SUMMARY

1. The pink solutions yielded by oestrone or oestriol in the Kober reaction with the phenol-sulphonic acid reagent are completely decolorized after heating at 100° for 1.5 hr.

2. The brown solutions yielded by ether-soluble phenolic fractions of human male urine in the Kober reaction undergo little change, as judged by absorption at c. 520 mμ., when similarly treated.

3. On the basis of these observations a simple method has been developed of correcting for the interfering brown colour which is developed from non-oestrogenic substances in the determination of oestrogens in human pregnancy urine.

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The Influence of *l*-Ascorbic Acid on the Rupture of the Benzene Ring of *l*-Tyrosine Consumed in High Doses by Guinea-pigs

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Kotake, Matsuoka & Okagawa (1922) observed that when a massive dose (10 g.) of *l*-tyrosine was administered to adult rabbits the aromatic part of the *l*-tyrosine molecule was not fully metabolized, since *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids were excreted in the urine. Recent work by Sealock & Silberstein (1939, 1940) and Levine, Marples & Gordon (1939, 1941*a*, 1941*b*) threw a new light on the rupture of the benzene ring of *l*-tyrosine by the animal organism. The former investigators found that when 0.5 g. or more of *l*-tyrosine was administered to guinea-pigs on a scorbutic diet, homogentisic, *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids were excreted in the urine. When, however, *l*-ascorbic acid was administered in sufficiently high doses the excretion of these aromatic compounds ceased. Levine and his co-workers made a similar observation on infants. They found that premature infants on diets containing 5 g./kg. body weight/day or more of protein excreted *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic, but not homogentisic acids.

On the other hand, full-term infants on a similar diet only excreted these aromatic amino-acids when they consumed 1 g./kg. body weight of either *l*-tyrosine or *l*-phenylalanine. In both cases the excretion of the aromatic amino-acids could be abolished by the consumption of *l*-ascorbic acid. These observations led to a tentative suggestion that *l*-ascorbic acid participated directly or indirectly in protein metabolism. The experiments described in this communication were primarily devised to test this hypothesis.

EXPERIMENTAL

Technique

Experimental animals and diet (see Penney & Zilva, 1946).

Collection of urine. The experimental animals were kept in metabolism cages and the urines were collected in tubes which, except in experiments of short duration, contained sufficient HCl to keep the urines acid to congo red. The urines to be tested for homogentisic acid, were collected under liquid paraffin.

Preparation of tissues for analysis. The animals were killed by stunning and bleeding and the blood was collected in oxalate. Metaphosphoric acid was used in the extraction of the tissues instead of trichloroacetic acid, as the latter interfered with some of the colorimetric determinations. The whole blood and the urine were precipitated with equal volumes of 10% metaphosphoric acid. The liver, muscle, and the small and large intestines, the latter after removal of the contents and subsequent washing with water and drying the tissue with filter-paper, were extracted twice with one part by weight of 8.5% metaphosphoric acid by grinding with sand and centrifuging. The kidney and stomach were similarly treated with 4 parts by weight of 5% metaphosphoric acid. The contents of the intestinal tract, to which were added any faeces present (although the latter contained only traces of hydroxyphenyl compounds), were diluted with the smallest practicable amount of water and extracted with $\frac{1}{3}$ vol. of 20% metaphosphoric acid. The metaphosphoric acid extracts of the large intestines and the liver were sometimes found to be turbid, but the extract could be cleared with small quantities of kieselguhr, a process which did not interfere with the analyses.

Determination of total hydroxyphenyl compounds. This was carried out by the method of Folin & Ciocalteu (1927) as modified by Medes (1932), except that a calibration curve was used instead of standards. All experimental values were expressed in terms of tyrosine.

Determination of *p*-hydroxyphenylpyruvic acid. This was carried out by the Penrose & Quastel (1937) test using a calibration curve, obtained from a solution of *p*-hydroxyphenylpyruvic acid in 2.5% metaphosphoric acid which was allowed to remain for 30 min. after being adjusted to pH 9.0 (see Penney & Zilva, 1943; Painter & Zilva, 1947). It is to be pointed out that this test, apart from being more specific in this instance, is also more delicate than the Millon test and that the 2,4-dinitrophenylhydrazones has been isolated from the urines of guinea-pigs on a scorbutic diet receiving an excess of *l*-tyrosine.

Glycyl-*l*-tyrosine. This was prepared by the method of Fischer (1904). Preparations obtained by a single precipitation with ethanol were used. The purity of these preparations was assessed by the Folin & Ciocalteu method and was found to be about 85%.

***l*-Tyrosine.** The commercial product was used for dosing but for standardizing purposes it was purified by the method of Folin & Ciocalteu (1927).

