The Influence of Bactericidal Agents and of the Absence of L-Ascorbic Acid on the Accumulation of Hydroxyphenyl Compounds in the Large Intestine of Guinea Pigs after the Consumption of High Doses of L-Tyrosine

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In a previous communication (Painter & Zilva, 1947) it was shown that very much larger quantities of hydroxyphenyl compounds* were found in the large intestine, mainly in the caecum, 3 or 5 hr. after the ingestion of high doses of L-tyrosine when guinea pigs were depleted of L-ascorbic acid, than when they received cabbage *ad lib.* with the basal diet. It was further observed that a similar accumulation in the large intestine could be found when sulphaguanidine was administered to guinea pigs subsisting on the basal diet with cabbage *ad lib.* In this paper experimental evidence is given which affords an explanation of this phenomenon, and observations have been made which are of fundamental theoretical interest and have possibilities of therapeutic application.

EXPERIMENTAL

Technique. For details of experimental animals and diet, see Penney & Zilva (1946). For methods of collection of urine, preparation of tissues for analysis, determination of total hydroxyphenyl compounds ('tyrosine'), and L-tyrosine, see Painter & Zilva (1947).

The samples of sulphaguanidine, succinylsulphathiazole and phthalylsulphathiazole used were commercial medicinal products.

Administration of the bactericidal agents. In the most intensive experiments of long duration (8–15 days), the compounds were administered as aqueous suspensions in quantities varying from 0.1 to 0.3 g., the doses being adjusted from day to day so as to prevent a loss in weight of the animals. In the case of the experiments of shorter duration (3 days) daily graded doses of 0.05, 0.1, 0.2 and 0.3 g. of sulphaguanidine were administered to the respective groups. Experiments have proved that guinea pigs on a diet containing cabbage *ad lib.*, even after dosage with sulphaguanidine for 8 days, were fully saturated in respect of L-ascorbic acid.

* Hydroxyphenyl compounds are usually determined by methods which are based on Millon's reaction. A negative reaction is then taken to indicate rupture of the benzene ring, although in reality it only indicates the disappearance of the phenolic group. For the sake of greater accuracy we abstain in this communication from referring to the rupture of the benzene ring in cases in which the Millon's reaction is absent.

RESULTS

The 'tyrosine' content of the large intestine of guinea pigs after the administration of L-tyrosine under different dietetic conditions.

Table 1 summarizes the results with sixteen groups, each of 12 guinea pigs. All the animals were killed 3 hr. after the administration of 0.5 g. of L-tyrosine and the contents of the large intestine, comprising the caecum, colon and rectum, were immediately analyzed for hydroxyphenyl compounds by Folin & Ciocalteu's method (1927). The values thus obtained are calculated as 'tyrosine'. No significant quantities of these compounds were found in the faeces passed during the 3 hr. between the administration of the L-tyrosine and slaughter. References in the text to animals depleted for a certain time signify the time which elapsed since the removal of the cabbage, previously administered *ad lib.*, from the basal scorbutic diet. The statistical treatment of the data was carried out as described by Snedecor (1937) for the determination of the *t* value.

The mean value for 'tyrosine' obtained with the animals receiving cabbage *ad lib.* in addition to the basal scorbutic diet during the pre-experimental period (group 1) was very low compared with that obtained with the animals which were depleted for 14 days (group 6). Group 1 also has a wide variation of the individual values. No other group, with the possible exception of group 16, showed this characteristic. This is particularly evident when the high ratio of the standard deviation to the mean is compared with similar ratios in the other groups. This observation is in accordance with expectation, if it be assumed that the quantity of 'tyrosine' found in the large intestine is controlled by the quantity of cabbage present there when the administered L-tyrosine reaches the large intestine in considerable quantities. The amount of the cabbage present would in such a case depend on the quantity eaten and on the time when it was consumed and this would vary with the individual animal. In all the other groups the conditions were more under the control of the experimenter and thus the fluctuations were not so marked.

