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THE FORMATION *IN VIVO* OF *NOR*ADRENALINE FROM 3 : 4-DIHYDROXYPHENYLSERINE (*NOR*-ADRENALINE CARBOXYLIC ACID)

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The amino-acid 3 : 4-dihydroxyphenylserine was first described by Rosenmund and Dornsaft (1919), who suggested that it might be a precursor of adrenaline. During the extensive studies of enzymic formation of pressor amines carried out in this laboratory, Blaschko, Holton, and Sloane Stanley (1948) found that the decarboxylase present in *Streptococcus faecalis* R was able to decarboxylate dihydroxyphenylserine *in vitro*, the amine formed being *l-nor*adrenaline. They did not, however, obtain any evidence that the mammalian amino-acid decarboxylase, present, for example, in guinea-pig's kidney, was able to bring about this reaction. Recently Beyer (1950) observed that mammalian tissue extracts could in fact convert *nor*adrenaline carboxylic acid into a pressor substance with evolution of CO_2, and jointly with Blaschko, Burn, and Langemann (1950) he summarized evidence that the pressor substance was *nor*adrenaline. The work in this laboratory (Blaschko, Burn, and Langemann, 1950) showed that decarboxylation occurs, but only at a very slow rate and with fairly large amounts of tissue extract present, which was the reason why it had not been observed earlier. The amine formed exerted a strong pressor action in the spinal cat and at the same time only a weak action on the normal nictitating membrane and thus resembled *nor*adrenaline. Calculation showed that only the laevorotatory stereoisomer was produced.

Dopa-decarboxylase was originally described as an enzyme present in mammalian kidneys (Holtz, Heise, and Lüdtke, 1938), and, although it has later been shown to exist in other organs, renal tissue seems to be by far the most active source. Bing (1941) and Bing and Zucker (1941) showed that ischaemic cat's kidneys were able to produce a pressor substance, presumably hydroxytyramine, by decarboxylation of dihydroxyphenylalanine both in perfusion experiments and in experiments *in vivo*. Holtz, Credner, and Koepp (1942) made experiments on various animals (and man), and found that after both oral and parenteral administration of Dopa the urine contained hydroxytyramine.

From these considerations the question arose whether the administration of dihydroxyphenylserine to experimental animals would lead to an analogous output of *nor*adrenaline in the urine.

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METHODS

Rabbits were chosen as the most suitable animals. A good diuresis can easily be obtained after giving water through a stomach tube without an anaesthetic.

The animals were given 100 ml. tap water in order to start diuresis. The next day the same amount of water was given in the evening and the animals were put in metabolism cages. The urine produced was collected over-night in beakers in which 0.5 ml. of hydrochloric acid (conc. HCl diluted 1 : 1) was placed to keep the urine acid. These samples were collected in order to determine whether pressor substances were present in considerable amount in the normal urine of the rabbit.

The next evening the animals were given dihydroxyphenylserine intravenously, usually 5 mg. per kg. body weight. The amino-acid was dissolved in a few ml. of saline. Immediately after this they were again given 100 ml. tap water by stomach tube and put in the cages. The urine was collected over-night, acid being added as described above. The *p*H of the urine, thus collected, was usually about 3–4.

The dihydroxyphenylserine used was a sample of the material prepared by Dalgliesh and Mann (1947), previously used by Blaschko and his co-workers.

In a few experiments adrenalectomized rabbits were used in order to find out if the removal of the suprarenals interfered with the decarboxylation of dihydroxyphenylserine.

The amount of urine formed by the rabbits was usually well over 100 ml. In preliminary experiments it was found that this urine did not exert any significant action when tested on the cat's blood pressure and nictitating membrane. The urine was therefore concentrated by two methods.

The first method was as follows: Two volumes of alcohol were added to the acidified urine and the mixture was put in the refrigerator for two hours. It was then filtered on a Buchner funnel and the filtrate evaporated *in vacuo* to a small volume, 1 ml. of the final extract usually corresponding to 5 or 10 ml. of urine.