The influence of the method of administering high doses on the degradation of l-tyrosine by the guinea-pig

In the majority of their experiments, Sealock & Silberstein incorporated the *l*-tyrosine in the basal diet. The substance was thus consumed intermittently. It seemed to us that the oral administration of *l*-tyrosine suspended in a little water and given in one dose might be more advantageous, and it was, therefore, decided to investigate the excretion of the aromatic degradation products of tyrosine in the two procedures.

One group of three guinea-pigs on a scorbutic diet received orally in one dose 0.5 g. of *l*-tyrosine suspended in water, and in a similar group the

l-tyrosine was incorporated in the basal diet. In both groups the amounts of total hydroxyphenyl compounds and of *p*-hydroxyphenylpyruvic acid excreted in the urine during 24 hr. were determined. The daily quantity of *l*-tyrosine consumed by animals receiving it in the diet varied from 0.5 to 0.8 g., and the amounts of excretion products determined were recalculated for a uniform consumption of 0.5 g. In Fig. 1 the results are shown as the means

Fig. 1. Influence of the method of administration on the degradation of 0.5 g. of *l*-tyrosine by the guinea-pig. — Tyrosine administered in one single daily dose. ---- Tyrosine added to basal diet. *M* = total hydroxyphenyl compounds. *K* = *p*-hydroxyphenylpyruvic acid.

for three animals. It will be seen that when the *l*-tyrosine was consumed in a single dose instead of intermittently a higher percentage of the tyrosine was excreted as aromatic compounds. For this reason we considered the single-dose technique more suitable for the investigation of our problem. In both cases the excretion of aromatic compounds commenced soon after the *l*-ascorbic acid was removed from the diet. When the *l*-tyrosine was given

in one dose the ratio K/M, i.e. *p*-hydroxyphenylpyruvic acid to total hydroxyphenyl compounds, was lower during the first 4 days of the *l*-ascorbic acid deprivation period, viz. 0.23 ± 0.14 for the first 4 days and 0.61 ± 0.10 for the next 10 days.

The influence of the magnitude of the dose on the excretion of aromatic intermediate products of l-tyrosine degradation

The aim of these experiments was to establish approximately the amounts of *l*-tyrosine which a guinea-pig on a scorbutic diet must consume, so that aromatic intermediate products of *l*-tyrosine degradation might appear in the urine. Single doses of 0.1, 0.2, 0.3, 0.4 and 0.5 g. of *l*-tyrosine were therefore administered orally to five groups of three guinea-pigs, 8 days and, again, 14 days after being put on a scorbutic diet. For comparison the amounts of total hydroxyphenyl derivatives and *p*-hydroxyphenylpyruvic acid excreted were determined for each animal on the day before the *l*-tyrosine was administered.

were deducted from the values obtained after administration of *l*-tyrosine. There was an increase in the excretion of *p*-hydroxyphenylpyruvic acid in only one of the guinea-pigs which received 0.1 g. of *l*-tyrosine after 8 days on a scorbutic diet, though there was a general small increase in the excretion of the total hydroxyphenyl derivatives on this dose. After 14 days of scorbutic diet there was a general increase in the excretion of both *p*-hydroxyphenylpyruvic acid and the total hydroxyphenyl derivatives in the group of animals given 0.1 g. of tyrosine. This increase was more marked in the animals receiving 0.2, 0.3 and particularly 0.4 and 0.5 g. of *l*-tyrosine after 8 or 14 days, and occurred in all the animals.

It would, therefore, appear that the proportion of *l*-tyrosine in which the benzene ring was ruptured *in vivo* became less with the rise in the dose. It is also to be noted that the benzene ring was opened in over 90 % of the *l*-tyrosine when the dose was 0.1 g., which, although a comparatively high dose, seems to be the border-line quantity for the excretion of aromatic intermediate products of degradation. In

Table 1. *The influence of the magnitude of the dose on the excretion of aromatic intermediate products of l-tyrosine degradation*

Days on scorbutic diet	0.1 g. <i>l</i> -tyrosine		0.2 g. <i>l</i> -tyrosine		0.3 g. <i>l</i> -tyrosine		0.4 g. <i>l</i> -tyrosine		0.5 g. <i>l</i> -tyrosine	
	K (mg.)	M (mg.)	K (mg.)	M (mg.)	K (mg.)	M (mg.)	K (mg.)	M (mg.)	K (mg.)	M (mg.)
8	0	12.6	4.6	70	61.0	84	43	63	—	—
	0	1.8	10.2	17	21.5	41	25	34	—	—
	3.0	4.2	81.0	127	24.0	35	107	159	—	—
	Mean 1.0	5.2	45.7	71.3	36.0	53	58.3	85	—	—
	S.D. 1.7	5.2	35.3	56	22.3	26.7	39.7	66	—	—
14	7.0	8.4	19.5	38	40.6	127	94	133	189	193
	1.7	3.1	16.0	21	46.5	63	138	180	62	110
	7.0	14.1	47.0	56	52.5	75	101	139	261	309
	Mean 5.2	8.5	27.5	38	46.5	88	111	151	171	204
	S.D. 3.0	5.5	16.5	17	6.0	34	23.6	25.6	102	100

In this and all the subsequent tables, K = *p*-hydroxyphenylpyruvic acid; M = total hydroxyphenyl compounds, calculated as tyrosine.

