells that are adapted to L-tryptophan alone are likewise adapted to L-kynurenin alone, showing an oxygen

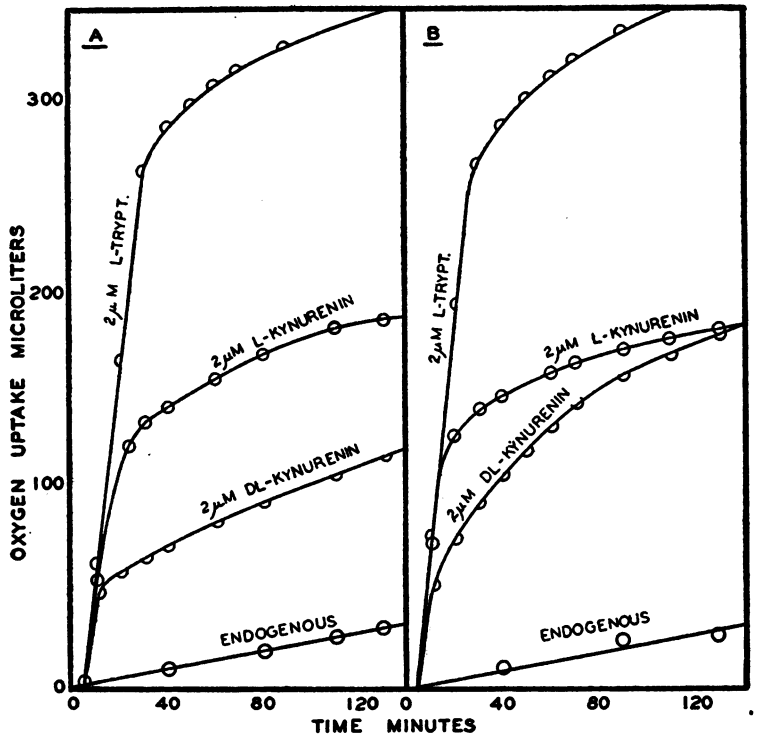


Figure 7. The effect of pre-established enzymatic specificity toward the stereoisomers of tryptophan on the oxidation of the stereoisomers of kynurenin by *Pseudomonas* sp. *A*, cells adapted to L-tryptophan; *B*, cells adapted to both L- and D-tryptophan.

consumption with DL-kynurenin that is only half the oxygen consumption with the L-isomer. One peculiar feature of this experiment deserves particular mention, since it provides indirect evidence for the postulated dual mechanism of kynurenin decomposition. When L-tryptophan-adapted cells act on DL-kynurenin, the total oxygen uptake remains *permanently* about half as great as with the equivalent molarity of pure L-isomer; no matter how long the experiment is continued after the initial rapid oxygen consumption has ceased, there is never any sign of a secondary adaptation to, and oxidation of, the D-isomer such as characterizes the attack on DL-tryptophan by similarly adapted cells. Inability to attack the D-isomer of kynurenin cannot, of course, provide an ex-

planation of the phenomenon, since cells adapted to DL-tryptophan clearly possess this ability. It suggests, rather, that the D-isomer disappears as a result of some other biochemical transformation, involving little or no oxygen uptake, during the time when adaptation to it would normally be taking place. Unfortunately, we did not have sufficient kynurenin to investigate this point further.

The adaptive patterns of cells specifically adapted to kynurenin and kynurenic acid are shown in figures 8 and 9. It will be seen that adaptation to kynurenic acid fails to bring about adaptation either to kynurenin or to tryptophan, whereas adaptation to kynurenin causes simultaneous adaptation not only to its presumed successor, kynurenic acid, but also to tryptophan.