As it was soon found that these concentrates of urine contained blood pressure lowering substances in amounts large enough to interfere with the successful estimation of the *nor*adrenaline present, the method of adsorbing catechols described by Euler (1948, 1949), Euler and Hellner (1950), and Goodall (1950) was adopted in the following manner: Enough sulphuric acid was added to the urine to bring the *p*H to about 1. A 20 per cent (w/v) solution of $Al_2(SO_4)_3$ was added to the filtered urine in an amount equal to 2 per cent of the urine volume and also 1 ml. of BDH " universal indicator." From a burette 0.5 per cent (w/v) NaOH was added drop by drop, stirring continuously until a *p*H of 7.5 was reached, as shown by the colour change. The precipitate of aluminium hydroxide thus formed was then allowed to settle and collected on a Buchner funnel. The precipitate was washed three times with distilled water and then dissolved in a small volume (5–10 ml.) of 2N-H_2SO_4. To this solution 1 per cent (w/v) NaOH was added to bring the *p*H to about 3. Four volumes of a mixture containing equal parts of alcohol and acetone were then added and the solution left over-night in the refrigerator. The precipitated salts were filtered off and the filtrate evaporated to a small volume *in vacuo*.

For reasons which will be discussed later the majority of the extracts were tested on the blood pressure and the nictitating membrane of the cat under chloralose with the suprarenals tied off and after the intramuscular administration of cocaine (3 mg./kg.), an antihistamine (2 mg. Lergigan/kg.), and atropine (0.5 mg./kg.).

RESULTS

Normal rabbit's urine.—The samples of normal urine treated with alcohol and concentrated were invariably found to cause a blood pressure fall when injected into the cat. Since Anrep, Ayadi, Barsoum, Smith, and Talaat (1944) have shown that

rabbit urine contains small amounts of free histamine it seemed probable that this fall was due to the presence of histamine. For this reason the antihistamine Lergigan, described by Halpern and Schmiterlöw (1951), was given in a dose of 2 mg./kg. After this the same samples still gave a slight drop in blood pressure although the effect of equiactive amounts of histamine was abolished. This small fall disappeared, however, when atropine was given, the extracts now having almost no effect on the blood pressure. Sometimes there was a very small rise, not amounting to more than a few mm. of Hg. It has been shown by several authors that normal urine from different species of animals contains pressor substances, but from these results the amount present in the concentrate from 10–20 ml. rabbit urine is negligible. In Fig. 1 is shown the effect of 1 ml. of normal urine concentrate, equivalent to 10 ml. of original urine, before and after the test animal (cat under chloralose) was treated with Lergigan and atropine. The figure shows that after the administration of the antihistamine the

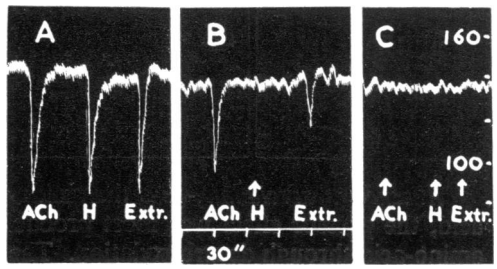

FIG. 1.—Cat; chloralose; blood pressure record. ACh = 1 μg. acetylcholine. H = 2 μg. histamine (base). Extr. = 1 ml. of urine extract (= 10 ml. of original urine) obtained by treating the urine with alcohol and subsequent concentration. (A) Before an antihistamine drug and atropine were given; (B) after 2 mg. of Lergigan per kg.; (C) after Lergigan and 0.5 mg. of atropine per kg.

effect of a dose of histamine, originally giving the same fall in blood pressure as 1 ml. of the urine concentrate, was now abolished, whereas the effect of an equiactive dose of acetylcholine was only diminished to a slight extent. After a further dose of atropine neither acetylcholine nor histamine nor the urine concentrate caused any effect on the blood pressure.

When the normal urine was treated by Euler's adsorption method the final concentrated extracts did not lower the blood pressure. This is in full accordance with the statement of Euler (1949) that this adsorption method gave " extracts with pure pressor activity." Evidently depressor substances are not adsorbed on the aluminium hydroxide. Neither did these extracts cause any significant rise of blood pressure (see Fig. 3, D).

Urine from rabbits given dihydroxyphenylserine.—When the rabbits received dihydroxyphenylserine intravenously their urine was found to contain a pressor principle.

At first the urine samples were treated with alcohol and concentrated. When these concentrates were injected into the chloralosed or spinal cat the effect on the blood pressure was an initial fall, followed by a slight secondary rise. After the administration of Lergigan and atropine to the test animal the urine samples no longer produced a fall in blood pressure but a conspicuous rise, which was enhanced by cocaine. Fig. 2 shows the difference in effect of the urine samples before and after the administration of the antihistamine.