The results are incorporated in Table 1. The figures obtained before dosing revealed that there was some individual variation in excretion of substances which react with Millon's and Penrose & Quastel's reagents and that the continuance of the animals on a scorbutic diet for longer than 8 days did not significantly influence this excretion. The level of excretion in the control period was very low, viz. mean value for the total hydroxyphenyl compounds was 4.2 mg., S.D. 1.7 and for substances reacting with the Penrose & Quastel reagent 2.2 mg., S.D. 1.0 (calculated as *p*-hydroxyphenylpyruvic acid) and was of a similar magnitude to that obtained in some later experiments with guinea-pigs on a mixed diet containing cabbage. These 'blank' values

other words, the amount of *l*-tyrosine in the diet has to be abnormally high before a significant excess of these compounds can appear in the urine of guinea-pigs deprived of *l*-ascorbic acid, and even then the quantity excreted corresponds only to a part of the *l*-tyrosine consumed.

The dietary equivalent of the additional *l*-tyrosine is illustrated by the fact that guinea-pigs of the above weight consume about 25–30 g. of basal diet containing about 0.13 g. *l*-tyrosine and about 0.09 g. of *l*-phenylalanine. Consequently the extra consumption even of the border-line dose of 0.1 g. of *l*-tyrosine would necessitate the raising of the food intake to the region of 40 g. The average animal of this age would not consume this quantity willingly

and, in all probability, even if it were possible to administer such a quantity forcibly, it would not conduce to the animal's well-being.

The course of the differential degradation of high doses of l-tyrosine by guinea-pigs deprived of l-ascorbic acid

The object of this experiment was not only to investigate the excretion of the aromatic intermediate products of degradation but also to throw some light on the distribution of these products in the various tissues of the animals at varying intervals after the consumption of the *l*-tyrosine and to compare the results with those obtained with guinea-pigs on the same diet with the addition of cabbage as a source of *l*-ascorbic acid.

A dose of 0.5 g. of *l*-tyrosine was administered *per os* to the two groups of animals and the tissue, urine and intestinal contents were examined 3, 5 and 24 hr. after the administration of the dose. The results are given in Table 2. As the figures after 5 hr. were very similar to those obtained after 3 hr. they are omitted from the table for economy of space. It is seen that the ketonic substances (*K*) present in the tissues and in the urine of the control animals, although generally somewhat higher in the case of the animals which received cabbage, were of the same low order. These were not deducted from the results obtained after administration of *l*-tyrosine. Only traces of other hydroxyphenyl compounds (*M* - *K*) could be detected in the tissues of the control animals and these results are omitted from the tables.

The first point which emerges from these experiments is that if the total hydroxyphenyl compounds found in the tissues, in the intestinal contents, and in the urine be taken into consideration, the benzene ring was not ruptured after 3 hr. in 69 mg. (14 %) of the *l*-tyrosine dose in the case of the guinea-pigs receiving cabbage and in 255 mg. (51 %) in the case of animals receiving no *l*-ascorbic acid. After 24 hr. the two groups excreted 15 mg. (3 %) and 205 mg. (41 %), respectively, of these intermediate products of tyrosine degradation with the benzene ring intact and only traces were found in the tissues and in the intestinal contents (Table 5). Not only were there more intermediate degradation products found in the urine of animals not receiving cabbage, but also the ratio $K/(M - K)$ was found to be much higher, i.e. 3.02 as compared with 0.19 in animals receiving cabbage. It is not surprising that in the animals deprived of *l*-ascorbic acid less of the aromatic intermediate compounds were accounted for in the tissues and urine together after 3 or 5 hr. than after 24 hr., since the absorption of *l*-tyrosine from the intestinal tract and the excretion of the aromatic intermediate products were still in progress after 3 hr. (cf. Table 5). The distribution of *p*-hydroxyphenyl-

pyruvic acid and of the other hydroxyphenyl compounds in the tissues shows that in the case of animals not receiving *l*-ascorbic acid these substances were significantly higher in the liver and in the muscle; in the kidney the *p*-hydroxyphenyl-pyruvic acid content only was higher. These observations unfortunately give no clear indication so far concerning the mechanism involved in the incomplete degradation of some of the *l*-tyrosine by the guinea-pigs deprived of *l*-ascorbic acid.