Table 1. *The 'tyrosine' content of the large intestine of guinea pigs 3 hr. after the administration of 0.5 g. L-tyrosine to animals previously maintained under various dietetic conditions*

Group no.	Addition to basal diet	Days	Treatment on day of L-tyrosine dosing	Mean 'tyrosine' content (expressed as mg. L-tyrosine)	S.D.	$\frac{\text{S.D.}}{\text{Mean}} \times 100$
1	Cabbage <i>ad lib.</i> in the pre-experimental period	0	—	24	29	121
2	Nil	1	—	94	39	42
3	Nil	3	—	134	51	38
4	Nil	5	—	104	49	47
5	Nil	8	—	106	30	28
6	Nil	14	—	159	46	29
7	25 mg. L-ascorbic acid daily	6	—	65	32	49
8	Nil	1	25 mg. L-ascorbic acid with the L-tyrosine	86	41	48
9	Nil	1	100 mg. L-ascorbic acid with the L-tyrosine	58	31	53
10	Nil	14	100 mg. L-ascorbic acid with the L-tyrosine	88	46	52
11	Nil	6	100 mg. L-ascorbic acid with the L-tyrosine	85	39	46
12	Nil	14	25 mg. L-ascorbic acid with the L-tyrosine	108	53	49
13	Nil	14	100 mg. L-ascorbic acid 1 hr. before, with, and 1 hr. after the L-tyrosine	76	47	62
14	Nil	14	Cabbage <i>ad lib.</i> immediately after the L-tyrosine	92	35	38
15	Nil	14	100 mg. L-ascorbic acid with the L-tyrosine and cabbage <i>ad lib.</i> immediately after	98	24	25
16	Nil	14	Cabbage <i>ad lib.</i> 2 hr. before the L-tyrosine, 100 mg. L-ascorbic acid with the L-tyrosine and cabbage <i>ad lib.</i> after	29	26	90

That the disappearance of the 'tyrosine' from the large intestine depends on the constant presence of cabbage is seen from the fact that after the removal of the cabbage from the diet for 24 hr. (group 2), the quantity of 'tyrosine' present in the large intestine became significantly higher than in group 1 ($t = 4.98$), but it still remained significantly lower than that found after 14 days' depletion (group 6, $t = 3.73$). Unfortunately, an attempt to find out how soon after the removal of the cabbage from the diet the maximum intestinal content of 'tyrosine' might be reached was not completely successful as experiments carried out 3, 5 and 8 days after the removal of the cabbage from the diet (groups 3-5) yielded values which were not always significantly different among themselves, and sometimes not even

so when compared with the mean obtained after 14 days' depletion (cf. groups 3 and 6, $t = 1.26$). This is possibly due to the great individual variation in the time during which the unaltered cabbage remains in the lower reaches of the intestinal tract.

The first attempt to ascertain whether the degradation of the phenolic group of the L-tyrosine in the large intestine was connected with the vitamin C content of the cabbage was made by administering daily 25 mg. of synthetic L-ascorbic acid in solution for 6 days to guinea pigs maintained on the basal scorbutic diet, the last dose of the vitamin being given with the L-tyrosine. It was found (group 7) that the mean of the contents of this group was of the same order as that of group 2 (animals depleted for

24 hr.), but was, nevertheless, significantly higher than that of group 1 which received cabbage *ad lib.* ($t=3.29$). The essential point, however, is that the means of groups 3-6 which were depleted for 3, 5, 8 and 14 days respectively ($t=3.97, 2.31, 3.24$ and 5.81 respectively) were markedly higher. In other words, the presence of L-ascorbic acid *per se* is capable of producing a speedier disappearance of the phenolic group of the 'tyrosine' in the intestine. It is of interest to note that after 1 day's depletion the 'tyrosine' values could not be lowered by the administration of 25 or 100 mg. of L-ascorbic acid with the L-tyrosine dose (*vide* groups 8 and 9). Yet after 6 or 14 days' depletion the 'tyrosine' values could be lowered to that obtained after 1 day's depletion by the administration with the L-tyrosine of 25, 100 and 300 mg. (in three successive doses of 100 mg.) of L-ascorbic acid (*vide* groups 10-13).

