ROLE OF ASCORBIC ACID IN ADRENAL MEDULLA

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It is widely known at the present that the endocrine glands in general contain significant amounts of ascorbic acid, but little information is available concerning the role played by the substance in the cellular physiology of respective incretory organs. Of the information concerning the occurrence of this substance in the adrenal medulla, such is also the case at the present status, excepting such works as those by Giroud and Martinet (1941) and Banerjee (1944, 1945).

In one of preceding studies of the respiratory enzyme systems in the thyroid, adrenal cortex and adrenal medulla of the pig, the author (Katsura, 1955) reported characteristic mode of oxygen consumption of the adrenal medulla, in which an abrupt rise of oxygen consumption followed a lag phase and an abrupt fall ensued in response to the addition of cytochrome c to tissue homogenate of the organ. In parallel with the rise of oxygen consumption, the reaction mixture began to develop a reddish color, which turned black brown toward the initiation of and during of the subsequent fall, suggesting the oxidation of endogenous adrenalin to adrenochrome, an intermediary oxidation product, which, owing to its activity as a hydrogen-transport system, is responsible for the abrupt oxygen consumption observed.

A work was carried out with the aim to inquire into the possibility of functional connection of the ascorbic acid to the said characteristic phenomenon in the adrenal medulla, results of which are rendered in the following accounts.

EXPERIMENTAL

Materials Freshly obtained pig adrenals were employed in the present study. Cortex was carefully stripped off, and the medulla was trimmed to be made 10% homogenate by means of Potter's homogenizer. In the same way, 10% homogenate of pig heart muscle was prepared from the ventricular apex. For the determination of ascorbic acid content of the adrenal medulla, adrenal cortex, skeletal muscle, heart muscle, liver, renal cortex and the thyroid of the pig and horse, these tissues were homogenized with the addition of 4% trichloroacetic acid. For the experiment on ascorbic acid deficiency 10 male guinea pigs weighing 300-350 g were used. They were divided into 3 groups of 4, 4 and 2 animals respectively. Each one from groups 1 and 2 were kept together in a cage, fed on the ascorbic-acid deficient diet of Wohlbach and Howe (1926) for 20 days. In group 2, which stood as a control lot, animals were daily given ascorbic acid, 10mg orally and another 10mg intraperitoneally in addition to the experimental diet. Animals of group 3, also controls, were normal animals supplied by local dealers. The deficient diet induced apparent signs of scurvy in 22 days, and the animals shortly before the development of signs of scurvy were used in the following experiments.

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Phosphate buffer of 0.1 M and pH 7.4 was prepared with recrystallized Na_2HPO_4 and HCl, both being of GR-brand. Water used for buffer and other reagents was twice distilled with a glass vessel. The pH was adjusted by means of a pH meter with glass electrode.

As adrenalin, powder Epirenamine of Sankyo Pharmaceutical Co., dissolved in 2×10^{-3} N HCl, was used. In two of the following trials, it was dissolved to 1% cattle serum albumin solution. It is to be noted here, adrenalin is easily soluble in the protein solution, and such a solution is not so autoxidizable as that dissolved in HCl solution, when compared at the same pH of 7.4 in reaction mixture.

Ascorbic acid was dissolved in a small amount of water and adjusted to pH 7.4 with 0.1 N and 0.01 N NaOH immediately before use, *i.e.*, after the completion of all the other preparations.

Cytochrome c was prepared from the horse heart muscle after Keilin and Hartree (1937, 1945).

Methods Determination of oxygen consumption was carried out at 37° C and pH 7.4 with Warburg apparatus. The composition of the reaction mixture will be described in respective accounts of experiments.