The blood pressure rise thus caused by the urine samples was matched against *nor*adrenaline. It was found that 10 ml. of urine (= 1 ml. of concentrate) gave an effect equal to that of about 2–3 μg. *l-nor*adrenaline.

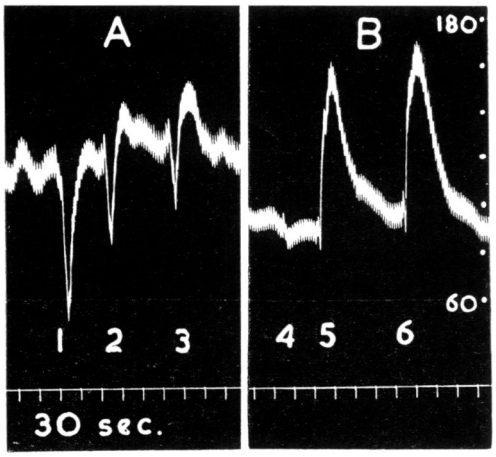

FIG. 2.—Cat; chloralose; blood pressure record. 1 and 4 = 2 μg. histamine (base). 2 and 5 = 1 ml. of concentrated urine (= 5 ml. of original urine) collected from rabbit given 5 mg. dihydroxyphenylserine per kg. intravenously. Urine treated with alcohol and concentrated. 3 and 6 = a similar sample from another animal. (A) Before and (B) after the administration of 2 mg. Lergigan per kg.

Absence of adrenaline.—As the amino-acid injected was *nor*adrenaline carboxylic acid, the pressor effect was most probably due to *nor*adrenaline, derived from the amino-acid through decarboxylation. The possibility exists, however, that adrenaline may also be formed *in vivo* from this compound, which would lead to the output of a mixture of adrenaline and *nor*adrenaline.

One method of estimating these substances in a mixture has been described by Burn, Hutcheon, and Parker (1950); in this a spinal cat is used and the blood pressure and the contraction of the normal nictitating membrane recorded. The method, however, requires a concentration of about 10 μg./ml. of the two amines, which is much greater than was present in the urine concentrates. The cat under chloralose was therefore used instead, the sensitivity of which is much higher especially when cocaine has been administered (3 mg./kg. by intramuscular injection). Cocaine, however, diminishes the difference between the effect of *nor*adrenaline and of adrenaline on the nictitating membrane, but the difference can be brought out again by giving atropine (0.5 mg./kg.). This method of testing is discussed elsewhere (Schmiterlöw, 1951). It was found satisfactory when applied to urine concentrates prepared by the adsorption method. When alcoholic extracts were examined it was necessary to tie off the adrenals and to give Lergigan and atropine as well as cocaine.

When the urine extracts were now tested it was found that their effect on the nictitating membrane corresponded exactly to the effect caused by equipressor doses of *l-nor*adrenaline. There was no sign of an admixture of adrenaline.

Yield of nor*adrenaline.*—In the early experiments varying doses of dihydroxyphenylserine were injected, 15, 10, and 5 mg. per kg., but no significant relationship between the injected dose and the output of *nor*adrenaline was observed. For this reason and because of shortage of the amino-acid, the lowest dose, 5 mg. per kg., was used throughout the rest of the experiments. In all, 22 experiments were done with this

dose in 12 rabbits. In all the experiments the urine contained a pressor constituent which behaved like pure *nor*adrenaline. The diuresis of the rabbits varied, the lowest output of urine during approximately fifteen hours (from the evening till next morning) being 75 ml., the highest 245 ml. The calculated average output of *nor*adrenaline was found to be 0.34 $\pm$ 0.03 (standard deviation $\pm$ 0.13) μg. per ml. of urine. If the total output of *nor*adrenaline was compared with the amount of dihydroxyphenylserine given (5 mg. per kg.) the yield of *nor*adrenaline from the amino-acid was on an average 0.39 $\pm$ 0.02 (standard deviation $\pm$ 0.11) per cent.

In Fig. 3 is shown the effect of one of the extracts (adsorption method) on the blood pressure and nictitating membrane in comparison with the effect of *nor*-adrenaline and adrenaline. The effect of normal urine from the same rabbit is also shown.