Various attempts to attain the low 'tyrosine' values observed in the case of guinea pigs on a diet containing cabbage *ad lib.* were made. This was accomplished only when cabbage was offered *ad lib.* to guinea pigs 2 hr. before the administration of 0.5 g. of L-tyrosine with 100 mg. of L-ascorbic acid and subsequent cabbage *ad lib.* (group 16). On the other hand, the administration to guinea pigs depleted for 14 days of (a) three successive doses of 100 mg. of L-ascorbic acid, one before, one with and one after the dose of L-tyrosine (group 13), (b) of cabbage *ad lib.* with the L-tyrosine (group 14) and (c) of 100 mg. of L-ascorbic acid in addition to cabbage *ad lib.* with the dose of L-tyrosine (group 15), only lowered the large intestine 'tyrosine' values to values approximating that of the mean of the group of animals depleted for 1 day (group 2). It will be noted that in group 16 the individual values are as widely distributed as in group 1, probably for the same reason.

The evidence in this series of experiments definitely supports the view that the consumption of vitamin C favours the disappearance of the phenolic group of L-tyrosine or of the hydroxyphenyl degradation products of this compound from the intestine. The higher efficiency of cabbage as an agent in this process of degradation as compared with L-ascorbic acid in solution cannot be fully explained at present. Two possible explanations are worth considering. One is that during the transit of the cabbage in the alkaline section of the intestine the vitamin is more protected, and that in the large intestine there is a gradual release of the L-ascorbic acid. In consequence, the total quantity of the vitamin is more efficiently utilized in the reaction in question than when the L-ascorbic acid is in solution, and is more accessible for destruction through other agencies. Alternatively, the cabbage, apart from its physical condition, may contain another sub-

stance which conduces to the breakdown of L-tyrosine in the intestine. We incline to the former view.

The influence of bactericidal agents on the degradation of L-tyrosine in the intestines of guinea pigs

The results of the above experiments strongly indicated the possibility that the accumulation of 'tyrosine' in the large intestine of the depleted guinea pigs was the consequence either of a change in the intestinal flora which was caused by the absence of L-ascorbic acid or of a change in the capacity of some of these organisms to metabolize the phenolic group of L-tyrosine and its degradation products. The obvious approach to the elucidation of the problem was, therefore, to study the influence of bactericidal agents known to modify the bacterial flora of the intestine. The action of sulphaguanidine, succinylsulphathiazole and phthalylsulphathiazole was accordingly investigated with this point in view. In each case it was found that these agents when administered *per os* to animals receiving cabbage *ad lib.* produced, as in the case of the depleted animals, an accumulation of 'tyrosine' in the large intestine, mainly in the caecum. A detailed and comparative study of guinea pigs receiving cabbage *ad lib.* and sulphaguanidine as the bactericidal agent on the one hand, and depleted guinea pigs on the other, has, however, revealed some differences between the changes brought about by the presence of the bactericidal agent and the absence of L-ascorbic acid from the diet. These differences, as will be seen later, did not, however, militate against the hypothesis that the absence of L-ascorbic acid and the presence of the bactericidal agent were capable of modifying the intestinal flora of the guinea pig so as to interfere with their degradation of the L-tyrosine present there.

The results of this group of experiments are summarized in Tables 2 and 3. The outstanding fact which emerges from Table 2 is that 3 hr. after the administration of L-tyrosine there is a great accumulation of 'tyrosine' in the large intestine of the depleted guinea pigs and of the animals which received cabbage *ad lib.* and sulphaguanidine, when compared with the content found in the case of the animals receiving only cabbage *ad lib.* Even a daily dose of 0.1 g. of sulphaguanidine given for 3 days previous to the administration of L-tyrosine already produced this effect. The data in Table 2 disclose a further point which, apart from its general interest, has a bearing on the present problem. It will be seen that the concentration and contents of the hydroxyphenyl compounds in the tissues of the entire intestinal tract in the groups of the depleted animals and of the animals receiving cabbage *ad lib.* were much higher than in the case of the groups which were dosed with sulphaguanidine. In the

Table 2. The distribution of 'tyrosine' in the body and urine of guinea pigs maintained on diets containing cabbage ad lib. with and without sulphaguanidine, and that of guinea pigs on a scorbutic diet 3 hr. after the administration of 0.5 g. L-tyrosine