For the determination of ascorbic acid, the dinitrophenylhydrazine method (Roe, 1954) was used, which determines the sum-total of ascorbic acid, dehydroascorbic acid and diketoglonic acid. For the determination of oxidizing rate of ascorbic acid, the 2, 6-dichlorophenol indophenol method (Roe, 1954) was used. This can determine the separate amount of ascorbic acid (reduced form), but involves the possibility of interference by adrenalin. Therefore, the standard ascorbic acid was preliminarily measured in the presence of adrenalin, and non-interference was confirmed. For the determination of adrenalin, the Persky's method (1955) based on the classic reaction of Vulpian was followed. (Since under the condition employed measurement was concerned with noradrenalin too, so it might be safe to infer that sum-total of catechol amines was observed in the present study. Nevertheless, the measurement is dealt with as "adrenalin-determination" for the sake of brevity of expression.)

Determination of the absorption spectra of adrenochrome and adrenolutine was made with Beckmann's spectrophotometer model DU, and maximum absorptions of these two substances were found at 480 and 410 $\text{m}\mu$ respectively. Therefore, to know change in adrenochrome concentration in reaction mixture, the optical absorption at 470 $\text{m}\mu$ was selected. In this case, if determinations were concerned with tissue homogenate, the reaction mixture was centrifuged at 3000 r.p.m. for 5 seconds and the supernatant, diluted 4 times or non-diluted, was subjected to the determination. Trichloroacetic acid was not added, since it was learned that the acid interfered variably with the development of coloration of adrenochrome. Instead, the mixture was refrigerated with ice.

RESULTS

Ascorbic Acid Content of Adrenal Medulla As mentioned in the introductory remarks, endocrine glands are generally reported to contain much ascorbic acid. In the present work, content of the substance in the adrenal medulla was first determined. To obtain comparative data, the same line of determination was also made with the skeletal muscle, heart muscle, liver, renal cortex, thyroid and adrenal cortex of the pig and horse. As shown in Table I, in both of the pig and horse organs, ascorbic acid content was extremely high in the medulla, *i.e.* 1990 and 2080 $\mu\text{g/g}$ wet weight, respectively, which are equivalent to the content in the adrenal cortex. They are followed by that in the thyroid. Ascorbic acid contents in the skeletal and heart muscles are of much lower levels.

Table 1. Contents of ascorbic acid in various tissues of pig and horse
($\mu\text{g/g}$ wet wt.)

Pig		Horse	
Skeletal muscle	30	Skeletal muscle	46
Heart muscle	37	Heart muscle	44
Liver	240	Liver	200
Renal cortex	152	Renal cortex	112
Adrenal cortex	1900	Adrenal cortex	2720
Adrenal medulla	1990	Adrenal medulla	2080
Thyroid	536	Thyroid	200

Oxygen Consumption in Homogenate of Adrenal Cortex and of Heart Muscle added with Adrenalin If the characteristic oxygen consumption in the adrenal medulla mentioned above is due to the presence of adrenalin, it may be presumed that same would be the case with other tissue homogenates. To confirm this presumption, the adrenal cortex, whose content of ascorbic acid is at the same order as the adrenal medulla and the heart muscle, which contains lesser amount of the acid, were experimented on. Five per cent homogenate of each of these tissues in an amount of 1.0 ml was placed in a flask together with 1.0 ml of phosphate buffer, 0.5 ml of 800 $\mu\text{g/ml}$ adrenalin, and 0.2 ml of H_2O , and after confirming no obvious change in pH, 0.3 ml of 6×10^{-5} M cytochrom c was further added to each. The flasks were set in a thermostat, and after 5 minutes of temperature equilibration, determination of oxygen consumption was made over a period of 100 minutes at 5-minute intervals, beginning at the end of the first 5 minutes, which was designated as zero time. As shown by Fig. 1., oxygen consumption in the heart muscle began to rise immediately after the commencement of the determination, attaining the maximum level in 25 minutes, whereas in the adrenal cortex it lagged for a prolonged period as compared with the former, attaining the maximum rate in 40 minutes. For the sake of comparison, the result with the adrenal medulla homogenate to which were added cytochrome c and phosphate buffer (curve C) is given in a scale of 3/2.

