# THE EFFECT OF CHLORAMPHENICOL AND CERTAIN OTHER DRUGS ON THE OXIDATION OF AROMATIC AMINO ACIDS BY A STRAIN OF PSEUDOMONAS AERUGINOSA

FREDERICK BERNHEIM AND WILLIAM E. DeTURK

*Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina*

Received for publication February 21, 1952

The structure of chloramphenicol suggests that it may interfere in the bacterial metabolism of aromatic amino acids. To test this possibility a strain of Pseudomonas aeruginosa originally obtained from Dr. Koppers was used. As shown below this organism readily oxidizes phenylalanine, tyrosine and phenylserine and the effect of chloramphenicol and other antibiotics and drugs was studied on the oxidation of these and certain other compounds. The effect on ammonia production and assimilation was also determined. It has previously been shown (Bernheim and DeTurk, 1951) that this organism rapidly assimilates ammonia in the presence of an oxidizable substrate.

*Experimental.* The bacteria were grown and prepared as previously described (Bernheim and DeTurk, 1951). The oxygen uptakes were measured in Warburg manometers in the usual way. The bacteria were suspended in 0.05 *M* K-Na-phosphate buffer at pH 6.0 or 7.8 and the drugs incubated with them 30–60 minutes befoıe the addition of the substrate. The final volume was 2.0 ml. At the end of the experiment 1.0 ml. of 10 per cent trichloroacetic acid was added, the bacteria centrifuged down and the free ammonia determined by the method of Speck (1949) and the amino nitrogen by the Van Slyke method. Only the natural isomers of phenylalanine and tyrosine were rapidly oxidized. The non-natural isomers were attacked slowly if at all. At pH 7.8, 12 atoms of oxygen were taken up per molecule of phenylalanine, 10 per molecule of tryosine and 10 per molecule of phenylserine on the basis of both isomers. The last was used in the *dl*-form and the ammonia determinations showed that both isomers were deaminated. On the basis of the differential effect of drugs and of hydrogen ion concentration phenylalanine is probably not oxidized through tyrosine. Dopa was oxidized very slowly so it cannot be an intermediate and the corresponding amines were not oxidized which indicates that oxidative decarboxylation of the side chain is not the first step. Carbon dioxide formation occurred, however, in all cases in amounts which indicated that the ring in each compound was broken.

The oxygen uptake curves for the three aromatic amino acids are shown in fig. 1. The oxidation rate of phenylalanine increased after about 40–60 minutes and then remained constant until the end. The oxidation rates of the other two amino acids showed no such rate change. A comparison of fig. 1 with fig. 2 shows that at pH 6.0 phenylalanine was oxidized much more slowly than at pH 7.8 whereas the oxidation rates of phenylserine and tyrosine were essentially

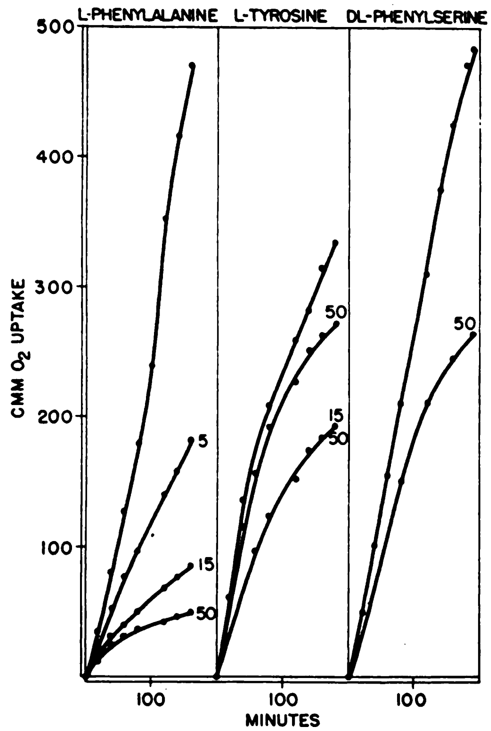


FIG. 1. The effect of chloramphenicol on the oxidation of 1.0 mgm. each phenylalanine, tyrosine and phenylserine at pH 7.8, 37°. The numerals are microgm./ml. of chloramphenicol added to the bacteria 30 min. before the amino acids. In this and subsequent figures the small autorespiration of the organisms has been subtracted.

