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THE FORMATION OF *NOR*ADRENALINE FROM DIHYDROXYPHENYLSERINE

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The occurrence of *nor*adrenaline in mammalian tissues first demonstrated by v. Euler is now well established. Evidence has also been obtained that in the suprarenal gland *nor*adrenaline is converted to adrenaline. Bülbring (1949) has shown that methylation occurs in minced suprarenal tissue in the presence of adenosine triphosphate and that the conversion is greatest after stimulation of the splanchnic nerve. Bülbring and Burn (1949b) have shown that a similar conversion takes place in the perfused suprarenal gland. Burn, Hutcheon, and Parker (1950) have found that when the suprarenal glands are first depleted by hypoglycaemia, the proportion of adrenaline to *nor*adrenaline in the recovery period falls below that in the normal glands.

The evidence of the occurrence and fate of *nor*adrenaline raises the question of how the compound is formed. Various pathways require consideration. One of these is discussed in this paper: the enzymic decarboxylation of 3: 4-dihydroxyphenylserine (which may be regarded as *nor*adrenaline carboxylic acid) in mammalian tissue extract. The enzymic decarboxylation of this amino-acid by a preparation from *Streptococcus faecalis R* has already been described (Blaschko, Holton, and Sloane Stanley, 1948). In this work it was shown that in the decarboxylation reaction laevo-rotatory *nor*adrenaline was formed; this is the isomer that occurs in the body. A few experiments with mammalian tissue extracts were also described, in which no formation of carbon dioxide was observed; no tests for pressor activity were carried out on this material. Beyer (1950), working with another specimen of dihydroxyphenylserine, found a formation of CO_2 and of a pressor agent on incubation with similar extracts, and it seemed therefore advisable to re-examine the question whether *nor*adrenaline can be formed from dihydroxyphenylserine by mammalian extracts.

MATERIAL AND METHODS

Two specimens of dihydroxyphenylserine were examined. The first was a sample of the material prepared by Dalgliesh and Mann (1947) which had already been used for the experiments with the bacterial enzyme, the other was a sample obtained from Messrs. Hoffman La Roche in Bâle which had been used in experiments by Guggenheim (1940). In addition, a few experiments were carried out with *N*-methyl-3: 4-dihydroxyphenylserine (adrenaline carboxylic acid), a compound first described by Dalgliesh and Mann (1947).

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The tissue extracts were made by grinding the fresh organs (kidney and liver) with sand and adding 1 ml. of 0.066 M-sodium phosphate buffer of $pH = 7.4$ for each g. of tissue. The extracts were centrifuged for about five minutes, and the supernatant was used. The incubation of the extracts with the amino- (or methylamino-) acid was carried out in conical manometer flasks at 37.5° C. and in an atmosphere of nitrogen. The flasks were set up with the extract in the main compartment and the amino-acid in the side arm. The reaction was begun by mixing the contents after a period of about fifteen minutes for temperature equilibration. The decarboxylating activity was checked by making parallel experiments with the same amount of tissue extract, but with L-DOPA as a substrate; in these experiments with DOPA, the retention of CO_2 by the extracts was determined by acidifying with sulphuric acid as previously described (Blaschko, 1942). The figures for CO_2 formed, which are given in the experimental part of this paper, are corrected for this retention.

At the end of the period of incubation the flasks were removed and the contents were immediately acidified by adding an equal volume of 0.1 N-HCl, heated in boiling water for two minutes, cooled and centrifuged. The supernatant was then stored in a refrigerator at −15° C. until used for the test for pressor activity.

These tests were carried out on the spinal cat; the arterial blood pressure and, in most experiments, the contractions of the nictitating membrane were recorded.

RESULTS

Experiments with dihydroxyphenylserine

The manometric experiments showed that gas was slowly evolved after dihydroxyphenylserine had been added to extracts of guinea-pig kidney under anaerobic conditions, indicating that carbon dioxide was formed. A slow but smaller evolution of CO_2 was also seen in the control flask containing the extract alone, without any amino-acid added. Table I gives the amounts of carbon dioxide evolved in both the control and experimental vessels in each of these experiments, nine in all.

