CLXXXVIII. THE RELATION BETWEEN VITAMIN C AND ADRENALINE

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It was shown by Daoud & Ayyadi [1936] that the rate of glycogenolysis caused by adrenaline, under constant conditions, ran parallel with the degree of saturation of the body with vitamin C. Two alternative suggestions were offered to explain this parallelism. The first was that the degree of saturation of the body with vitamin C determines directly the rate of glycogenolysis, though hyperglycaemia and glycosuria are produced in states of saturation with the vitamin only when the peripheral utilization of the blood sugar is interfered with by adrenaline. The second suggestion was that the vitamin in the reduced form protects adrenaline from rapid oxidation and thus increases glycogenolysis.

As regards the second suggestion, such a relationship has been suggested by different investigators owing to the co-existence of both substances in the suprarenal and to the reduction in the amount of both stated by some to occur in scurvy. The stabilizing effect of the vitamin on adrenaline may be inferred from the work of Szent-Györgyi [1928], Heard & Raper [1933], and Euler & Klusmann [1933]. More recently Heard & Welch [1935], working on the Ringer-Locke perfusate of the suprarenal glands, found that as long as a small amount of reduced ascorbic acid remained in the perfusate there was no diminution in its pressor activity. They also found that the O_2 uptake of the perfusate was characteristic of an oxidation-reduction system. They stated that the perfusate contained neither sulphhydryl compounds nor any reducing substances known to occur in the gland other than ascorbic acid, but did contain an agent which minimized the rate of autoxidation of ascorbic acid.

Against the results of these investigators may be set those of Deutsch & Schlapp [1935] who found a reduction in the adrenaline content of the suprarenals of guinea-pigs in inanition but no significant change in their ascorbic acid content, provided that sufficient green food were given in the diet. It thus appeared that inanition was an important factor in the reduction of the adrenaline content of the suprarenals in scorbutic animals. They came to the conclusion that there was no close physiological relation between ascorbic acid and adrenaline.

The relation of these two substances, even in the suprarenal which is rich in both, thus remains obscure. The suprarenal is concerned in the elaboration and storage of adrenaline and it is natural to suppose that it is provided with some system which protects this hormone from destruction; alternatively, it may be deficient in the agents elsewhere responsible for the destruction of adrenaline. This view is strengthened by the fact that the liver is also an organ with a high vitamin C content, yet, according to Markowitz & Mann [1929], Elliott [1905] and Giragossintz & Mackler [1929], the liver is particularly active in the destruction of adrenaline. The work on the suprarenal or its perfusate will

therefore give no clue to the part which the vitamin alone plays in its physiology, especially if we bear in mind, from the data reported by Wiltshire [1931], Kellie & Zilva [1935], Mawson [1935], Hopkins & Morgan [1936] and Heard & Welch [1935], that most of the substances and tissue extracts which exert a protective effect on the vitamin are also protective for adrenaline, and that substances which protect the vitamin are found in the perfusate of the suprarenals. These protective substances are widely distributed in the body, so, even if we admit a protective influence of the vitamin on the adrenaline in the suprarenals or in pure mixtures of the two substances, it will still be questionable whether this protective effect is of particular significance in the body as a whole, where numerous other protective substances are present and where certain organs appear to be more active in the destruction of adrenaline than others.

These considerations made it desirable to find out how far the work done on isolated suprarenals or on adrenaline *in vitro* applied to the functions of vitamin C in the body as a whole and to decide whether the effect of the vitamin in augmenting the rate of glycogenolysis caused by adrenaline is due to a stabilizing effect of the vitamin on adrenaline or is due to a direct glycogenolytic effect of the former.

During the course of this investigation, the effect of the vitamin on the blood pressure was studied in pithed animals.

EXPERIMENTAL

Since experiments carried out *in vitro* or on isolated organs are suspected of being unhelpful as a criterion for the state of events in the body as a whole, it was decided to work on the whole animal. We first compared the effects produced by certain doses of adrenaline injected before and after the gradual increase of the vitamin C content of the body.