A striking feature of this experiment is the higher content of the total hydroxyphenyl compounds found 3 and 5 hr. after the administration of *l*-tyrosine in the large intestine of the animals deprived of *l*-ascorbic acid. The mean was 149 ± 60 mg. as compared with 21 ± 34 mg. in animals receiving cabbage. The statistical treatment of these data as described by Snedecor (1937) for the determination of *t* gave a *t* value of 4.59. This shows that the difference was significant.

In another experiment in which a daily dose of *l*-ascorbic acid instead of cabbage was given to 12 guinea-pigs, the quantities of total hydroxyphenyl derivatives found in the large intestine contents 3 hr. after the administration of 0.5 g. of *l*-tyrosine were compared with those in 12 animals receiving *l*-ascorbic acid in the form of cabbage *ad lib.* and in 12 animals on a scorbutic diet. It was found that the mean in the first case was 65 ± 32 mg., in the second 24 ± 29 mg. and in the third 159 ± 46 mg. The *t* values showed that the differences between all the groups were significant. The quantities of total hydroxyphenyl derivatives found in the urine of the animals of the above groups were 6.7, 8.9 and 40 mg. respectively, the difference in the case of the first two groups not being significant. This proves that the accumulation of total hydroxyphenyl derivatives in the large intestine of guinea-pigs on a scorbutic diet is due to the absence of *l*-ascorbic acid *per se* from the diet and that this accumulation is accompanied by a higher elimination of the substances from the kidney. Another point that emerges from this experiment is that there is a greater accumulation in the large intestine when the *l*-ascorbic acid is administered in solution than when it is given in the form of cabbage. This is probably due to the fact that in the former case the *l*-ascorbic acid passes through the intestine more quickly than in the latter case.

The parenteral production of aromatic intermediate products of l-tyrosine degradation by guinea-pigs subsisting on a scorbutic diet

The observation that there was a higher content in the large intestine of hydroxyphenyl compounds 3-5 hr. after the ingestion of 0.5 g. of *l*-tyrosine in the case of guinea-pigs not receiving *l*-ascorbic acid,

Table 2. *The distribution of hydroxyphenyl compounds in the body and urine of guinea-pigs maintained on diets with and without l-ascorbic acid after administration of 0.5 g. l-tyrosine*

		Scorbutic diet for 14 days											
		Mixed diet containing cabbage						Control, no tyrosine administered, 3 animals					
		3 hr. after l-tyrosine administration, 5 animals			3 hr. after l-tyrosine administration, 6 animals			3 hr. after l-tyrosine administration, 4 animals			24 hr. after l-tyrosine administration, 4 animals		
		M - K		K	M - K		K	M - K		K	M - K		K
		Conc. (mg./100 g.)	Content (mg.)	Conc. (mg./100 g.)	Conc. (mg./100 g.)	Content (mg.)	Conc. (mg./100 g.)	Conc. (mg./100 g.)	Content (mg.)	Conc. (mg./100 g.)	Conc. (mg./100 g.)	Content (mg.)	Conc. (mg./100 g.)
		Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Stomach tissue:	Mean	—	—	—	—	—	—	—	—	—	—	—	—
	S.D.	—	—	—	—	—	—	—	—	—	—	—	—
Stomach contents:	Mean	0.2	6.6	0.23	—	7.0	Trace	—	Trace	—	Trace	0.1	—
	S.D.	0.0	5.0	0.3	—	7.7	—	—	—	—	—	0.1	—
Small intestine tissue:	Mean	0.23	3.6	0.14	1.2	5.2	Trace	0.15	1.2	Trace	Trace	0.16	1.4
	S.D.	0.06	2.8	0.09	—	2.1	—	0.1	—	—	—	0.06	—
Small intestine contents:	Mean	0.12	2.6	0.4	—	2.4	Trace	0.35	—	Trace	—	0.16	—
	S.D.	0.13	1.5	0.2	—	0.8	—	0.2	—	—	—	0.2	—
Large intestine tissue:	Mean	0.11	3.2	0.14	1.4	3.9	0.51	0.18	1.6	Trace	Trace	Trace	Trace
	S.D.	0.07	2.6	0.1	—	1.7	—	0.11	—	—	—	—	—
Large intestine contents:	Mean	1.5	20.0	1.1	—	147.6	—	1.6	—	Trace	—	1.6	—
	S.D.	0.9	34.0	0.9	—	59	—	1.4	—	—	—	1.4	—
Blood:	Mean	0.17	7.2	0.53	2.6	10.8	Trace	0.77	3.8	Trace	Trace	Trace	Trace
	S.D.	0.05	4.5	0.3	—	3.5	—	0.5	—	—	—	—	—
Liver:	Mean	0.33	2.4	0.34	2.8	4.7	0.39	0.53	4.2	Trace	Trace	0.76	5.2
	S.D.	0.22	2.1	0.19	—	1.2	—	0.25	—	—	—	0.18	—
Kidney:	Mean	0.09	1.6	0.08	3.8	1.8	0.05	0.20	7.0	Trace	Trace	0.07	2.7
	S.D.	0.1	1.6	0.07	—	0.8	—	0.08	—	—	—	0.04	—
Muscle:	Mean	1.6	5.9	0.55	1.1	27.2	1.7	2.5	4.5	Trace	Trace	0.55	1.0
	S.D.	0.1	5.1	0.15	—	13.0	—	3.0	—	—	—	0.30	—
Urine:	Mean	0.25	9.6	1.5	—	14.3	—	21.8	—	—	—	154	—
	S.D.	0.1	10.0	1.1	—	13.0	—	12.8	—	—	—	82	—