TABLE I

DECARBOXYLATION OF DIHYDROXYPHENYLSERINE BY EXTRACT OF GUINEA-PIG'S KIDNEY

In experiments 3A and 4A the amino-acid prepared by Guggenheim was used.

Exp. No.	Time of incubation in min.	μl. CO_2 formed			μg. *l-nor*adrenaline	
		Control flask	Experimental flask	Difference experimental minus control	Expected	Found
1	300	45	87	42	317	266
2	300	97	142	45	340	322
3	320	54	98	44	332	324
3A	320	—	—	—	—	165
4	300	18	61	43	324	320
4A	300	18	52	34	256	240
5	300	83	149	66	498	264
6	265	49	79	30	226	209
7	242	30	73	43	324	275
8	260	28	77	49	370	143
9	240	49	86	37	279	252

It will be seen that in every experiment the vessel which contained the amino-acid had a higher rate of CO_2 formation than the control vessel. This observation strongly suggested that a decarboxylation of dihydroxyphenylserine was occurring at a slow rate during the incubation period.

This was proved when the reaction mixture was injected intravenously into the spinal cat preparation. The contents of the manometer flasks, control as well as experimental, were treated as described under Methods; the solutions were used suitably diluted with saline. Injection of the control fluids, extracts incubated without added amino-acid, caused no significant change of the arterial blood pressure, but the injection of samples from flasks in which dihydroxyphenylserine had been incubated with the extracts was invariably followed by a rise in blood pressure. Injection of an aqueous solution of the amino-acid, incubated under exactly the same conditions as the experimental samples, was without effect on the arterial blood pressure.

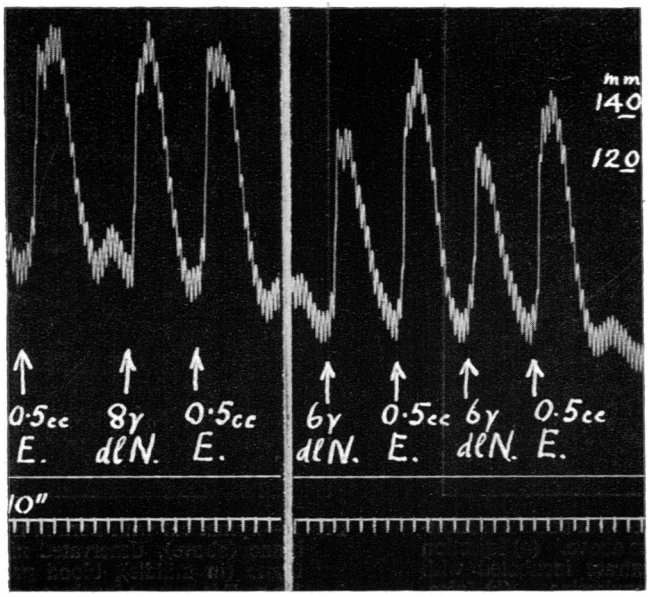


FIG. 1.—Spinal cat. Blood pressure. Extract of guinea-pig kidney incubated with dihydroxyphenylserine (*E*) produced CO_2 equivalent to the simultaneous production of 324 μg. of *l-nor*adrenaline. The figure shows that one-eightieth of the total amount of extract (equivalent to 4.05 μg. of *l-nor*adrenaline) had a pressor activity equal to that of 8 μg. of *dl-nor*adrenaline (*dl-N*), and greater than that of 6 μg. *dl-nor*adrenaline.