A pithed cat was injected intravenously with different doses of 1/10,000 adrenaline hydrochloride solution and the maximum rise in blood pressure and the duration of the rise caused by each dose were recorded. Ascorbic acid (B.D.H.) was then given and the effects of the previous doses of the adrenaline hydrochloride were again tried. Ascorbic acid and adrenaline hydrochloride were then administered alternately to determine the effect of adrenaline after different amounts of ascorbic acid had been administered.

It was noticed that ascorbic acid injected in doses of 100 mg. into a 2700 g. cat had no effect on the blood pressure, but when the dose was increased to 200 mg. a rapid drop in blood pressure occurred. The maximum drop was 24–28 mm. and lasted for 93–95 sec. Reinjection of the same doses of adrenaline which had been given before the administration of ascorbic acid caused a much smaller and less sustained rise in blood pressure than before. Adrenaline injected in considerably larger doses than those given before the ascorbic acid administration caused a rise of much smaller extent and shorter duration than would be expected. Simultaneous administration of the two substances produced the same small effects.

The effect of ascorbic acid in lowering the blood pressure might have been due to its acidity rather than to any specific influence of the vitamin. The observation that adrenaline had less effect on the blood pressure after the ascorbic acid injections might possibly be explained by the fact that the acidity caused the production of substances which antagonized the action of adrenaline. The experiment was therefore repeated on a pithed cat weighing 2.5 kg., the ascorbic acid being neutralized with *N*/100 NaOH immediately before injection. In this experiment the magnitude and duration of the rise in blood pressure

caused by adrenaline were the same after as before the administration of the neutralized ascorbic acid; further, doses of 200 mg. of the neutralized acid caused neither rise nor fall in blood pressure as shown in Table I.

Table I

Substances in the order of injection	Blood pressure (mm. Hg)		Duration of rise or fall sec.
	Maximum rise	Maximum fall	
0.1 ml. adrenaline*	52	—	130
0.2 " "	96	—	165
0.1 " "	58	—	130
0.2 " "	91	—	190
0.1 " "	54	—	145
0.2 " "	88	—	165
100 mg. ascorbic acid	—	—	—
100 " "	—	—	—
200 " "	—	—	—
0.2 ml. adrenaline	91	—	165
0.1 " "	56	—	132
100 mg. ascorbic acid	—	—	—
100 " "	—	—	—
100 " "	—	—	—
100 " "	—	—	—
100 " "	—	—	—
0.1 ml. adrenaline	52	—	135
0.1 " "	58	—	140
0.2 " "	89	—	187
0.1 ml. adrenaline + 100 mg. ascorbic acid	57	—	137
200 mg. ascorbic acid	—	—	—
200 " "	—	—	—
0.2 ml. adrenaline	88	—	190
0.1 " "	56	—	140
0.1 " "	53	—	136
0.1 " "	57	—	145
0.2 " "	90	—	185

* 1/10,000 adrenaline hydrochloride solution.

The injection of a total quantity amounting to 1.4 g. of ascorbic acid, adjusted to the physiological pH, into a cat of 2.5 kg. body weight neither augmented nor prolonged the effect of adrenaline on the blood pressure.

The above results, although instructive, do not disprove the existence of a protective effect of ascorbic acid on adrenaline, since the destruction of adrenaline in the body appears, from the work of Weiss & Harris [1904], not to coincide with the return of the blood pressure after the injection of adrenaline to its original level. These investigators found that when a cat was injected with adrenaline the transfusion of its blood taken after the blood pressure had fallen to its original level into a second cat caused a rise of blood pressure in the latter. We therefore attempted to find out the time necessary for the destruction of a certain amount of adrenaline in animals saturated with the vitamin and in normal controls in the following manner.

Two cats were chosen of exactly the same weight, 2800 g., and were kept on the same diet. One of the cats received 7 daily injections of 70 mg. ascorbic acid neutralized with Na_2CO_3 immediately before injection. On the 8th day the two cats were anaesthetized with intraperitoneal injections of dial. 5 ml. samples of arterial blood were taken from each cat and injected alternately into a third pithed cat and the small rise in blood pressure was noted in each case.