Oxidation of Adrenalin in the Adrenal Medulla of Ascorbic-Acid Deficient Guinea Pig On the 20th day after the commencement of ascorbic-acid deficient regimen, the animals were killed by decapitation, the adrenal medulla collected and homogenized to be diluted with H_2O at a rate of 30 mg of the tissue per 2.9 ml of the dilution. To it was further added 1.0 ml of phosphate buffer and 0.6 ml of 10^{-5} M cytochrome c to the final volume of 4.5 ml. The reaction mixture was distributed into tubes, incubated at 37°C , and optical density at $470\text{ m}\mu$ was measured at 15-minute intervals over a period of 120 minutes. As shown by Fig. 2, the absorption increased in a similar way to that of oxygen consumption in Fig. 1. But in ascorbic-acid deficient guinea pigs, initial time lag was remarkably shortened, the extinction increasing from the beginning at near by a maximum rate, whereas for the animals administered of ascorbic acid, it takes as long as 60 minutes to attain the maximum rate. In ordinarily fed animals, the oxidation of adrenalin proceeded nearly in the same way as in the ascorbic-acid treated controls.

Changes in Amount of Adrenalin and Ascorbic Acid with Oxygen Consumption of Medulla

Fig. 1. Oxygen consumption in homogenate of adrenal cortex and of heart muscle to which was added adrenalin. For experimental conditions see text. A: heart muscle; B: adrenal cortex; C: adrenal medulla, result is in a scale of 3/2.

Homogenate Results above described clearly indicate that the characteristic oxygen consumption in the medulla homogenate resulted from the inhibition of adrenalin oxidation by endogenous ascorbic acid in the medulla. To confirm this further, the determination of the optical densities at $470\text{ m}\mu$, of the amount of adrenalin, total vitamin c (ascorbic acid, dehydroascorbic acid and diketoglonic acid) and ascorbic acid were simultaneously made with that of oxygen consumption. For this purpose, 10 ml of 10% adrenal medulla homogenate, 20 ml of phosphate buffer, 6 ml. of 6×10^{-5} M cytochrome c and 24 ml of H_2O were mixed, and with 3 ml of this mixture, placed in a Warburg flask, oxygen consumption was determined. The remainder was placed in an Erlenmeyer flask of 100 ml capacity, shaken in the same Warburg thermostat. Oxygen consumption was determined at 30-minute intervals, taking the setting time in the thermostat as O-time. Then immediately the reaction mixture in the Erlenmeyer flask was transferred in an amount of 1 ml, 1 ml and 3 ml into three centrifuging tubes, in which preliminarily was kept refrigerated 3 ml of H_2O , 1 ml of 8% trichloroacetic acid plus 8 ml of 4% trichloroacetic acid, and 3 ml of 8% trichloroacetic acid respectively. The first mixture with H_2O was used for the determination of extinction at $470\text{ m}\mu$. The next sample was used for the determination of total vitamin c and the last, after being centrifuged, for the determination of ascorbic acid and adrenalin

Fig. 2. Oxidation of adrenalin in the adrenal medulla of ascorbic-acid deficient guinea pig. For experimental conditions see text. A: ascorbic acid deficient; B: ascorbic acid treated control; C: normal control.

amount. As shown by Fig. 3, for the first 30 minutes, oxygen consumption did not increase, and the absorption at $470\text{ m}\mu$, which indicates production of adrenochrome did not increase, but ascorbic acid abruptly decreased during this time, becoming so little in 60 minutes that the determination by this method was not answered by any definite data. After this time, the reaction mixture colored so reddish that it was difficult to determine the end point of the reaction. About this time of near complete exhaustion of ascorbic acid, adrenalin level began to decline abruptly in coincidence with the abrupt rise of the rate of oxygen consumption together with the rise of absorption at $470\text{ m}\mu$. In this case the oxidation of the greater part of ascorbic acid did not proceed beyond the formation of diketogluconic acid. The results of this experiment clearly indicate that so long as ascorbic acid is present, even in an extremely small amount, adrenalin can not be oxidized in the adrenal medulla homogenate, to which cytochrome c is added.