The blood pressure response after the injection of an experimental sample was like that of adrenaline or *nor*adrenaline. This is shown in the experiment of Fig. 1, where the blood pressure response to the experimental fluid was compared with that to *dl-nor*adrenaline. The experimental fluid, 4.0 ml. of the incubated and acidified extract, was diluted tenfold. The figure shows that the injection of 0.5 ml. of this diluted fluid caused a response equal to that of 8 μg. and greater than that of

6 μg. of *dl-nor*adrenaline. We have also calculated from the observed CO_2 formation the amount of *nor*adrenaline to be expected. This was a total of 324 μg. The amount of amine injected was therefore expected to be $\frac{324 \times 0.5}{4 \times 10} = 4.05$ μg. In fact it was equal in effect to 8 μg. *dl-nor*adrenaline, and thus was twice as active; this would be expected if *l-nor*adrenaline were formed.

In a similar experiment the contractions of the nictitating membrane were also recorded (Fig. 2). In this preparation the injection of 10 μg. of *l*-adrenaline caused

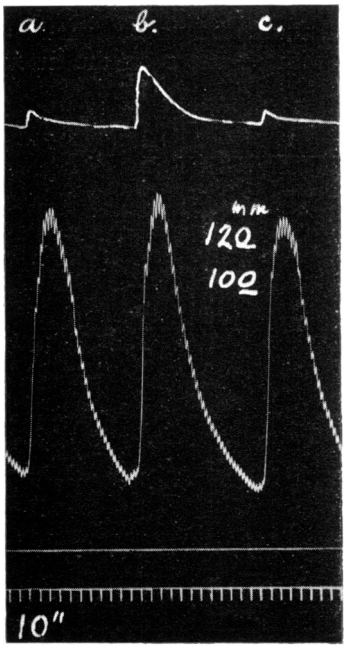


FIG. 2.—Spinal cat with normal nictitating membrane above. (*a*) Injection of kidney extract incubated with dihydroxyphenylserine. (*b*) Injection of 10 μg. *l*-adrenaline. (*c*) Injection of 20 μg. *dl-nor*adrenaline; note the similarity of (*a*) and (*c*) and the difference from (*b*).

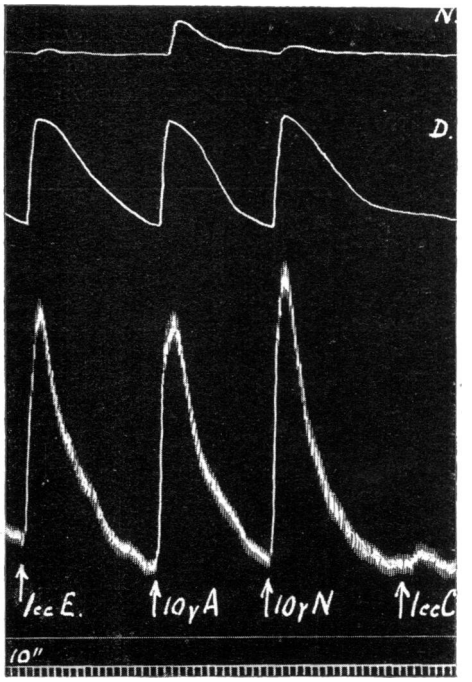


FIG. 3.—Spinal cat with normal nictitating membrane (above), denervated nictitating membrane (in middle), blood pressure (below). 1 c.c. *E* is extract incubated with dihydroxyphenylserine and diluted 5 times. Its effect on all three records is similar to that of 10 μg. *l-nor*adrenaline and unlike that of 10 μg. *l*-adrenaline on the normal nictitating membrane. 1 c.c. *C* is control extract incubated without amino-acid. It had no effect.

not only a blood pressure rise but also a contraction of the nictitating membrane. Injections of the experimental fluid as well as of *dl-nor*adrenaline gave similar blood pressure responses, but they had a very slight effect on the nictitating membrane.

Bülbring and Burn (1949a) have shown that after denervation the sensitivity of the nictitating membrane to *nor*adrenaline is increased. We have therefore also

examined the action of the incubated extract on the denervated nictitating membrane. In the experiment of Fig. 3 the contractions of the normal and the denervated nictitating membranes and the arterial blood pressure are recorded. The figure shows that the effect of the substance formed during incubation was *nor*adrenaline-like in that it caused no contraction of the normal membrane, whereas it had a marked effect on the denervated organ. An approximately equipressor dose of adrenaline was followed by a contraction of both the normal and the denervated membranes. The control extract incubated without addition of the amino-acid had no effect.