Each of the two cats was injected 3 times intravenously with 1 ml. adrenaline hydrochloride 1/1000, allowing 5 min. between each injection. Samples of 5 ml. of arterial blood taken after definite intervals of time had elapsed since the last adrenaline injection in each case were immediately injected into the femoral vein of the pithed cat. The rise in blood pressure above the initial rise caused by the blood of each cat before the adrenaline injection was noted in each case. The transfusion of blood was continued till the samples of blood taken from each cat caused no greater rise than that caused by the blood of this particular cat before it had received adrenaline. The time between the last injection of adrenaline and the disappearance of its effect on the blood pressure of the pithed cat was taken as the time necessary for the destruction of adrenaline in the cats under experiment as indicated in Table II.

Table II

Time after last adrenaline injection min.	Rise in blood pressure above initial mm. Hg	
	Control cat	Cat injected with ascorbic acid
5	108	—
20	86	47
40	52	32
60	30	20
80	7	5
90	4	Nil
95	Nil	—

It will be noticed that in this experiment the rise in the blood pressure of the pithed cat caused by the blood of the cat injected with ascorbic acid was less than in the case of the control cat, but this was not always so. The main observation is that the time taken by the two cats to destroy the same amount of adrenaline is practically the same, being, in fact, 5 min. less in the case of the cat previously injected with ascorbic acid. In another experiment a total dose of 2 ml. of 1/1000 adrenaline hydrochloride was injected into each of two cats weighing 2700 g.; the destruction of adrenaline took place in 70 min. in both cases and there was no appreciable difference in the magnitude of rise of blood pressure caused by the two bloods. These two experiments are especially recorded because the blood taken from the two cats before adrenaline injections did not show a depressor effect on the pithed cat, as sometimes happened, and the experiments were successfully carried out to the end.

The above result seems to indicate that, in the intact animal, ascorbic acid has no protective effect on adrenaline. Since it has been shown by Sandiford [1920] and by Cori & Cori [1928] that adrenaline causes a rise in metabolism, it was decided to compare the time necessary for the disappearance of the effect of adrenaline on the metabolism of rats saturated with ascorbic acid with the time necessary in the case of unsaturated rats. For this purpose a number of male rats were chosen weighing 250–255 g. They were divided into two batches and all were kept on a scorbutic diet for 7–10 days. Each of the members of one batch then received daily injections of 7 mg. of ascorbic acid for 3–9 days before the adrenaline administration. Food was removed at 4 p.m. on the day preceding an experiment. On the days of experiments, one pair of rats taken from each batch was injected with 0.1 ml. physiological saline and the O₂ consumption by the pair was then determined every 20 min. in a closed system.

In the first 20 min. a high consumption was always noticed, which was undoubtedly due to the effect of handling; the observations were continued until steady consumptions were attained in the subsequent 20 min. periods. Usually the amount of O_2 consumed in the second or third 20 min. agreed with the subsequent readings. The constant amount of O_2 consumed was taken as the basal amount for the pair in 20 min. Each rat then received an injection of 0.1 ml. 1/1000 adrenaline hydrochloride solution and the readings were again taken until the quantity of O_2 consumed in 20 min. returned to the basal amount. The time between the adrenaline injection and the end of the last 20 min. during which there was still a rise in O_2 consumption above the basal, was taken as an estimate of the time necessary for the wearing away of the effect of a definite amount of adrenaline in the bodies of the saturated and unsaturated rats. In a very few cases, continuous fluctuations in the O_2 consumption in the 20 min. periods were noted, especially after adrenaline injections, but such results were rejected. Many experiments were performed in the manner described; for the sake of brevity, the results obtained with six pairs of rats are given in Table III.

Table III

Diet	Time for disappearance of adrenaline effect (min.)					
	I	II	III	IV	V	VI
Scorbutic	140	120	160	140	120	120
Scorbutic + ascorbic acid injections	120	120	140	140	160	140

The time necessary for the disappearance of the effect of the adrenaline in the two groups was of the same order and varied from 120 to 160 min. This result agreed with that of the blood transfusion experiments in suggesting that ascorbic acid had no protective effect on adrenaline *in vivo*.