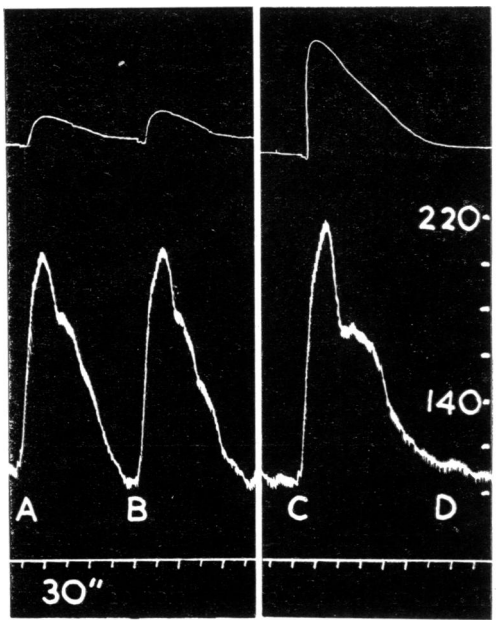

FIG. 3.—Cat; chloralose; normal nictitating membrane above, blood pressure below. Cocaine (3 mg. per kg.) and atropine (0.5 mg. per kg.) given. A = 1 ml. of urine concentrate (= 10 ml. of original urine) obtained by the adsorption method; urine from rabbit which had been given 5 mg. dihydroxyphenylserine per kg. B = 3 μg. *l-nor*adrenaline. C = 7.5 μg. *l*-adrenaline. D = 1 ml. of urine concentrate (= 10 ml. of urine) obtained by the adsorption method; normal urine from the same rabbit as in A.

In two rabbits under urethane the effect of the intravenous injection of dihydroxyphenylserine on the blood pressure was recorded. No alteration in the blood pressure could be observed. If the amino-acid was injected into the cat there was no rise in blood pressure even if a dose of 2 mg. was given.

Presence of adrenal glands.—The appearance of *nor*adrenaline in the urine from rabbits given dihydroxyphenylserine was not influenced by the removal of the suprarenals, the output still being approximately the same as before. This was so whether the experiment was performed the day after the operation or eight days later.

Adrenaline carboxylic acid.—In four rabbits the effect of N-methyl-β-(3 : 4-dihydroxyphenyl) serine (= adrenaline carboxylic acid) was also tested, the same amount of this amino-acid being used as of the *nor*adrenaline carboxylic acid. The

urine from these rabbits, treated according to Euler's adsorption method, gave a slight rise in blood pressure (Fig. 4, C). This effect is, however, of little significance, since the compound used was contaminated with adrenaline. It is shown in Fig. 4 that the injection of 2 mg. of this amino-acid sample caused a rise in blood pressure equal to that caused by 5 μg. adrenaline, but a greater contraction of the nictitating membrane.

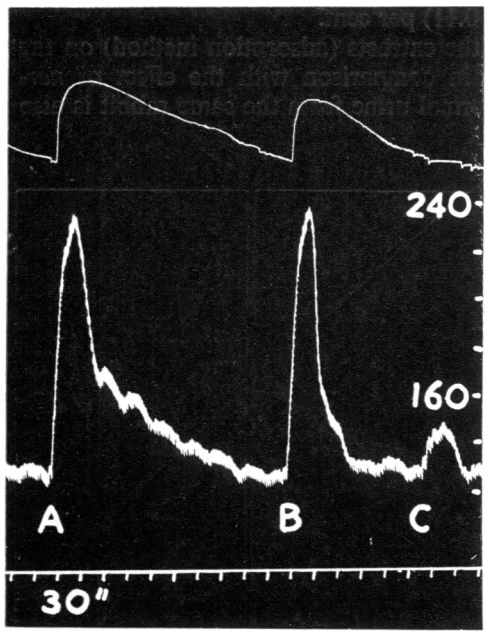

FIG. 4.—Cat; chloralose; normal nictitating membrane above, blood pressure below. Cocaine (3 mg. per kg.) and atropine (0.5 mg. per kg.) were given. A = 2 mg. adrenaline carboxylic acid. B = 5 μg. /-adrenaline. C = 1 ml. of urine concentrate (= 10 ml. of original urine) obtained by the adsorption method; urine from rabbit which had been given 5 mg. adrenaline carboxylic acid per kg. intravenously.