In nine of the eleven experiments in which the biological assay was carried out, the amino-acid prepared by Dalgliesh and Mann (1947) was used; in one experiment with the specimen studied by Guggenheim (1940) the result was essentially the same: carbon dioxide was formed on incubation and the pressor action observed indicated that the amine formed was also *laevo-nor*adrenaline. This shows that the two preparations of dihydroxyphenylserine had the same steric configuration.

It is known that the kidney of the guinea-pig is an organ particularly rich in L-DOPA decarboxylase (Holtz, Heise, and Lüdtke, 1938). It was for this reason that our experiments were chiefly done with extracts of this organ, but we have also used some extracts from other tissues. These are summarized in Table II. There

TABLE II

EXPERIMENTS WITH OTHER TISSUES

Tissue	Time of incubation min.	μl. CO_2			μg. *nor*adrenaline	
		Control	Experimental	Difference	Expected	Found
Guinea-pig liver	240	104	140	36	272	26
Guinea-pig liver	240	27	6	−21	—	126
Dog liver ..	225	120	157	37	—	—
Dog liver ..	300	46	48	2	—	—
Dog liver ..	115	−12	+10	22	—	—
Dog liver ..	240	0	11	11	—	—
Dog kidney ..	275	51	57	6	—	—
Dog kidney ..	63	10	8	−2	—	—
Dog small intest.	240	−4	−9	—	—	—

are two experiments with extracts from guinea-pig's liver. In both these experiments a test on the spinal cat was carried out and it was established that a pressor substance had been formed during incubation. There was, however, no agreement between the amount of CO_2 formed and the pressor activity. If we assume that L-DOPA decarboxylase is responsible for the decarboxylation reaction this is not difficult to understand, for it is known that in the guinea-pig the enzymic activity of the liver is much less than that of the kidney. In the dog, where enzymic activity is altogether very low, the liver is more active, weight by weight, than the kidney. This is borne out by our observations: with dog liver extracts we observed a small CO_2 formation in 3 out of 4 experiments, whereas in two experiments with dog kidney extracts no significant evolution of CO_2 was observed.

Experiments with N-*methyldihydroxyphenylserine*

This substance was first prepared by Dalgliesh and Mann (1947); in contrast to the corresponding amino-acid this methylamino-acid is not decarboxylated by acetone-dried preparations of *Streptococcus faecalis R* (Sloane Stanley, unpublished).

In three experiments with guinea-pig's kidney extracts we found no evidence of decarboxylation. In three experiments, the amounts of CO_2 formed during incubation were 27, 27, and 10 μl. respectively, but in the third of these experiments an aqueous solution of the compound, incubated under the same conditions as the compound and extract, also gave 10 μl. CO_2 in 5 hr. Tests showed that there was the same pressor activity after incubation with and without extract. Expressed in terms of adrenaline, the pressor activity corresponded to about 3 per cent of the amount of adrenaline carboxylic acid added. A sample of the substance, examined without incubation, was found to have a pressor activity corresponding to a content of about 0.9 per cent of adrenaline.