Immediately after the determination of extinction, the first mixture with H_2O was diluted with acetate buffer at pH 8.4 and poured on a alumina column. After purification by the method of Weil-Malherbe and Bone (1952), Lund's fluorometric determination of adrenalin was followed (*cf.* Persky, 1955). Although data are not present in this paper, resultant value of adrenalin was less than expected. In all probability it might be due to that removal of oxidative enzymes by the addition of trichloroacetic acid was not the case, and that further oxidation proceeded, and that adrenochrome was removed and did not participate in the

Fig. 3. Changes in the amount of adrenalin and ascorbic acid with oxygen consumption of adrenal medulla homogenate. For experimental conditions see text. A: μ l oxygen consumed; B: extinction at 470 m μ ; C: mg/3 ml of adrenalin; D: μ g/3 ml of ascorbic acid; D': μ g/3 ml of total vitamin c.

adrenalin determination. However, the alumina method of Weil-Malherbe and Bone was inadequate for the present purpose, since acetate has no buffering action at pH 8.4, and adjustment of pH of such solution by glass electrode took much time.

Effect of Addition of Ascorbic Acid It is clear from the above results that ascorbic acid deficiency leads to adrenalin oxidation. Below is shown the results which were obtained when ascorbic acid was added to the adrenal medulla homogenate (Fig. 4).

When 300 μ g/ml (final concentration) of ascorbic acid was added to the adrenal medulla homogenate, oxygen consumption which was high from the beginning of incubation, gradually declined, became very low in 80 minutes, but regained rapid rise toward 110 minutes. About this time the reaction mixture for the first time began to assume red color. On the contrary, the initial increase in oxygen consumption was not conspicuous in the case of the addition of 30 μ g/ml of ascorbic acid, and time lag seemed prolonged, *i. e.*, rise in oxygen consumption reached the maximum rate with the delay of 15-20 minutes as compared with that in the controls. The initial high oxygen consumption in the former case may be attributed to the oxidation of ascorbic acid itself. When ascorbic acid was added to a mixture of the heart muscle homogenate and adrenalin, time lag was prolonged, presenting a curve resembling that for the adrenal medulla (Fig. 5.)

Fig. 4. Effect of addition of ascorbic acid upon oxygen consumption of adrenal medulla homogenate. Incubation medium contained 1.0 ml of phosphate buffer, 1.0 ml of 5% adrenal medulla homogenate, 0.3 ml of 6×10^{-5} M cytochrome c, 0.2 ml of ascorbic acid (final concentrations 30 $\mu\text{g/ml}$ and 300 $\mu\text{g/ml}$) and 0.5 ml of H_2O . A: control; B: 30 $\mu\text{g/ml}$; C: 300 $\mu\text{g/ml}$ of ascorbic acid was added.

When cytochrome c was added to adrenalin solution, oxidation took place also in the presence of even very small amount of tissue homogenate representing the activity of cytochrome oxidase. It was investigated whether or not ascorbic acid could inhibit the oxidation of adrenalin in such a case. Since adrenalin underwent so rapid autoxidation in the absence of protein that details of reaction could not be observed, adrenalin was dissolved in 1% albumin, and 0.1 ml of a 0.06% heart muscle homogenate of the mouse was added as cytochrome oxidase. The results of addition of 8.3–133.3 $\mu\text{g/ml}$ of ascorbic acid were reproduced by Fig. 6. The higher the ascorbic acid concentration the more was retarded adrenalin oxidation. The higher oxygen consumption than in controls would be resulted from addition of oxygen amount required for ascorbic acid oxidation.