Mixed diet containing cabbage												Scorbutic diet	
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latter animals, although the size of the dose and the time during which it was administered did not influence the content of 'tyrosine' in the large intestine to an appreciable extent, it did, nevertheless, influence the content and concentration of the hydroxyphenyl compounds in the walls of the intestinal tract. In the animals dosed intensively (0.1–0.3 g. sulphaguanidine for 12–15 days) only traces of 'tyrosine' could be found in the intestinal tissues, but low concentrations, bearing a roughly inverse proportion to the dose, could be observed in the animals treated less drastically with the bactericidal agent (0.1, 0.2 and 0.3 g. sulphaguanidine for 3 days). The latter 'tyrosine' values were, however, considerably lower than those found in the case of the animals receiving no sulphaguanidine. The difference in the 'tyrosine' content and concentration between the animals dosed with the sulphaguanidine and the other guinea pigs is even more marked in the case of the blood, liver, kidney and muscle. All these differences could be explained by an impaired passage of the 'tyrosine' into the blood stream brought about by the consumption of the bactericidal agent.

This view was strengthened by further evidence. In Table 3 are given the 'tyrosine' contents of (a) the intestinal tract and faeces, (b) the tissues, and (c) the urine of all the animals 3 hr. (summarized from Table 2) and 24 hr. after the administration of the dose of L-tyrosine. It is seen that while the greater part of the 'tyrosine' in the tissues and large intestine found 3 hr. after the administration of the L-tyrosine to the depleted guinea pigs was eliminated by the kidney during the following 21 hr. (169 out of 219 mg.), only about one eighth was accounted for in the urine of the sulphaguanidine-treated guinea pigs. This fact taken in conjunction with the observation that the tissues of these animals, as mentioned above, contained little or no 'tyrosine' when compared with the tissues of the guinea pigs which did not receive sulphaguanidine, supports the view that the consumption of sulphaguanidine greatly impaired the passage of the 'tyrosine' into the blood stream. That some of these compounds did pass into the circulation is seen from the fact that the mean of the 'tyrosine' content of the urine values of the animals receiving sulphaguanidine for 12–15 days was 24.7 ± 15.6 mg. (Table 3). This value was significantly higher than the mean we have previously obtained from a control group of 12 guinea pigs not receiving L-tyrosine (6.3 ± 4.1 mg.; $t = 3.95$). Furthermore, we have found that, by the prolonged administration of L-tyrosine to guinea pigs receiving sulphaguanidine, 'tyrosine' values reaching 60 mg./day could be attained. Therefore, although some of the 'tyrosine' did enter the system, the best part of it seems to have disappeared from the gut, but at a very much slower rate than in the case of guinea

pigs receiving cabbage *ad lib.* without sulphaguanidine. In the case of these animals only 57.9 mg. of 'tyrosine' out of the 500 mg. of L-tyrosine received was present in the large intestine and in the tissues 3 hr. after the administration of L-tyrosine against 154 mg. present, entirely in the large intestine, in the case of guinea pigs which received 100–300 mg. of sulphaguanidine daily for 12–15 days. After a further 21 hr. not a trace was found in the intestines and tissues in the former group of animals, whilst in the latter 21.3 mg. 'tyrosine' was still present in the intestinal contents and in the faeces. The evidence indicates that the bactericidal agent had significantly diminished the intestinal bacterial population which is capable of attacking the phenolic group in L-tyrosine, and in consequence the phenolic group of the L-tyrosine or of the hydroxyphenyl degradation products was destroyed to a smaller extent during the first few hours than in the case of the animals on the same diet which did not receive sulphaguanidine.

DISCUSSION

The evidence of this investigation leaves little doubt that the accumulation of 'tyrosine' in the large intestine 3 hr. after the administration of L-tyrosine in guinea pigs on a diet not containing cabbage is due to the absence of L-ascorbic acid *per se* and that the accumulation of 'tyrosine' in the gut of the depleted animals is primarily responsible for the incomplete breakdown of the phenolic group in some of the animals receiving a massive dose of L-tyrosine. The reason why the administration of cabbage is more effective than L-ascorbic acid in obviating the accumulation of 'tyrosine' in the large intestine is not quite clear, but in all probability it is due to the greater protection of the L-ascorbic acid when the cabbage is passing through the alkaline region of the small intestine and to the gradual release of the vitamin from the plant tissue. It was further seen that a similar accumulation of 'tyrosine', but without the appearance in the urine of an excess of hydroxyphenyl compounds, can be produced by the administration to guinea pigs of bactericidal agents such as sulphaguanidine, succinylsulphathiazole and phthalylsulphathiazole even when the animals consume cabbage *ad lib.* In order to obtain a clear understanding of the problem it is necessary to correlate the present results with those described by us in a previous communication (Painter & Zilva, 1947).