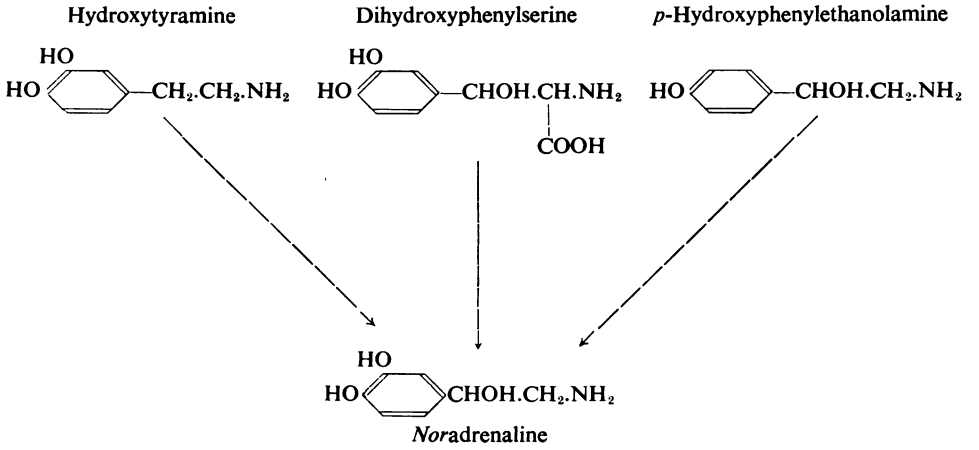
Discussion

The experiments described in this paper show that a slow formation of *laevo-nor*adrenaline from dihydroxyphenylserine occurs when the amino-acid is incubated with extracts of guinea-pig's kidney and liver under anaerobic conditions. These two tissues contain L-DOPA decarboxylase, and it is therefore possible that one and the same enzyme is responsible for the decarboxylation of both DOPA and dihydroxyphenylserine. The rate of the reaction with L-DOPA is much greater, and it is for that reason that the decarboxylation of the serine derivative was not noticed in earlier experiments (Blaschko, Holton, and Sloane Stanley, 1948); the amounts of tissue extract then used were too small, and the time of incubation too short, for a significant amount of CO_2 to be formed. Moreover, no tests for pressor activity were carried out. Tests of this kind were made of the amine produced from dihydroxyphenylserine by the decarboxylase of *Streptococcus faecalis R*, and the quantitative evaluation showed that the amine formed was laevorotatory *nor*-adrenaline. The present experiments indicate that the product of the reaction studied is also *l-nor*adrenaline. In view of the relatively large CO_2 formation in the enzyme blanks, and of the inaccuracy inherent in the determination of the CO_2 retention, the accuracy of the CO_2 measurement is not very high, but it is sufficient to indicate that the amine formed was much more active than *dl-nor*adrenaline.

Beyer (1950) has recently found that another sample of dihydroxyphenylserine, incubated with mammalian tissue, gave rise to a pressor substance and CO_2. Too little material was available to establish the identity of his material with the two samples used in our work, but in view of our results it seems very probable that the product was also *nor*adrenaline (Beyer, Blaschko, Burn, and Langemann, 1950).

These experiments raise the question: is decarboxylation the reaction by which *nor*adrenaline normally arises in the animal body? This question cannot at present be answered. Of the possible immediate precursors of *nor*adrenaline three may be considered; they are hydroxytyramine, dihydroxyphenylserine, and *p*-hydroxyphenylethanolamine (see formulae on page 437).

A full discussion of the formation of *nor*adrenaline in the animal body will only be possible after all these reactions have been studied. It may be mentioned that the



third pathway, that from *p*-hydroxyphenylethanolamine, is of interest in view of the recent statement by Werle and Peschel (1949) that *p*-hydroxyphenylserine is slowly decarboxylated by tissue extracts.

In our experiments the corresponding *N*-methyl amino-acid was not decarboxylated to form adrenaline. This is of interest as it shows that the serine derivatives conform to the general rule that methylamino-acids are not decarboxylated. It seems therefore unlikely that adrenaline can be formed from an immediate amino-acid precursor by decarboxylation.

SUMMARY

1. When dihydroxyphenylserine is incubated anaerobically with minced guinea-pig kidney, carbon dioxide is evolved and *nor*adrenaline is formed.

2. The formation of *nor*adrenaline has been demonstrated in the spinal cat by its pressor action, by its stimulant action on the denervated nictitating membrane, and by its very slight action on the normal nictitating membrane when compared with adrenaline.

3. Quantitative agreement between the amount of CO_2 and the amount of *nor*adrenaline formed shows that only the laevorotatory stereoisomer is produced.

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