as compared with those which did, made it necessary to ascertain whether the opening of the benzene ring in the excess *l*-tyrosine took place parenterally and not solely in the intestine. In order to settle this point the urinary excretion of guinea-pigs receiving the scorbutic diet with and without the addition of cabbage was investigated after the injection of a series of single graded doses of glycyl-*l*-tyrosine. The urines were examined (a) from the animals which were maintained on the basal diet with cabbage *ad lib.* for 6 days previous to the injection, (b) after they were deprived of cabbage for 8 days, (c) after a further 5 days on the *l*-ascorbic acid deficient diet and finally, (d) 2 days after cabbage was reintroduced into the diet. The dipeptide was injected intraperitoneally. The urinary excretion of the aromatic products of degradation was found to be complete 24 hr. after injection and the urine was analyzed then.

It will be seen from Table 3 that there can be little doubt that the opening of the benzene ring can take place, at least in the main, outside the intestinal tract and that the mechanism of this change is similar whether the *l*-tyrosine is introduced orally or parenterally. These experiments also bring out the fact that when the dose of *l*-tyrosine is not too high, viz. 0.3 g., the abnormal degradation becomes evident only after the guinea-pig organism has been more thoroughly depleted of ascorbic acid, i.e. after 13 days on the scorbutic diet; with the higher doses this phenomenon was already to be observed after 8 days of *l*-ascorbic acid depletion. We are inclined to believe that this also operates when the *l*-tyrosine is consumed orally, but owing to the large individual variation and the comparatively few animals involved in all these experiments it cannot be demonstrated statistically every time. Also in these experiments an increase in the excretion of total hydroxyphenyl compounds by the ascorbic acid deficient animals was accompanied by an increase in the excretion of *p*-hydroxyphenylpyruvic acid. The border-line dose in these experiments seems to be somewhat higher than that found in the experiments when the *l*-tyrosine was administered orally. Another deviation in the above results from those obtained with the animals which received the *l*-tyrosine orally, namely that the quantity of the total hydroxyphenyl compounds excreted was higher when the 0.5 g. of *l*-tyrosine was injected (cf. Table 1). This is in all probability due to the fact that the amount of *l*-tyrosine circulating as the soluble dipeptide, or even as degradation products of *l*-tyrosine, in the body in the early stages after the injection, is much higher than that present at any time when a single dose is consumed orally and this in consequence leads to a more intense clearance by the kidney. This view is supported by the fact that the increased excretion has also been observed when

Table 3. The parenteral production of aromatic intermediate products of *l*-tyrosine degradation by guinea-pigs subsisting on a scorbutic diet

Animal no.	Equivalent <i>l</i> -tyrosine injected (g.)	Scorbutic diet + cabbage (6 days)				Scorbutic diet (8 days)				Scorbutic diet (13 days)				Scorbutic diet + cabbage (2 days)			
		M		K		M		K		M		K		M		K	
		(mg.)	(%)	(mg.)	(%)	(mg.)	(%)	(mg.)	(%)	(mg.)	(%)	(mg.)	(%)	(mg.)	(%)	(mg.)	(%)
1	0.55	53	9.7	6	1.1	189	34.2	108	19.6	282	51.3	174	31.5	64	11.6	6	1.1
2	0.58	40	7.0	4	0.7	342	59.0	142	24.5	336	57.8	177	29.5	30	5.2	13	2.1
3	0.29	50	18.0	5	1.7	15	5.1	2	0.7	131	45.0	62	21.3	34	11.8	5	1.7
4	0.29	31	10.6	2	0.7	36	12.4	3	0.9	145	49.9	70	24.1	14	4.9	2	0.6
5	0.29	33	11.4	1.5	0.5	49	16.9	7	2.2	109	37.5	41	14.1	29	10.0	4	1.3
6	0.29	40	13.7	4	1.3	15	5.1	3	0.9	34	11.7	4	1.3	29	10.0	3	1.0
7	0.17	21	12.1	2	1.2	15	8.7	2	1.2	24	13.8	3	1.7	8.0	4.6	1	0.5
8	0.17	33	19.1	3.5	2.0	19	10.9	2	1.2	16	9.2	3	1.7	17	9.9	4	2.1
9	0.18	12	6.7	2	1.1	18	10.0	3	1.6	11	6.1	2	1.1	30	16.7	3	1.5
10	0.06	9	16.3	2	3.5	7	12.7	2	3.5	10	18.1	2	3.5	14	25.4	3	5.1
11	0.06	4	7.2	2	3.5	9	16.3	2	3.5	6	10.9	3	5.1	19	34.5	3	5.1