It has already been mentioned that Daoud & Ayyadi [1936] gave two alternative suggestions in explanation of the parallelism between the degree of saturation of the body with vitamin C and the rate of glycogenolysis caused by adrenaline. The present work rules out the suggestion of a protective effect of vitamin C on adrenaline.

Effect of vitamin C on adrenaline in vitro

Adrenaline is known to undergo rapid autoxidation in atmospheric oxygen. Although there is a tendency at present to consider that adrenaline is destroyed in the body by enzymes, yet the rapidity of autoxidation suggests at least that this may be partially responsible for the destruction. This, together with the apparent similarity between the mechanisms of autoxidation and some of the enzymic oxidations, made it desirable to examine the effect of the vitamin, when isolated from other systems, on the oxidation of adrenaline.

Heard & Welch [1935], in their work on the effect of atmospheric oxygen on the perfusate of the suprarenals already referred to, concluded that the stabilization of adrenaline was effected by means of an oxidation-reduction system, and that ascorbic acid in the reduced form was the agent responsible for the protection of the catechol group of adrenaline from oxidation to *o*-quinone. When the ascorbic acid was completely oxidized, irreversible oxidation of adrenaline, manifested by the appearance of a red coloration and diminution in pressor activity, commenced; thereafter the rate of adrenaline oxidation was dependent on the amino-acid content of the perfusate.

It was planned to study the problem in solutions containing mixtures of the two pure substances. Since adrenaline is more stable in acid than in neutral or alkaline solutions, the effect of the acidity of ascorbic acid was eliminated by buffering the solutions at pH 7 with phosphate. The relative proportions of adrenaline hydrochloride and ascorbic acid in the solutions were of the order reported by Heard & Welch to be present in some of the perfusates of the suprarenals; the ratio of ascorbic acid to adrenaline was increased in some of the experiments for the purpose of comparison.

It is generally accepted that the appearance of the red colour in adrenaline solutions is an indication of irreversible oxidation of adrenaline and is accompanied by diminution in pressor activity. The red-coloured substance was considered by Green & Richter [1937] to be adrenochrome, which is inactive as a vasoconstrictor.

In the present work the commencement of irreversible oxidation was judged at first by the appearance of this red colour. Equal volumes of a solution of adrenaline hydrochloride in phosphate buffer were placed in a number of bottles of 250 ml. capacity. The bottles were all brown in colour with the exception of one colourless bottle which was used for comparing the effect of light on the decomposition of adrenaline. The total volume of solution in each bottle was always 100 ml.; all solutions were buffered at pH 7 and contained the concentrations of adrenaline alone or adrenaline and ascorbic acid indicated in Table IV. In addition, control experiments were made in which only buffered ascorbic acid (B.D.H.) was included. All bottles were tightly stoppered with glass stoppers and were shaken every now and then for the same length of time on a mechanical shaker to facilitate oxidation by the air enclosed above the liquid. After each period of shaking the brown bottles were placed in the dark and the colourless bottle was placed in the light.

In most of the experiments the reddish tinge appeared in the different solutions in the following order: adrenaline alone kept in light, simultaneously in the different solutions of adrenaline + ascorbic acid, adrenaline alone kept in dark; no red colour appeared in the solutions containing ascorbic acid alone. In some cases, however, the appearance of the red tinge in the solutions containing adrenaline and ascorbic acid together took place at the same time as in the solutions of adrenaline alone kept in the light (Exp. 2, Table IV). There was

Table IV

Comparative intensities of reddish colour

Exp.	Time of observation hr.	In light 2 mg. adr.	In coloured bottles in dark			
			2 mg. adr.	2 mg. adr. + 1 mg. asc.	2 mg. adr. + 2 mg. asc.	2 mg. adr. + 4 mg. asc.
1	3	Trace	-	-		
	24	+	-	-		
	48	++	-	+		
	72	++	-	++		
	96	++ *	+	++ *		
2	20	+	-	+	+	
	44	++	+	++	++	
3	24	Trace	-	-	-	-
	48	+	-	-	-	-
	72	++	-	+	+	+
	96	++	+	++	++	++ *
	110	++ *	++	++ *	++ *	++ *

* Yellow tint.

adr. = adrenaline hydrochloride.

asc. = ascorbic acid.

no definite rule as regards the time of first appearance of colour in the solutions kept in the light. The rate at which the reddish colour appeared and increased in intensity in the different solutions is indicated by the results of 3 experiments recorded in Table IV.