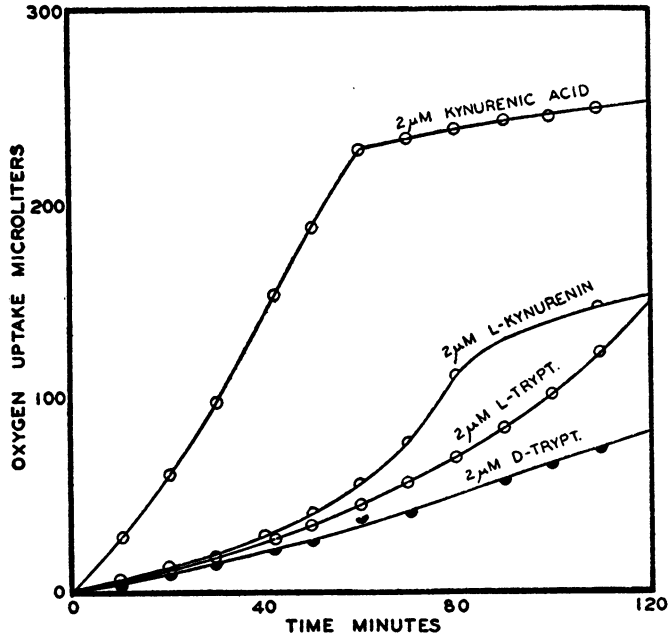


Figure 8. The adaptive patterns established in *Pseudomonas* sp. by preadaptation to kynurenic acid.

In addition to oxidation through kynurenin to kynurenic acid, a second pathway for tryptophan breakdown via kynurenin has been shown recently by the work of Beadle, Mitchell, and Nyc (1947) and Mitchell and Nyc (1948) on a mutant strain of *Neurospora crassa*. This involves an oxidation via kynurenin to 3-hydroxyanthranilic acid, from which nicotinic acid is formed by subsequent transformations of an unknown nature. Kynurenic acid does not participate in this reaction chain, the kynurenin being attacked presumably by substitution of a hydroxy group in the 3-position on the benzene ring. Although the evidence presented above pointed to the participation of kynurenic acid in the oxidation of tryptophan by *Pseudomonas* sp., it seemed desirable to check the possibility that the reaction sequence shown in *Neurospora* might be operative. Accord-

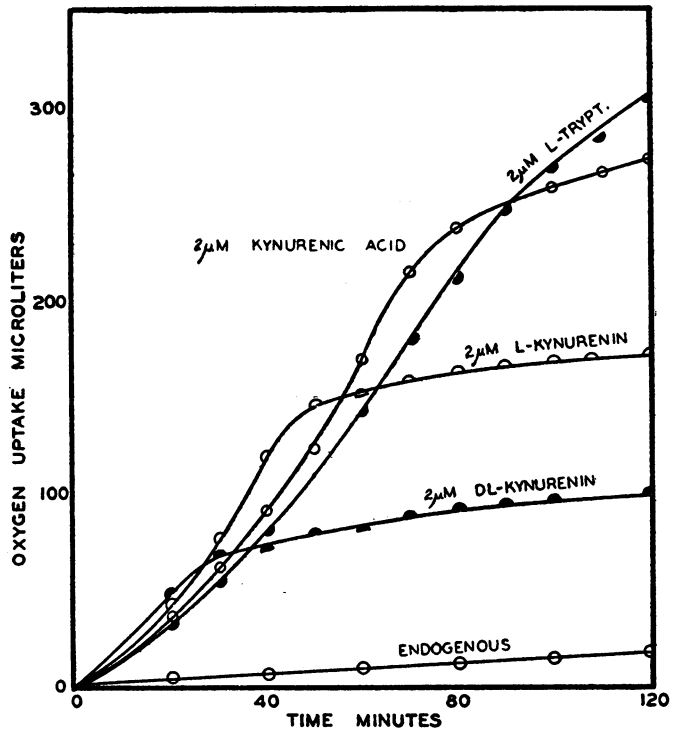


Figure 9. The adaptive patterns established in *Pseudomonas* sp. by preadaptation to L-kynurenin.