the animals received cabbage. It will be noticed that when the guinea-pigs, after being depleted of *l*-ascorbic acid for 13 days, were offered cabbage for 48 hr. their capacity for dealing with high doses of *l*-tyrosine became similar to that observed in the preliminary cabbage period. In other words, their ability for opening the benzene ring in the entire dose of *l*-tyrosine was restored immediately on becoming 'supersaturated' with *l*-ascorbic acid (cf. Penney & Zilva, 1946). Experiments after prolonged cabbage feeding showed that once circulation of the excess of *l*-ascorbic acid was attained no further change occurred in the degradation of *l*-tyrosine within the limits of the doses employed.

It may be mentioned here that an attempt was made to ascertain whether the injection of much higher doses of *l*-tyrosine in guinea-pigs receiving cabbage *ad lib.* would bring about an abnormal excretion of the total hydroxyphenyl compounds, particularly *p*-hydroxyphenylpyruvic acid. Unfortunately we encountered certain technical difficulties in the attempt. An indication was however obtained that it may be so. In the only successful experiment, in which 2 g. of glycyl-*l*-tyrosine was injected, an excretion of total hydroxyphenyl compounds equivalent to 25% and of *p*-hydroxyphenylpyruvic acid equivalent to 5% of the injected *l*-tyrosine was found in the urine. These percentages, although lower than those which were found in the case of animals subsisting on a scorbutic diet were significantly above those so far observed in animals receiving cabbage *ad lib.* and injected with smaller doses of glycyl-*l*-tyrosine.

The absence of homogentisic acid from the urines of guinea-pigs receiving high doses of tyrosine on a scorbutic diet

In view of the fact that Sealock & Silberstein (1940) established the presence of homogentisic acid in the urine of guinea-pigs receiving *l*-tyrosine on a diet free of vitamin C, we tested the urine of some of our experimental animals for the presence of this acid. Tests were carried out on urines obtained from a group of six guinea-pigs maintained on a scorbutic diet for 14 days and receiving a daily dose of 0.5–0.8 g. of *l*-tyrosine orally, and also from a group of three animals which received 0.5 g. of *l*-tyrosine on three consecutive days and 1.0 g. on the fourth day, after previously subsisting on a scorbutic diet for 8 days and during the four subsequent days when dosed. As the coloration with ferric chloride and the reduction of ammoniacal silver nitrate are also given by *p*-hydroxyphenylpyruvic acid, it was not surprising that these tests were given by the urines of all animals receiving *l*-tyrosine on the scorbutic diet. On the other hand, the reaction with neutral silver nitrate and the darkening on aeration of the alkaline urines which are characteristic of homogentisic acid,

were not observed, nor could a benzoyl derivative be isolated. On addition of 25 mg. of pure homogentisic acid, kindly supplied by Prof. Claude Rimington, to 10 ml. of a control urine, however, the characteristic reactions for the acid were observed in the above tests. No attempt was made to isolate the benzoyl derivative in this case. The above reactions were also negative in extracts of the intestinal contents and of the liver of guinea-pigs on a scorbutic diet 3 hr. after receiving a dose of 0.5 g. of *l*-tyrosine. Also, contrary to Sealock's observations, the quantities of *p*-hydroxyphenylpyruvic acid found by the Briggs reaction in the urines of three of our animals in which the comparison was made corresponded closely to those given by the Penrose & Quastel test and consequently did not indicate the presence of homogentisic acid.

DISCUSSION

The results obtained in these experiments leave little doubt that the excretion of hydroxyphenyl compounds by guinea-pigs receiving high doses of *l*-tyrosine after *l*-ascorbic acid has been withdrawn from the diet, is in the main due to an abnormality in the degradation of the excess of this compound by the general system, since this excretion was observed whether the *l*-tyrosine was administered orally or parenterally. Furthermore, this abnormal excretion was characterized by the presence of *p*-hydroxyphenylpyruvic acid in the urine in both cases.