In the first investigation we demonstrated that when a high enough dose of L-tyrosine was injected in depleted guinea pigs intraperitoneally as glycyl-L-tyrosine, i.e. when the concentration of L-tyrosine in the blood was raised to a certain critical level, there was an increase in the amount of the total hydroxyphenyl compounds eliminated by the kidney and *p*-hydroxyphenylpyruvic acid was excreted in the

urine at the same time.* The phenolic group in a part of the L-tyrosine was, therefore, not broken down in the system. Very much higher doses of glycyl-L-tyrosine had to be injected into animals receiving cabbage to bring about this incomplete degradation of the L-tyrosine, evidently because of the much higher concentration of L-ascorbic acid in the blood of these animals. It was, therefore, not unwarranted to assume that a critical concentration of 'tyrosine' in the blood, similar to that obtained by injection of high doses of glycyl-L-tyrosine, could also be attained by permeation from the large intestine of depleted guinea pigs. The accumulation in the lower reaches of the intestinal tract caused by the absence of L-ascorbic acid from the diet of these animals must, therefore, be considered primarily responsible for the partial degradation of the phenolic group in the large dose of L-tyrosine. The fate of the 'tyrosine' accumulated in the large intestine of the guinea pigs which received cabbage and sulphaguanidine was different. It will be recalled that the intestinal tissues, blood, liver, kidney and muscle did not contain any appreciable quantities of 'tyrosine', and that only about one eighth of the accumulated 'tyrosine' was accounted for in the urine. This strongly suggested that little of these hydroxyphenyl compounds entered the blood stream and that the highest concentration thus reached was below the critical concentration necessary to produce an abnormal degradation of the compounds in question attained in the depleted guinea pigs. Consequently, no *p*-hydroxyphenylpyruvic acid was formed under these conditions, not even when L-tyrosine was administered on several successive days. Since, however, the greater part of the accumulated 'tyrosine' disappeared within the next 21 hr. (*vide* Table 3) it may be assumed that these compounds have been slowly destroyed in the intestine, as compared with the animals which received cabbage without sulphaguanidine, especially as some 'tyrosine' was, under these circumstances, still present in the intestinal contents and in the faeces. We are, therefore, faced with the following outstanding facts: (a) A quick destruction of 'tyrosine' in the case of the guinea pigs receiving L-ascorbic acid, since the amount present in the large intestine after 3 hr. was small, and although this entered freely into the blood stream containing 'saturated values' of L-ascorbic acid it could not reach the critical concen-

tration above which the system was incapable of metabolizing these compounds normally. Only 3 % of the 0.5 g. of L-tyrosine was accounted for after 24 hr., the remainder having been destroyed. (b) A much slower destruction of the 'tyrosine' with a resultant accumulation in the large intestine after 3 hr. in the case of the guinea pigs receiving cabbage and sulphaguanidine. The bulk of this was slowly destroyed in the lower reaches of the intestine during the following 21 hr. In this case 4-9 % of the consumed L-tyrosine was accounted for (Table 3), most of it being destroyed in the intestine. (c) A slow destruction of the 'tyrosine' in the depleted animals with an accumulation in the large intestine. In this case, however, the hydroxyphenyl compounds enter the circulation freely, and the concentration in the blood is such that in the presence of little ascorbic acid the phenolic group cannot be degraded in all the 'tyrosine', and at the same time some phenolic compounds are produced which are evidently intermediate in the degradation of the L-tyrosine. After 24 hr. 41 % of the L-tyrosine was accounted for in the urine as hydroxyphenyl compounds (Table 3).