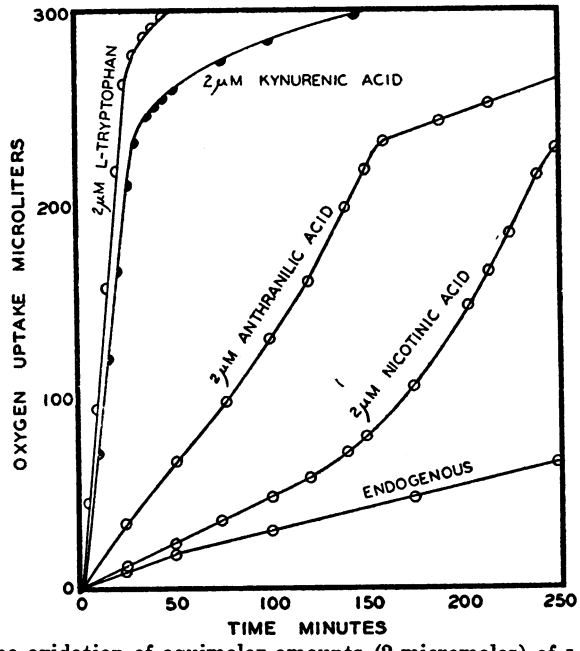
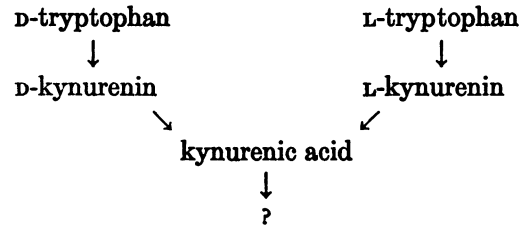


Figure 10. The oxidation of equimolar amounts (2 micromoles) of L-tryptophan, kynurenic acid, anthranilic acid, and nicotinic acid by *Pseudomonas* sp. adapted to L-tryptophan.

ingly, the adaptive response of tryptophan-adapted cells to nicotinic acid, anthranilic acid, and two hydroxy derivatives of the latter compound was determined. As shown in figure 10, *Pseudomonas* sp. can oxidize both nicotinic and anthranilic acids, but the low rates with these substrates after growth on tryptophan preclude the possibility that they participate in the dissimilation of tryptophan. The two derivatives of anthranilic acid (3-hydroxy- and 3,4-dihydroxyanthranilic acids) proved completely unoxidizable.

SUMMARY AND CONCLUSIONS

Studies on the oxidation of D- and L-tryptophan by an unidentified organism, *Pseudomonas* sp., have provided evidence to suggest that the initial steps in the reaction occur as follows:



The participation of two enzyme systems in the initial attack on the two isomeric forms is clearly shown by experiments on adaptive specificity. The L-tryptophan-oxidizing system is specifically activated by, and specific in its action upon, the L-isomer, the D-isomer being oxidized only after a considerable adaptive lag by cells previously exposed to the L-isomer. The steric specificity in tryptophan oxidation is not absolute, however, since exposure to D-tryptophan produces cells capable of attacking both stereoisomers without a lag. The pattern of steric specificity set up by exposure to L-tryptophan carries over to kynurenin, such cells being unadapted to D-kynurenin, although they are simultaneously adapted to L-kynurenin.

The evidence for the participation of kynurenin in the foregoing scheme is weakened by the fact that the total oxygen uptake per mole with this substrate is always anomalously low in comparison to the oxygen uptakes with tryptophan and kynurenic acid. Since the oxygen uptakes with kynurenin were very similar irrespective of whether natural L-kynurenin or synthetic DL-kynurenin was used (allowing, of course, for the effects of stereoisomerism with the latter), it is improbable that the low oxygen consumption reflects the presence of impurities in the material used. A possible explanation, for which there is some indirect evidence, is that kynurenin can be broken down by another path involving a low oxygen uptake in addition to the path through kynurenic acid, and that the latter mechanism operates efficiently only when kynurenin is present in trace amounts, as would be the case during tryptophan oxidation.

The experiments on simultaneous adaptation show clearly that alternative possible mechanisms for the oxidation of tryptophan, such as a primary attack on the side chain or a rupture of the molecule with formation of indole, cannot be

operative. By this means it was also possible to exclude the sequence through kynurenin to 3-hydroxyanthranilic and nicotinic acids.

The oxidation of tryptophan proceeds far beyond kynurenic acid, as shown by both oxygen uptake and carbon dioxide production, but the occurrence of oxidative assimilation makes it difficult to determine whether the reaction represents a complete combustion. Although the oxygen uptake per mole of tryptophan is considerably increased by DNP, the figure even under these circumstances in our experiments was never greater than 80 per cent of that theoretically required for complete oxidation to carbon dioxide, ammonia, and water.

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