If all the experimental evidence be brought into focus two factors appear to dominate the problem. In the first place very high doses of *l*-tyrosine must be consumed by guinea-pigs on the scorbutic diet before the failure to rupture the benzene ring in a part of the ingested compound can be observed. So much is this the case, that, in order to ensure the consumption in the form of a natural diet even of the border-line dose of 0.1 g. of *l*-tyrosine, which is just capable of producing the abnormal excretion, guinea-pigs must eat quantities of food so large as to make likely a deleterious effect on their health. The second outstanding point to emerge from the experimental results is that this abnormal degradation of high doses of *l*-tyrosine becomes effective very soon after the *l*-ascorbic acid is removed from the diet. Sealock & Silberstein (1940) found the maximum excretion of hydroxyphenyl compounds to occur as early as 24–48 hr. after the animals were deprived of the vitamin. In our experiments also the excretion of these compounds was observed to begin as early as the first day, although the maximum was not reached until a few days later (Fig. 1). In other words, rupture of the benzene ring does not take place in the whole of the *l*-tyrosine if the dose is high even when the guinea-pig tissues are almost

entirely saturated with *l*-ascorbic acid. The following considerations illustrate the point. Penney & Zilva (1946) have shown that the tissues of guinea-pigs are 'supersaturated' during the first few hours after the removal of cabbage from the animals' diet, owing to the 'flushing' of the saturated tissues by the excess of *l*-ascorbic acid still circulating in the system following the absorption of considerable quantities of *l*-ascorbic acid from the cabbage. In Table 4 the

Table 4. *Percentage loss of vitamin C in small intestine after l-ascorbic acid depletion*

No. of days depleted	Percentage loss calculated on supersaturated value	Percentage loss calculated on saturated value
1	35.8	6.5
2	51.0	28.6
3	60.8	42.8
4	66.7	51.5
5	72.6	60.0
6	77.4	67.2

losses of *l*-ascorbic acid (calculated as percentages of the initial 'supersaturated' ascorbic acid concentration, as well as of the theoretical saturated values) in the small intestine, which is representative of most of the other tissues, are given for the first few days after the removal of cabbage from the diet. It is seen from these figures that the abnormal degradation of part of the *l*-tyrosine, when administered in a dose as high as 0.5 g. daily, begins almost simultaneously with the disappearance of the excess of *l*-ascorbic acid which 'flushes' the tissues and at a time when the organism is almost fully saturated, i.e. 93.5%, and that at this failure to break down the benzene ring in a part of the dose which reaches a maximum at 3-4 days, only about a half of the *l*-ascorbic acid which saturated the body has disappeared. In addition we have found an indication that when the dose is high enough this abnormality may be observed even when the animal is 'supersaturated'. It is not surprising therefore that Kotake *et al.* (1922) observed that when large quantities of *l*-tyrosine were administered to rabbits, animals capable of synthesizing *l*-ascorbic acid in sufficient quantities for normal metabolic purposes, they excreted *p*-hydroxyphenylpyruvic acid and were unable to rupture the benzene ring in the whole administered *l*-tyrosine when the dose was excessive. Other examples of how abnormal consumption of aromatic amino-acids may lead to an excretion of intermediate products of degradation are (a) an experiment performed by Abderhalden (1912) in which a subject, though not alkaptonuric, excreted homogentisic acid after the consumption of 50 g. of *l*-tyrosine *per os*, and (b) one by Papageorge & Lewis (1938) who induced alkaptonuria in normal rats by the administration of high doses of *l*-phenyl-

alanine. We therefore do not consider that the necessity for the presence of an excess of *l*-ascorbic acid in the guinea-pig system in order that the benzene ring shall be ruptured in all the *l*-tyrosine consumed when the dose is abnormally high, is an indication of the existence of a connexion between the normal function of *l*-ascorbic acid and the normal metabolism of aromatic, and still less other, amino-acids. The apparent interaction *in vivo* between *l*-ascorbic acid and *l*-tyrosine under these conditions suggests to us a physiological response to an unusual situation.