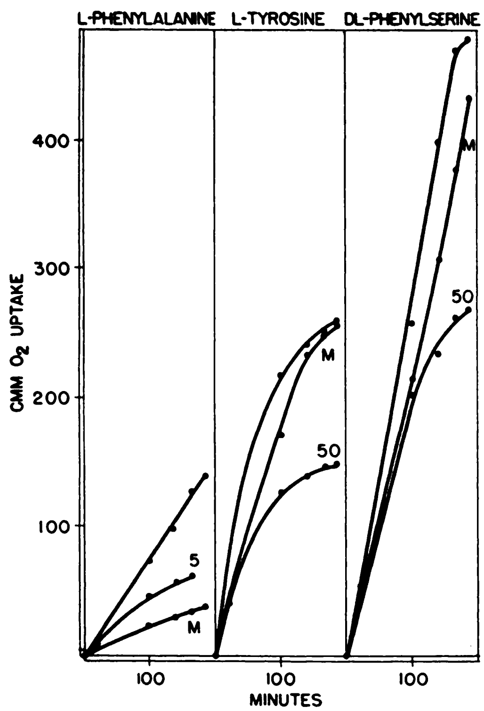


FIG. 2. The same as fig. 1 except at pH 6.0. M is 1.0 mgm. *dl*-mandelic acid/ml. and the numerals are microgm./ml. of chloramphenicol.

independent of pH. Fig. 1 also shows that chloramphenicol is much more effective in inhibiting the oxidation of phenylalanine than that of the other two but this is also true for streptomycin, terramycin and aureomycin. The effect of these drugs on the oxidation of phenylalanine is shown in fig. 3. Mandelic acid which is only active in the acid range has little effect on the oxidation of tyrosine and phenylserine but inhibits that of phenylalanine (fig. 2) and finally thienylalanine is equally effective on the oxidation of phenylalanine and phenylserine and has little effect on that of tyrosine (fig. 4). Higher concentrations of all these

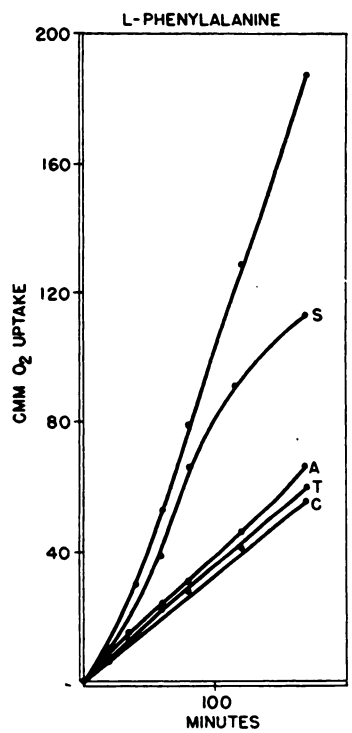


FIG. 3

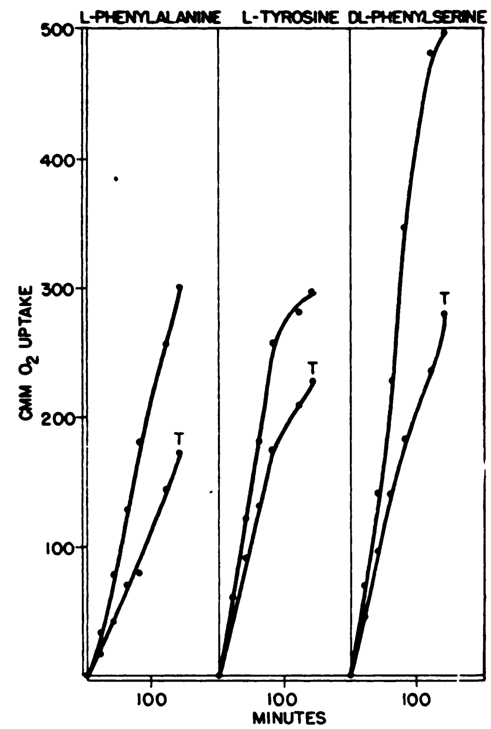


FIG. 4

FIG. 3. The effect of 5 microgm./ml. of aureomycin (A), terramycin (T), chloramphenicol (C) and 25 microgm./ml. of streptomycin (S) on the oxidation of 0.5 mgm. phenylalanine at pH 7.8, 37°.