Effect of Ascorbic Acid upon Autoxidation of Adrenalin As described above, ascorbic acid inhibited the oxidation of adrenalin by cytochrome system, which was endogenous in the adrenal medulla or added to the heart muscle homogenate, or in extremely low tissue concentration. It inhibited autoxidation of adrenalin in the absence of cytochrome system too. Figure 7 shows the effect of 133.3 μg , 33.3 μg and 8.3 $\mu\text{g/ml}$ of ascorbic acid upon the autoxidation of 133.3 $\mu\text{g/ml}$ of adrenalin at pH 7.4. As shown in this figure, rapid increase of extinction at 470 $\text{m}\mu$ was observed with the control from the onset of determinations. In the case with 8.3 $\mu\text{g/ml}$ of ascorbic acid, oxidation of adrenalin was observed to proceed at a very low rate for the first 60 minutes, but was suddenly enhanced afterwards. In the case of 33.3 $\mu\text{g/ml}$ oxidation did not take place in any significant measure

Fig. 5. Effect of ascorbic acid upon oxidation of adrenalin which was added to the heart muscle homogenate. Experimental conditions were the same as in Fig. 4 excepting that 0.5 ml of 800 $\mu\text{g}/\text{ml}$ adrenalin substituted H_2O . A: control; B: ascorbic acid was added.

for as long as 195 minutes, and then suddenly increased. And in the case of 133.3 $\mu\text{g}/\text{ml}$ oxidation was almost suppressed for as long as 375 minutes.

Effects of Ascorbic Acid upon Hydrogen Transfer by Adrenochrome Ascorbic acid inhibited adrenalin oxidation, but such an action was not observable when production of adrenochrome reached a certain level. As shown by Fig. 8, oxygen consumption, instead of being depressed, began to increase remarkably on addition of ascorbic acid to a mixture of adrenal medulla homogenate and cytochrome c at the end of 20 minutes, when the oxidation began to increase slightly, and the mixture tinged reddish. The increase resulted from the simultaneous oxidation of ascorbic acid by adrenochrome. When adrenochrome was added to ascorbic acid in the absence of adrenalin and tissue, ascorbic acid was oxidized rapidly (Fig. 9). When ascorbic acid concentration was overwhelmingly higher than adrenochrome concentration, the red color of adrenochrome faded away but oxygen consumption did not decline. Measurement of optical absorption of the discolored mixture revealed the maximum value at 410 $m\mu$, and irradiation at 416 $m\mu$ developed fluorescence, which looked like to indicate the production of adrenolysis. The reducing power of ascorbic acid did not seem to reduce adrenochrome to adrenalin.

Fig. 6. Effect of various concentration of ascorbic acid upon the oxidation of adrenalin which was added cytochrome c and very diluted heart muscle homogenate. Incubation medium contained 1.0 ml of phosphate buffer, 1.5 ml of 266.7 $\mu\text{g/ml}$ adrenalin solution dissolved in 1% albumin, 0.2 ml of 9×10^{-5} M cytochrome c, 0.1 ml of 0.06% heart muscle homogenate and 0.2 ml of ascorbic acid. A: control; B: 8.3 $\mu\text{g/ml}$; C: 16.7 $\mu\text{g/ml}$; D: 33.3 $\mu\text{g/ml}$; E: 66.7 $\mu\text{g/ml}$; F: 133.3 $\mu\text{g/ml}$ of ascorbic acid was added.

Fig. 7. Effect of ascorbic acid upon autoxidation of adrenalin. Incubation medium contained 1.0 ml of phosphate buffer, 1.0 ml of ascorbic acid and 1.0 ml of 400 $\mu\text{g/ml}$ adrenalin which was added at last. A: control; B: 8.3 $\mu\text{g/ml}$; C: 33.3 $\mu\text{g/ml}$; D: 133.3 $\mu\text{g/ml}$ of ascorbic acid was added.

