Some Catechol Compounds other than Noradrenaline and Adrenaline in Brains

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This report gives the evidence for statements (Montagu, 1957, 1959) that a basic catechol compound other than noradrenaline and adrenaline is present in considerable amounts in the brains of different animals, and that it is probably 3-hydroxytyramine. Human brain contained, in addition, 3,4-dihydroxyphenylalanine and an acidic catechol compound. The presence of 3-hydroxytyramine in brain was also reported in 1957 by Carlsson, Rosengren, Bertler & Nilsson (see Bertler & Rosengren, 1959), and has since been confirmed by others (see the Discussion section).

METHODS

Extraction and estimation of catecholamines. The whole brains of male rats, guinea pigs, rabbits and chicks were extracted within 60 min. of death with acid methanol and treated as described by Montagu (1956, 1959). The catechol compounds were adsorbed on columns of alumina and eluted with dilute acid; the catecholamines were isolated from the eluate by treatment, at pH 6.5, with Amberlite IRC-50 resin equilibrated with 0.5M-phosphate buffer, pH 6.5. Up to 20 ml. of extract containing up to 100 µg. of catechol compounds was passed through 2 g. of the resin, from which the basic fraction was eluted with N-HCl. Noradrenaline and adrenaline were estimated in this eluate, together with an unidentified substance, compound X, whose concentration was expressed in terms of hydroxytyramine (Montagu, 1959).

Identification of catecholamines by paper chromatography. After the tissue extracts had been treated with alumina and Amberlite IRC-50 resin, they were again treated, at pH 8.4, with alumina. The final eluate was concentrated *in vacuo* at 35° to 1 drop; 5 ml. of acid acetone (1 ml. of conc. HCl/100 ml.) was added, the mixture centrifuged and the supernatant concentrated *in vacuo* just to dryness. This was repeated until acid acetone failed to produce a precipitate. The solution was then divided into two unequal parts; each was concentrated just to dryness. The smaller fraction was dissolved in 10 ml. of aq. 0.3N-HCl and the concentrations of catecholamines were estimated. The other, in 0.5 ml. of acid acetone, was applied along a 4 cm. line to Whatman no. 1 paper. A mixture of noradrenaline, adrenaline and 3-hydroxytyramine was similarly applied. Chromatograms were run for 18–32 hr. at room temperature, either in *n*-butanol-acetic acid-water (4:1:5, by vol.) by

the descending technique (Partridge, 1948), or in phenol-water (3:1, w/v) in air saturated with 5N-HCl by the ascending technique (Weil-Malherbe & Bone, 1954). They were then dried and photographed with ultraviolet light (Markham & Smith, 1949, 1951). The guide strips were sprayed with ethylenediamine-aq. 10% (w/v) ammonia (1:4, v/v) and the positions of the three catecholamines defined by their fluorescence in ultraviolet light. The corresponding sections of the chromatograms were cut out and extracted overnight at 4° in 10 ml. of N-HCl. Portions (2 ml.) were used for the estimation of noradrenaline, adrenaline and compound X.

Neutral and acidic catechol compounds. A normal human brain was refrigerated for 22 hr. after death and was then immersed in 0.1N-HCl (1 vol.). After a further 8 hr. 1 vol. of water was added and the brain was homogenized; the homogenate was centrifuged, and the supernatant was concentrated and passed at pH 8.4 through alumina. The adsorbed catechol compounds were eluted with acid. A small part of the eluate was passed through Amberlite IRC-50 resin; the catecholamines were eluted with N-HCl and estimated fluorimetrically. They did not account for all the fluorogenic material present before treatment with the resin. Half of the eluate from the alumina, corresponding to 615 g. of brain, was then filtered through Amberlite IRC-50 resin at pH 6.5. The filtrate was concentrated nearly to dryness and extracted with acid acetone, and the extract applied to chromatography paper with 3,4-dihydroxyphenylalanine and 3,4-dihydroxyphenylacetic acid as markers. The chromatograms were run overnight in *n*-butanol-acetic acid-water, dried and photographed in ultraviolet light.

The other half of the eluate was passed through a column of 2 g. of Zeo-Karb 225 resin (H⁺ form) as described by Weil-Malherbe & Bone (1957). The column was washed with 150 ml. of 0.2M-acetic acid and eluted with 50 ml. of 0.2M-BaCl₂. The eluate from this resin would contain neutral and basic catechol compounds; BaSO₄ was precipitated with 10N-H₂SO₄ and the supernatant passed through a column of alumina. The eluate from this column was concentrated and treated with acid acetone as though for paper chromatography. The final residue was dissolved in 0.2 ml. of 0.01N-HCl and applied to paper previously washed with HCl followed by EDTA. Electrophoresis was carried out in 0.04M-acetate buffer, pH 5.6, as described by Weil-Malherbe & Bone (1956), with a current of 7 ma for about 2 hr. The guide strip, containing noradrenaline, adrenaline, 3-hydroxytyramine and 3,4-dihydroxyphenylalanine, was sprayed with ethylenediamine-ammonia mixture, and the positions of the catechol compounds were defined. The appropriate zones of the paper were cut out and eluted overnight in 10 ml. of 0.01N-HCl.

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Part (46 g.) of a second human brain, consisting mainly of the midbrain, pons and medulla oblongata, which in cats and dogs contain the highest concentrations of adrenaline and noradrenaline (Vogt, 1954), was removed 30 hr. after death and extracted and treated in the same way as the whole brains of other animals.