FIG. 4. The effect of 0.5 mgm./ml. thienylalanine (T) on the oxidation of 1.0 mgm. each of phenylalanine, tyrosine and phenylserine pH 7.8, 37°.

drugs are necessary to inhibit effectively the oxidation of alanine and serine. Sulfanilamide in a concentration of 1.0 mgm./ml. has no effect on the oxidation of the three aromatic amino acids.

Table 1 shows that when phenylalanine, tyrosine or phenylserine was incubated with these organisms deamination occurred and ammonia appeared in the medium. In the presence of chloramphenicol the deamination of phenylalanine was inhibited 25 per cent in this experiment but the oxygen uptake was inhibited 63 per cent. Thus the drug inhibited the oxidation of the molecule as a whole more than it did the oxidative deamination. An even more striking example of this is seen in table 2 where the deamination was inhibited less than 10 per cent by streptomycin and the oxygen uptake was inhibited 66 per cent. Table 1 also shows that if the amino nitrogen remaining is added to the ammonia

TABLE 1

*The effect of chloramphenicol on the oxidation and deamination of 1.0 mgm. each of phenylalanine, tyrosine and phenylserine at pH 7.8, 37°*

The figures are in micrograms. The theoretical values for the total nitrogen added are 85, 77 and 77 micrograms, respectively. The per cent inhibition of deamination was calculated from the $NH_2$-N remaining.

| AMINO ACID | $NH_2 - N$ | $NH_3 - N$ | TOTAL | % INHIBITION OF DEAMINATION | % INHIBITION OF OXIDATION |
|---|---|---|---|---|---|
| phenylalanine | 51.4 | 28.9 | 80.3 | | |
| + 5 microgm./ml. chloramphenicol | 72.3 | 18.9 | 91.2 | 25 | 63 |
| tyrosine | 5.9 | 26.7 | 32.6 | | |
| + 100 microgm./ml. chloramphenicol | 32.4 | 38.0 | 70.4 | 34 | 45 |
| phenylserine | 2.7 | 29.6 | 32.3 | | |
| + 100 microgm./ml. chloramphenicol | 37.7 | 40.5 | 78.2 | 44 | 35 |

TABLE 2

*The effect of several antibiotics on the $NH_3$-N recovered from phenylalanine, tyrosine and phenylserine after incubation at pH 7.8, 37°*

The drugs were present in a concentration of 100 microgm./ml.

| AMINO ACID | DRUG | $NH_3 - N$ | % INHIBITION OF OXIDATION |
|---|---|---|---|
| phenylalanine | — | 29.7 | |
| | terramycin | 13.2 | 81 |
| | aureomycin | 13.4 | 83 |
| | streptomycin | 27.0 | 66 |
| tyrosine | — | 44.9 | |
| | terramycin | 51.6 | 45 |
| | aureomycin | 50.3 | 48 |
| | streptomycin | 57.2 | 41 |
| phenylserine | — | 44.2 | |
| | terramycin | 52.2 | 47 |
| | aureomycin | 55.7 | 56 |
| | streptomycin | 67.3 | 42 |

nitrogen produced the total equals, within experimental error, the theoretical amount of nitrogen added. This is not true for tyrosine and phenylserine. In the absence of the drugs the total nitrogen recovered was always much less than the theoretical. This suggests that the ammonia produced by the deamination is in part assimilated during the oxidation of the rest of the molecule. In the presence of the antibiotics this assimilation was inhibited, more nitrogen remained as ammonia, and as shown in table 1 for chloramphenicol, the total nitrogen recovered approached the theoretical. It thus appears that no ammonia is assimilated during the oxidation of phenylalanine and the drugs inhibited the oxidation and to a lesser extent the deamination. For tyrosine and phenylserine the drugs inhibited oxidation, deamination and the subsequent assimilation of ammonia as well (table 2).