The observation made in the course of this inquiry that large quantities of hydroxyphenyl compounds are found 3 hr. after the ingestion of 0.5 g. of *l*-tyrosine in the large intestine of guinea-pigs which have been existing on a scorbutic diet for some days, is not without interest. For convenience the results given in Table 2 are summarized in Table 5. It will

Table 5. *Summary of the distribution of hydroxyphenyl compounds in the body and urine of guinea-pigs maintained on diets with and without l-ascorbic acid after the administration of 0.5 g. l-tyrosine; obtained from Table 2 (mg.)*

	Mixed diet containing cabbage		Scorbutic diet for 14 days	
	3 hr. after tyrosine dose	24 hr. after tyrosine dose	3 hr. after tyrosine dose	24 hr. after tyrosine dose
Tissues	27	Trace	60	Trace
Large intestine contents	21.1	Trace	149.1	Trace
Remainder of intestinal tract	9.8	Trace	9.7	Trace
Urine	11.1	15	36	205
Total	69	15	254.8	205

be seen from this summary that 3 hr. after the administration of *l*-tyrosine only 69 mg. of hydroxyphenyl compounds out of the 500 mg. of *l*-tyrosine administered could be accounted for in the case of guinea-pigs receiving cabbage. These compounds were distributed amongst the various tissues, the intestinal tract, and the urine excreted during the 3 hr. After 24 hr. the hydroxyphenyl compounds had disappeared from the body of animals receiving the same diet, having apparently been degraded in the system, since the quantity present in the urine was of the same order as that found after 3 hr. In the case of animals which did not receive cabbage the position was different. An equivalent of about as much as a half of the *l*-tyrosine dose could be accounted for after 3 hr., and 149 mg. of this was still present in the large intestine although the content in the remainder of the intestinal canal was of the same order as that observed in the case of

animals receiving cabbage. That most of these hydroxyphenyl compounds could enter the blood stream and be excreted without the rupture of the benzene ring to any great extent is seen from the fact that as much as 205 mg., found entirely in the urine, could be accounted for after 24 hr. in a similar group of animals not receiving cabbage. The hypothesis that the *l*-tyrosine in the large intestine was broken up by bacteria before it entered the system to a greater extent when the guinea-pigs were consuming *l*-ascorbic acid than when they were deprived of it was put to the experimental test. Guinea-pigs receiving cabbage were dosed with sulphaguanidine for several days previous to receiving the *l*-tyrosine dose in order to sterilize partially the contents of the large intestine. It was found that after this treatment there was indeed an accumulation of hydroxyphenyl compounds in the large intestine similar to that found in the case of the guinea-pigs which were deprived of *l*-ascorbic acid but, unlike the latter animals, they did not excrete significant amounts of these compounds in the urine even after 24 hr. It is evident that this problem calls for further inquiry.

SUMMARY

1. Guinea-pigs deprived of *l*-ascorbic acid and receiving 0.5 g. or more of *l*-tyrosine daily, excreted in the urine hydroxyphenyl compounds which included *p*-hydroxyphenylpyruvic acid. The excretion began soon after the removal of *l*-ascorbic acid and reached a maximum in 4–5 days.

2. When the *l*-tyrosine was consumed in a single daily dose instead of being ingested intermittently in the basal diet, more hydroxyphenyl compounds were excreted, but the proportion of *p*-hydroxyphenylpyruvic acid was lower during the first 4 days after the *l*-ascorbic acid was removed from the diet.

3. The total hydroxyphenyl compounds and the *p*-hydroxyphenylpyruvic acid excreted by guinea-pigs deprived of *l*-ascorbic acid increased as the

dose of *l*-tyrosine increased. The abnormal excretion became just noticeable when the dose was 0.1 g.

4. The examination of the tissues, intestinal contents, and urine of guinea-pigs, on a scorbutic diet with and without cabbage, 3, 5 and 24 hr. after the oral administration of 0.5 g. of *l*-tyrosine gave no clear indication of the mechanism involved in the incomplete degradation of *l*-tyrosine. After 3 or 5 hr. the proportion of *l*-tyrosine no longer recognizable as hydroxyphenyl compounds was 49% in animals receiving no *l*-ascorbic acid and 86% in those receiving cabbage, as compared with 59 and 97%, respectively, after 24 hr. The ratio of *p*-hydroxyphenylpyruvic acid to total hydroxyphenyl compounds excreted was much higher in animals not receiving *l*-ascorbic acid.

5. Similar results were obtained when *l*-tyrosine was administered parenterally.

6. Very much larger quantities of hydroxyphenyl compounds were found in the large intestine of guinea-pigs 3 or 5 hr. after ingestion of 0.5 g. of *l*-tyrosine when they were deprived of *l*-ascorbic acid than when they received the vitamin. 24 hr. after the consumption of the *l*-tyrosine none of these compounds could be found in the large intestine, but there was a marked increase in the urine. No significant increase was observed after 24 hr. in the urine of guinea-pigs receiving cabbage.

7. Homogentisic acid could not be found in the urine even after continued daily doses of 0.5–0.8 g. of *l*-tyrosine to guinea-pigs on a scorbutic diet.

8. The dependence of complete degradation of *l*-tyrosine in high doses on the presence of excess *l*-ascorbic acid in the guinea-pig system does not necessarily indicate a connexion between the normal function of *l*-ascorbic acid and the normal metabolism of aromatic amino-acids.

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