If the possibility of a very rapid passage into the blood stream is excluded, the evidence appears to justify the assumption that the L-tyrosine in the intestine of the guinea pig is normally quickly destroyed by intestinal organisms and that the rate of this destruction can be slowed down to a considerable extent either by administering bactericidal agents or by withholding L-ascorbic acid from the diet. In the former case the explanation is fairly clear; the bactericidal agent diminishes the population of the intestinal organisms including those destroying L-tyrosine. In the latter case other possibilities may have to be considered. The presence of L-ascorbic acid may favour organisms, capable of attacking the phenolic group, which are suppressed in the absence of the vitamin. The vitamin may even act as a necessary link in a chain of reactions concerned in this degradation. The literature on the subject does not throw much light on the problem. The action of various organisms on L-ascorbic acid has been investigated by several workers (Stepp & Schröder, 1935; Kendall & Chinn, 1938; Esselen & Fuller, 1939; Young & James, 1942; Young & Rettger, 1943). Certain strains of these organisms under the requisite conditions can oxidize L-ascorbic acid, mostly beyond the reversible stage, but some, such as *Escherichia coli*, which is actually capable of reducing dehydro-L-ascorbic acid, can act in a protective capacity. Fortunately, according to Crece-lius & Rettger (1943), the normal flora of the guinea pig intestine is relatively simple, consisting mainly (about 80 %) of a non-sporulating rod form of *Lactobacillus*. The remainder of the flora is composed of yeasts and of soil and air bacteria. The investigation of the action of these organisms on phenolic

* In our description of the method of determination of *p*-hydroxyphenylpyruvic acid (Painter & Zilva, 1947) we stated inadvertently that the solution of the compound in 2.5 % metaphosphoric acid used for the calibration curves was allowed to remain 30 min. after being adjusted to pH 9.0. This should have read that the solution of *p*-hydroxyphenylpyruvic acid was adjusted to pH 9.0, allowed to remain for 30 min., after which metaphosphoric acid was added so that the final concentration was 2.5 %.

amino-acids in the presence and in the absence of L-ascorbic acid may supply information not only of fundamental theoretical interest but also lead to therapeutic applications. The observation that some bactericidal agents are capable of modifying under these abnormal conditions the passage of 'tyrosine' into the blood stream is also of fundamental theoretical and practical interest. As was seen, this impairment of the passage was only partial, since some of the hydroxyphenyl compounds have been shown to enter the blood stream. It may also be noted that the guinea pigs receiving the bactericidal agent were found to be saturated in respect of L-ascorbic acid. This problem requires further investigation.

SUMMARY

1. Three hours after the administration of 0.5 g. of L-tyrosine more hydroxyphenyl compounds were found in the large intestine, particularly in the caecum, of guinea pigs which were depleted of L-ascorbic acid and in that of guinea pigs receiving sulphaguanidine, succinylsulphathiazole or phthalylsulphathiazole with cabbage *ad lib.* than in guinea pigs receiving vitamin C in the form of cabbage or L-ascorbic acid in solution without the bactericidal agents.

2. A number of experiments are described in which the influence of the mode of administration and of the quantity of the added L-ascorbic acid was investigated, and it is concluded that L-ascorbic acid as present in cabbage is more effective than as the synthetic compound in solution.

3. A great part of the hydroxyphenyl compounds present in the large intestine of the depleted guinea pigs 3 hr. after the administration of the L-tyrosine

was found in the urine of the animals 21 hr. later. Only a small portion of these accumulated compounds was present in the urine of the guinea pigs receiving cabbage *ad lib.* and sulphaguanidine.

4. The concentrations of hydroxyphenyl compounds in the walls of the intestine, the blood, the liver, the kidney and the muscle were much higher in the depleted animals and in the animals receiving cabbage *ad lib.* without sulphaguanidine than in the corresponding tissues of animals receiving the bactericidal agent.

5. Excluding a very rapid passage into the blood stream, it may be assumed that the lower content of hydroxyphenyl compounds in the large intestine of the animals receiving cabbage *ad lib.* or synthetic L-ascorbic acid in solution without sulphaguanidine is due to the influence of the intestinal bacterial flora which is modified by either the absence of L-ascorbic acid from or the presence of bactericidal agents in the diet of the guinea pigs. In the first case, owing to the free passage into the circulation, the concentration in the blood reaches levels which are too high for these hydroxyphenyl compounds to be metabolized normally. In the second case the impaired passage into the blood stream brought about by the consumption of the bactericidal agents prevented the production of such a critical concentration of hydroxyphenyl compounds in the circulation, and the greater part of these compounds was destroyed slowly during passage along the large intestine.

6. The fundamental and practical potentialities of these observations are stressed.

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