Fig. 8. Effect of ascorbic acid added immediately after the onset of abrupt increase of oxygen consumption in adrenal medulla homogenate. Experimental conditions were the same as in Fig. 4 excepting that ascorbic acid was added from the side-arm to main chamber immediately after checking oxygen consumption at the end of 20 minutes. A: ascorbic acid was added; B: control; C: autoxidation of same concentration of ascorbic acid as A.

DISCUSSION

In the present experiment, it was made clear that an extremely large amount of ascorbic acid was found in the adrenal medulla, and that the oxidation of adrenalin to adrenochrome was inhibited by ascorbic acid under various conditions. Such observations are implicable that ascorbic acid would work so as to protect the hormonal substance in the adrenal medulla from oxidative breakdown to the effect that loss of the hormone is prevented.

Ascorbic acid is known to be found extensively in mammalian tissues, especially richly in the nervous system and endocrine glands (Williams, 1943; Suzuki *et al.*, 1957). Of the latter, the adrenal cortex and the pituitary gland, and of the former, the hypothalamus, including a number of neurosecretory cells, are said to contain significant quantities (Diehl and Neumann, 1939; Schiebeler, 1951; *cf.* Scharrer and Scharrer, 1954).

Fig. 9. Effect of adrenochrome upon oxidation of ascorbic acid. A: 0.1 ml of 180 $\mu\text{g/ml}$ adrenochrome was added from the side-arm to the mixture of 1.0 ml of phosphate buffer, 1.0 ml of 900 $\mu\text{g/ml}$ ascorbic acid and 0.9 ml of H_2O 10 minutes after the onset; B: autoxidation of ascorbic acid; C: autoxidation of adrenochrome.

In the adrenal medulla, however, ascorbic acid had been rather overlooked, since the histochemical tinctorial reaction of the substance had failed in the tissue in question. Later, Harris and Ray (1933), Huszák (1933), *etc.*, succeeded in demonstrating the substance and suggested the presence of a certain substance which inhibits the staining reaction. Recently, Hagen (1954) reported that the beef adrenal medulla is much enriched with ascorbic acid, and that the most part of it could be fractionated in the supernatant by high-speed centrifugation. Suzuki *et al.* (1957) determined the ascorbic acid contents of various tissues of the beef and 1.4 mg/g wet weight was reported as the value for the beef adrenal medulla. In the present experiments, ascorbic acid content was determined in various tissues of the pig and horse to find the value in the adrenal medulla equivalent to that of the cortex.

Despite such a high concentration of ascorbic acid in tissues in general, its physiological significance has remained almost undetermined. For example, while it is well known fact that ascorbic acid in adrenal cortex decreases extremely under the condition of stress, relation between corticoid secretion and ascorbic acid metabolism is not yet clear. Generally, ascorbic acid is assumed to be used for

corticoid production. In this connection, there is a different view that ascorbic acid inhibits corticoid production and that corticoid production becomes possible only when ascorbic acid decreases (Meiklejohn, 1951). Kersten *et al.* (1956) stated that ascorbic acid combined with mitochondria of the adrenal cortex turned into an intermediate oxide, monodehydroascorbic acid, which accelerated oxidation as an electron transport system in the production of corticoid.

Also relation between ascorbic acid and thyroid function is not elucidated sufficiently. Suzuki (1957) made studies on the effect of ascorbic acid upon iodine metabolism in the thyroid of the beef and guinea pig, but without any definite results.

Concerning the physiological significance of ascorbic acid in the adrenal medulla, as has been inferred in the introductory remarks, little information has been published. A synergism of adrenalin and ascorbic acid was postulated by Kasahara *et al.* (1937, 1938) who injected adrenalin and ascorbic acid, separately or together, into normal and scorbutic guinea pig with resultant hyperglycemia in the deficient animals only when two were given together, and it was confirmed by Shimamura (1938) (*cf.* Morgan, 1951). From the author's point of view, this result is attributed to the prevention of injected adrenalin from being broken down through the agency of simultaneously given ascorbic acid. On the other hand, Giroud and Martinet (1944), and Banerjee (1944, 1945), recognized an inverse relationship between ascorbic acid and adrenalin contents in adrenal medulla of guinea pig (Morgan, 1951), which is incompatible with the view postulated by the author on the physiological significance of medullar ascorbic acid.