COMMENTS

The present investigation has shown that the urine from rabbits which have been given dihydroxyphenylserine (*nor*adrenaline carboxylic acid) contains *nor*adrenaline. This observation is an extension of the recent *in vitro* findings that this amino-acid can serve as a substrate for the mammalian amino-acid decarboxylase (Beyer, 1950; Beyer, Blaschko, Burn, and Langemann, 1950; Blaschko, Burn, and Langemann, 1950). It is now evident that this decarboxylation in fact also takes place *in vivo*.

During recent years extensive studies have been carried out on the presence and physiological role of *nor*adrenaline in various tissues and body fluids. Little is known, however, about the biosynthesis of this substance. In a recent review Blaschko (1950) gives the different pathways along which adrenaline and *nor*adrenaline may be formed in the body. It is tempting to assume, in the light of both *in vitro* and *in vivo* studies now available, that *nor*adrenaline carboxylic acid may serve as a precursor to *nor*-adrenaline. The main objection against such an assumption is, of course, that this amino-acid is at present a synthetic compound which has not yet been shown to exist in the body as a natural constituent. It seems to be of great interest to study this

question. Another objection could be that the amount of *nor*adrenaline formed from dihydroxyphenylserine *in vivo* in the present experiments was very small, the yield of *nor*adrenaline being only about 0.3 per cent of the injected amount of amino-acid. This figure is considerably less than that found in earlier experiments *in vitro*. It must be remembered, however, that the experiments *in vitro* were carried out under anaerobic conditions which prevented the further oxidation of the *nor*adrenaline formed. Even if a fairly large amount of *nor*adrenaline was in fact produced from the amino-acid *in vivo* it could hardly be expected that all this would appear in the urine, since it must be presumed that most of the amine would be rapidly oxidized in the body. For this reason little significance should be attributed to the quantitative estimation of the *nor*adrenaline recovered from the urine, but emphasis should rather be put on the fact that it occurs at all as a result of a decarboxylation process. Further investigations may show whether the output increases when the kidneys are rendered ischaemic or other precautions are taken to diminish oxidative processes to a minimum.

The results of testing the urine samples on the blood pressure and nictitating membrane of the cat under chloralose indicated that the pressor substance found was *nor*adrenaline only; they did not indicate whether the amine formed was the laevorotatory compound or the racemic form. In the *in vitro* experiments only *l-nor*adrenaline was formed (Blaschko, Burn, and Langemann, 1950). It is interesting to note that there was no evidence of the formation of adrenaline from the dihydroxyphenylserine, especially in view of the finding by Bülbring (1949) that suprarenal tissue is able to methylate *nor*adrenaline. Adrenaline if formed might have been destroyed before excretion.

When adrenaline carboxylic acid was given instead of *nor*adrenaline carboxylic acid the urine samples only caused a very slight rise in blood pressure. This rise was explained by a contamination of the amino-acid used with adrenaline. It was shown by Blaschko, Burn, and Langemann (1950) that adrenaline carboxylic acid does not serve as a substrate for amino-acid decarboxylase *in vitro* and it was therefore not to be expected that the administration of this amino-acid would lead to an output of adrenaline.

SUMMARY

1. When dihydroxyphenylserine, which is also known as *nor*adrenaline carboxylic acid, was administered to rabbits by intravenous injection in a dose of 5 mg. per kg., varying amounts of *nor*adrenaline were found in the urine.

2. The experiments were performed by making the injection when diuresis was established. The urine was collected, concentrated, and freed from depressor substances by Euler's method. The extract was tested on the blood pressure and nictitating membrane of the cat under chloralose. The test made it possible to show that the active material was *nor*adrenaline alone and that no adrenaline was present.

3. Normal rabbit urine extracted in this manner contained no pressor substances in the doses used.

4. The transformation still occurred in animals after adrenalectomy.

5. The injection of the corresponding adrenaline carboxylic acid was not followed by the appearance of any pressor substance in the urine.

6. The possibility that *nor*adrenaline carboxylic acid is a natural precursor to *nor*adrenaline has been recently supported by experiments *in vitro*. The present investigation shows that this decarboxylation occurs *in vivo*, and this finding throws some light on the biosynthesis of *nor*adrenaline.

It is a great pleasure for me to express to Professor J. H. Burn my sincere gratitude for his great hospitality and never-failing support during my stay in his department. I am also greatly indebted to Dr. H. Blaschko and Dr. E. Bülbring for their continual help and advice during this investigation.

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