It is clear that light accelerates the coloration of adrenaline solutions and that when the effect of this factor is eliminated the solutions containing adrenaline and ascorbic acid become coloured earlier than similar solutions containing no ascorbic acid. If the coloration of adrenaline solutions is accepted as an indication of oxidative destruction, this result would mean an acceleration of the destruction of adrenaline by ascorbic acid. When, however, the pressor activity of the solutions was actually tested, a contradictory result was obtained.

Solutions were prepared as before, some being made up one day before the others, for the purpose of studying the correlation between the intensity of colour and the pressor activity. The pressor activity of all the solutions was compared with that of a freshly prepared adrenaline hydrochloride solution buffered at pH 7 by measuring their effect on the blood pressure of one and the same pithed cat. Equal doses of 0.5 ml. were injected. The results of a number of such experiments are given in Table V.

Table V

Soln. no.	Contents of soln. mg. per 100 ml.	Condition	Age of preparation days	Intensity of colour	Mean rise in blood pressure mm. Hg	Strength relative to fresh soln. adr.
1	2 adr.	In light	3	++	28	0.25
2	2 adr.	In dark	3	+	40	0.36
3	2 adr.	In light	2	+	40	0.36
4	2 adr.	In dark	2	—	54	0.48
5	2 adr. + 1 asc.	In dark	2	+	54	0.48
6	2 adr. + 2 asc.	In dark	2	+	54	0.48
7	2 adr. + 4 asc.	In dark	2	+	54	0.48

adr. =adrenaline hydrochloride.

asc. =ascorbic acid.

Observations obtained after allowing the solutions to stand more than 3 days are not recorded owing to complications which occurred after this period, such as the development of a yellow tint.

It can be seen that under the specified conditions of concentration and pH all solutions, whether coloured or not, were less active than a similar but freshly prepared adrenaline solution. Even the colourless solution (4) lost some of its pressor activity. According to Ball & Chen [1933] the first oxidation product, the *o*-quinone compound corresponding to adrenaline, is not coloured. This is supported by the work of Raper [1926; 1927] on the oxidation of the related substance dihydroxyphenylalanine. If we assume that in solution (4) a complete or partial transformation into the *o*-quinone compound did take place, it follows that autoxidation of the OH groups of the catechol ring results in the formation of a compound of lower activity than the original adrenaline. The comparison of (4) with (3) does not support the view that the *o*-quinone compound is quite devoid of activity.

In the solutions containing adrenaline alone at pH 7, the earlier production of colour in the light than in the dark was accompanied by greater diminution in pressor activity (3, 4). The further increase of colour intensity was accompanied by further loss of pressor activity (1, 2, 3). Although there was an earlier development of colour in the light than in the dark, when the intensity of coloration became the same in both cases the solutions had the same activity (2, 3).

According to Green & Richter [1937], the red-coloured substance adrenochrome is produced by further oxidation of the primary *o*-quinone product of adrenaline oxidation and involves the removal of one hydrogen atom from the —NH— group of the side chain and one from the *o*-quinone ring, with the formation of an indole ring. According to them the first oxidation product is extremely unstable and at pH 7 undergoes an almost instantaneous change into the red-coloured adrenochrome which is physiologically inactive. The association of colour production with the formation of an indole ring from the *o*-quinone derivative of dihydroxyphenylalanine has also been pointed out by Raper [1926; 1927]. The red-coloured adrenochrome, therefore, is the second product of autoxidation of adrenaline although it constitutes the first visible stage in its oxidation. It is clear from the present work that this latter stage is photocatalysed, since there is an earlier development of colour in solutions of adrenaline alone kept in the light than in solutions prepared at the same time but kept in the dark, in which the original adrenaline proved to have undergone a change which is responsible for most of the total lowering of activity. This explains the further diminution in pressor activity as the colour develops (3, 4) and the proportionality of this diminution to the intensity of coloration (1, 2, 3, 4). It also explains why, when the intensity of coloration of solutions kept in the light and in the dark becomes the same, they have the same pressor activity, for the amount of active and inactive substances at this point will be the same in both cases.