There are three ways of oxidation of adrenalin, *viz.*, autooxidation, oxidation through the agency of cytochrome system and that through adrenochrome. It is easily presumable and effectively demonstrated in the present experiments that autooxidation is inhibited by ascorbic acid. Further, it was observed that the oxidation was not inhibited when ascorbic acid was added to a medulla homogenate after the production of adrenochrome by incubation, *i. e.*, the oxidation of adrenalin by autoxidizable adrenochrome can not be inhibited by ascorbic acid. In this case ascorbic acid is rapidly oxidized by adrenochrome, a fact observed also by Radsma and Golterman (1954). In their experiments, such an oxidation did not decline even when cytochrome system was inactivated with KCN. This was confirmed by the present experiments. Moreover, Radsma and Golterman observed under the same conditions that adrenalin did not accelerate the oxidation of ascorbic acid. This can be explained on the basis of the results of the present experiments as follows: Despite the co-existence of high concentration of cytochrome c with rat liver homogenate, the oxidation of adrenalin, and consequently the production of adrenochrome was inhibited in the presence of a large amount of ascorbic acid.

Adrenalin turned into adrenochrome through oxidation by cytochrome system. Huszák (1942) reported that neither cytochrome c nor cytochrome oxidase was present in the adrenal medulla, but Suzuki *et al.* (1957) recently reported the presence of both.

As a consequence of the above discussion, the author wants to assume that the role of ascorbic acid is to inhibit not only the autooxidation but also the oxidation by

cytochrome system of adrenalin.

Physicochemical mechanism of this inhibition still remains in obscurity. Then oxidation-reduction potentials of adrenalin and ascorbic acid were compared, the following results were obtained: Ascorbic acid, $E_0: +0.051$ (pH 6.32). Adrenalin, $E_0: +0.345$ (pH 7.66) (*cf.* Hewitt, 1950). In this way, the former is extremely lower than the latter. Yamamoto (1955) observed that electrode potential of ascorbic acid solution under aerobic condition was extremely low. From this fact it can be inferred that ascorbic acid has higher escaping tendency of the electrons than adrenalin, consequently raising electron activity of the medium and in this way inhibiting the escape of the electrons from adrenalin, *i. e.*, its oxidation into adrenochrome, or it may be considered that easier linking of ascorbic acid with cytochrome system competitively inhibits the same of adrenalin.

Taking into account such results of the present experiments and above discussion, the author is of opinion that ascorbic acid which presents at high concentration in the adrenal medulla is adaptive to the necessity in that it protects adrenalin from oxidation. However, such a consideration does not exclude other interpretations basically. So, it is considered naturally that ascorbic acid may have some other relationships to the biosynthesis and discharge of adrenalin.

SUMMARY

1. Measurement of ascorbic acid content of adrenal medulla of the pig and horse rendered the values of 1.99 and 2.08 mg per g wet wt. respectively. These values are comparable with the values for the adrenal cortex, and represent the highest among the values in all the tissues submitted to measurement.

2. It was learned that, so far as a certain amount of reduced ascorbic acid is present in the adrenal medulla homogenate, oxidation of adrenalin does not take place.

3. Autoxidation and oxidation of adrenalin through the agency of cytochrome system are inhibited by the addition of ascorbic acid, and the rate of inhibition is parallel with the concentration of the latter.

4. Data were presented and discussed to the effect that ascorbic acid found abundantly in the adrenal medulla would possibly be concerned with protection from oxidation of high concentration of adrenalin at its site of elaboration.

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