Previously it has been shown (Bernheim and DeTurk, 1951) that streptomycin inhibits the assimilation of ammonia by these organisms during the oxidation of succinate and certain other acids of the Krebs' cycle. At pH 6.0 the oxidation of these compounds is not inhibited but assimilation is. At pH 7.8 both oxidation

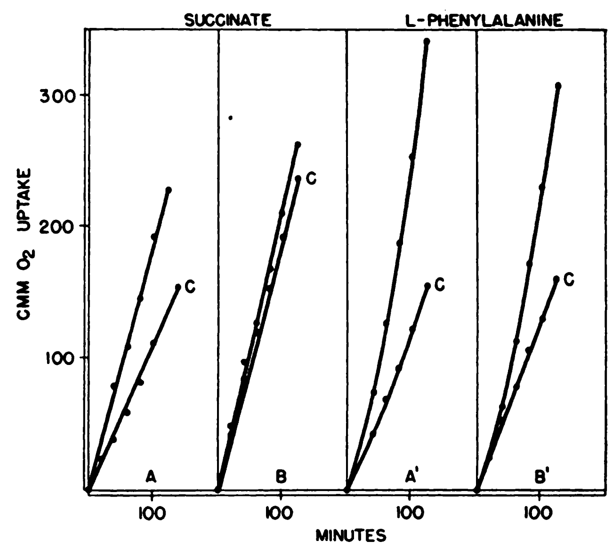


FIG. 5. The effect of 5 microgm./ml. of chloramphenicol on the oxidation of 2.0 mgm. succinate and 1.0 mgm. phenylalanine. In A and A[1] the drug was added 10 min. before the substrate; in B and B[1] 10 min. after the substrate. pH 7.8, 37°.

and assimilation are inhibited. Chloramphenicol behaved in the same way. In low concentrations at pH 7.8 it inhibited oxidation and assimilation, at pH 6.0 only assimilation. Its effect on the oxidation of succinate is shown in fig. 5. If added before the succinate, inhibition occurred but if added after succinate it had little effect. On the other hand the previous addition of phenylalanine failed to protect the enzyme from the action of the drug. This is also shown in fig. 5.

*Discussion.* On the basis of general structural similarity, chloramphenicol and mandelic acid might be expected to inhibit the oxidation of phenylserine to a greater extent than that of phenylalanine, and thienylalanine to be more effective on phenylalanine than on phenylserine. Actually the reverse is true for the first two drugs and the last is almost equally effective on both amino acids. A differential rate of penetration of the drugs to the sites of the various enzymes involved in the oxidations is apparently not a possible explanation since pre-incubation of the bacteria with the drugs for varying lengths of time did not alter the relative effects. The antibiotics regardless of their structure appear able to inhibit the oxidation of phenylalanine and succinate, for instance, to a greater extent than they inhibit tyrosine or phenylserine. In fact all the antibiotics tested, including thienylalanine but not mandelic acid, seem able to interfere directly or indirectly with the assimilation of ammonia which occurs in this organism simultaneously with the oxidation of a suitable substrate. This suggests that they interfere with some phosphorylating mechanism involved in the assimilation. Because of the change in rate during the oxidation of phenylalanine, which did not occur in the oxidation of tyrosine and phenylserine, it is possible that phosphorylated compounds are necessary for the oxidation of phenylalanine but not for the oxidation of the other two amino acids. This use of high energy phosphate compounds for the further oxidation of phenylalanine might take preference over their use for assimilation and this would offer a possible explanation for the fact that no ammonia was assimilated during the oxidation of phenylalanine as well as the fact that the oxidation of this amino acid is the most sensitive to the action of the antibiotics tested.

## SUMMARY

1. Chloramphenicol, streptomycin, terramycin and aureomycin inhibit the oxidation and to a lesser extent the deamination of phenylalanine, tyrosine and phenylserine by a strain of Pseudomonas aeruginosa. They also inhibit the oxidation of succinate and certain other dicarboxylic acids.

2. The oxidation of phenylalanine is most sensitive to the action of these drugs. During its oxidation no ammonia is assimilated. During the oxidation of tyrosine and phenylserine part of the ammonia produced is assimilated and this assimilation is inhibited by the drugs.

3. It appears that the antibiotics tested interfere directly or indirectly with the formation of compounds which may be necessary for the assimilation of ammonia or in the case of phenylalanine for its oxidation.

4. Thienylalanine inhibits the oxidation of phenylalanine and phenylserine and to a lesser extent tyrosine. It also inhibits the assimilation of ammonia during the oxidation of succinate. Mandelic acid has no effect on this latter reaction and is only effective in inhibiting the oxidation of phenylalanine in the acid range.

## REFERENCES

Bernheim, F., and DeTurk, W. E.: This Journal, **103**: 107, 1951.
Speck, J. F.: J. Biol. Chem., **179**: 1387, 1949.