Although there was an early development of colour in the solutions of adrenaline containing ascorbic acid, these showed an activity similar to that of a colourless solution prepared at the same time and kept under the same conditions, and a greater activity than a coloured solution also prepared at the same time but kept in the light. When the solutions 4–7 (Table V) were allowed to stand for another day, nos. 5–7 containing ascorbic acid developed maximum red colour and solution 4 developed a slight coloration; but while the pressor activity of nos. 5–7, when again compared with that of a freshly prepared solution, kept constant as before, that of no. 4 had diminished. These experiments show that ascorbic acid at pH 7 has a limited protective effect on the activity of adrenaline. This limited effect was still more prominent when 1 in 10^6 solutions of adrenaline alone and adrenaline containing the same amount of ascorbic acid were allowed to oxidize in the presence of air and light; it was found that after 24 hr. the solutions containing adrenaline alone completely lost their activity, while those containing ascorbic acid were still active, though they had a lower activity than freshly prepared solutions of the same concentration and reaction. The inactivation of solutions of adrenaline alone, in the light or in the dark, is proportional to the colour development, but no such proportionality exists in the solutions containing ascorbic acid as well. In the latter solutions, both the development of colour and the preservation of activity are independent of the amount of ascorbic acid present.

We can find no explanation of these experimental facts except by assuming (1) that the red-coloured product obtained with solutions of adrenaline containing ascorbic acid is different from the inactive adrenochrome produced in solutions of adrenaline alone; it might result from a reaction between the *o*-quinone primary oxidation product and ascorbic acid; its association with colour is an indication that it would also be an *o*-quinone; (2) that the protective effect of ascorbic acid hinders the transformation of the *o*-quinone primary oxidation product of adrenaline into the inactive adrenochrome. These assumptions explain (1) the preservation of an activity equal to that of the colourless

primary oxidation product by the solutions containing adrenaline and ascorbic acid, (2) the earlier appearance of coloration in these solutions than in those of adrenaline alone under the same conditions, (3) why further increase in colour intensity can take place without further loss of pressor activity, contrary to what occurs in solutions of adrenaline alone, since the increased intensity in the presence of ascorbic acid may simply mean the transformation of the colourless *o*-quinone into a coloured one of the same activity. According to Green & Richter [1937] ascorbic acid prevents the catalytic oxidation of adrenaline to adrenochrome.

Modified dichlorophenol-indophenol titrations showed that at *pH* 7 the rate of disappearance of the reduced form of ascorbic acid was rapid and was the same in solutions containing adrenaline as in similar concentrations of ascorbic acid alone. Complete disappearance took place in a few hours in both cases. The accelerated appearance of coloration in solutions containing adrenaline and ascorbic acid cannot therefore be due to the reduced form of ascorbic acid since the latter disappears long before the appearance of colour; the oxidation products of ascorbic acid may be responsible. These observations prove that the continued presence of the reduced form of ascorbic acid itself is not essential for the maintenance of the activity previously indicated to take place in the solutions of adrenaline with ascorbic acid, since it has been shown that the preservation of an activity equal to that of the primary oxidation product can continue after the development of the colour and, as can be seen, after the disappearance of the reduced form of ascorbic acid. Whether the coloured substance formed in the solutions which had contained ascorbic acid is concerned with the preservation of activity remains to be investigated.

The loss of potency in adrenaline solutions containing ascorbic acid (indicated in Table V) when compared with a freshly prepared solution of adrenaline alone was shown by further experiments to be demonstrable even before the complete disappearance of the reduced form of ascorbic acid, while at *pH* 7 a freshly prepared solution of adrenaline containing ascorbic acid had the same potency as a freshly prepared solution of adrenaline alone. It appears, therefore, that at *pH* 7 there is at first a simultaneous oxidation of the reduced form of ascorbic acid and of adrenaline which is changed to the corresponding *o*-quinone, thereafter there is a protection of the *o*-quinone compound from further oxidation.

These *in vitro* experiments were concerned with the protective effect of ascorbic acid on the pressor activity of adrenaline when the former was deprived of its acidic properties. In such experiments there is a rapid oxidation of ascorbic acid; a better protective effect on adrenaline might be expected in animal tissues, owing to their tendency to keep ascorbic acid in the reduced form.

The catalytic oxidation of adrenaline to the inactive adrenochrome was shown by Green & Richter [1937] to take place in the animal body by (1) a cyanide-insensitive system present in heart and skeletal muscle and (2) the cytochrome-indophenol oxidase present in all tissues. In the present work, as well as in the experiments of the above investigators, the hindrance of adrenochrome formation by ascorbic acid is indicated. In the intact animal, even if an increased concentration of ascorbic acid is effective in hindering adrenochrome formation, it is evident from the present work that the influence of this factor is combated by other factors, so that the same destruction of adrenaline is produced in normal animals as in those saturated with ascorbic acid. The possibility of adrenaline destruction in the body by mechanisms other than adrenochrome formation can be imagined from the different modes of adrenaline oxidation reported by previous investigators. Thus Weinstein & Manning [1937]

found a substance having the properties of protocatechuic acid in the urine of rabbits in which adrenaline had been injected, which indicates an oxidative removal of the side chain of the adrenaline molecule. Blaschko *et al.* [1937] have reported the presence of adrenaline oxidase in mammalian liver and other tissues; this was subsequently found by Richter [1937] to be an amine oxidase which induced an oxidative deamination in the side chain with the production of an aldehyde and methylamine.

It seems that any interference with the side chain of the adrenaline molecule by oxidative splitting or closure to form an indole ring results in complete loss of pressor activity, while autooxidation of the hydroxyl groups in the catechol ring simply lowers the activity.

SUMMARY

1. Small doses of unneutralized vitamin C injected into a pithed cat have no effect on the blood pressure, but large doses have a depressor effect. When the vitamin is adjusted to the physiological pH it has no action. The effect of the large doses of the unneutralized vitamin is ascribed to its acidity rather than to its real influence.

2. At the physiological pH, vitamin C augments neither the magnitude nor the duration of the adrenaline effect on the blood pressure, but the acidic vitamin diminishes both its magnitude and its duration.

3. The destruction of adrenaline is the same in normal intact animals as in those saturated with vitamin C. Any protective effect of the latter on adrenaline is combated by other destructive factors operating in the organism.

The parallelism previously observed between the rate of glycogenolysis caused by adrenaline and the degree of saturation of the body with vitamin C cannot be ascribed to a prolongation of the effect of the former through a protective effect of the latter.

4. *In vitro* at pH 7 ascorbic acid has a limited protective effect on the pressor activity of adrenaline. It appears that this protection consists in a temporary hindrance of further autooxidation of a primary oxidation product of adrenaline which has a lower pressor activity than the original compound. It is suggested that this primary product is the colourless *o*-quinone corresponding to adrenaline. The continued presence of the reduced form of ascorbic acid is not essential for this protection.

5. The fact that coloration occurs earlier in mixtures of ascorbic acid and adrenaline than in solutions of the latter alone cannot be taken as a sign of earlier destructive oxidation as in the case of adrenaline alone; it may be due to some sort of reaction between the primary oxidation product of adrenaline and the products of ascorbic acid oxidation. The coloured substance produced in these mixtures is suggested to be an *o*-quinone possessing a pressor activity equal to that of the colourless *o*-quinone compound.

6. The effect of light on the destruction of adrenaline appears to be concerned particularly with hastening the second step of autooxidation which is associated with the development of colour.

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