RESULTS

Table 1 shows the mean concentrations of noradrenaline, adrenaline and compound X found in the brains of different animals. In each, compound X calculated as hydroxytyramine was the most abundant of the catecholamines. The R_f value of compound X in phenol-water was 0.47 and in *n*-butanol-acetic acid-water 0.34. An average of 64 % of compound X applied to paper from brain extracts was recovered from sections with these R_f values. The corresponding R_f values for noradrenaline were 0.31 and 0.24, for adrenaline 0.55 and 0.29, and for pure 3-hydroxytyramine 0.47 and 0.32 respectively. Some paper chromatograms, run in *n*-butanol-acetic acid-water, of extracts of rat and human brain were exposed to iodine vapour followed by ammonia; there was fluorescence in the positions of noradrenaline and adrenaline; a band, in the position of hydroxytyramine, appeared only after a week. Chromatograms of pure solutions of the three catecholamines behaved similarly.

In the first human brain the estimated concentrations of the catecholamines accounted for only 45 % (Table 1) of the fluorescence produced by reaction with ethylenediamine dihydrochloride in acid eluates from the alumina columns. A further 16% was present in the filtrate from IRC-50 resin. A paper chromatogram of such a filtrate run in *n*-butanol-acetic acid-water showed a compound with the R_f (0.2) of 3,4-dihydroxyphenylalanine and another with an R_f (0.9) similar to that of 3,4-

dihydroxyphenylacetic acid (0.8). Part of the eluate from the alumina columns was filtered through Zeo-Karb 225 resin. After condensation with ethylenediamine dihydrochloride, the filtrate, like 3,4-dihydroxyphenylacetic acid, fluoresced about equally in saturated aqueous sodium chloride solution and in isobutanol. A compound eluted from Zeo-Karb 225 resin and separated from the catecholamines by paper electrophoresis was obtained from the zone corresponding to that of 3,4-dihydroxyphenylalanine. Like 3,4-dihydroxyphenylalanine it formed a fluorescent derivative in the reaction of Euler & Floding (1955), and its fluorescent derivative formed after condensation with ethylenediamine dihydrochloride was five times as soluble in saturated aqueous sodium chloride as in isobutanol.

In the sample of the second human brain (Table 1), 76% of the total catechol material was present in the eluate from the IRC-50 resin. A non-basic compound was not adsorbed by the resin. Its reactions and solubility in the ethylenediamine method and in the reaction of Euler & Floding (1955), as well as its R_f in *n*-butanol-acetic acid-water, were similar to those of 3,4-dihydroxyphenylacetic acid.

DISCUSSION

3-Hydroxytyramine is present in considerable concentrations in the brains of different animals. Similar amounts have been found with other methods by Bertler & Rosengren (1959) and by Holzer & Hornykiewicz (1959) in rat and guinea-pig brain, but the amounts found here in rabbit brain are lower than those reported by Carlsson, Lindquist, Magnusson & Waldeck (1958) and Bertler & Rosengren (1959).

Table 1. *Concentrations of catechol compounds in brains of different animals*

The results are expressed as $\mu\text{mg./g.}$ wet weight of tissue $\pm$ s.e. for five animals. The amounts of each catecholamine as percentages of the sum of the concentrations of all three are given in parentheses. Only part of the second human brain was used. —, Substance not estimated.

Animal	Noradrenaline	Adrenaline	Hydroxytyramine	3,4-Dihydroxyphenylalanine	Acidic catechol	Percentage of total fluorogenic material eluted with acid from IRC-50 resin
Cockerel	62.7 $\pm$ 7.0 (28 %)	36.0 $\pm$ 5.5 (16 %)	123 $\pm$ 22.1 (56 %)	—	—	82.7
Rabbit	121 $\pm$ 16.1 (37 %)	29.4 $\pm$ 8.0 (9 %)	174 $\pm$ 32.1 (54 %)	—	—	81.6
Rat	188 $\pm$ 30.4 (24 %)	53.4 $\pm$ 7.9 (7 %)	526 $\pm$ 103 (69 %)	—	—	95.3
Guinea pig	110 $\pm$ 5.4 (21 %)	38.9 $\pm$ 4.8 (7 %)	385 $\pm$ 58.5 (72 %)	—	—	96.5
Man	21.2 (18 %)	4.4 (4 %)	94.2 (78 %)	200	90	45.3
	19.1 (43 %)	5.5 (12 %)	20.0 (45 %)	—	100	76.4

Schumann (1959) identified the amine in seven organs of dog, sheep and ox, and found that the concentration of 3-hydroxytyramine relative to that of noradrenaline in brains of dogs was exceeded in liver and intestine, whereas Montagu (1957, 1959) and Bertler & Rosengren (1959) found only smaller and 'very small' amounts respectively in tissues of non-ruminant animals other than brain, suggesting a special role in this organ. Unlike noradrenaline and adrenaline (Vogt, 1954), the concentration of hydroxytyramine is highest in the corpus striatum (Bertler & Rosengren, 1959; Ehringer & Hornykiewicz, 1960).

3,4-Dihydroxyphenylalanine and the acidic catechol compound have not been identified in other than human brains or in rat tissues, but even quite large amounts, especially of 3,4-dihydroxyphenylalanine, could escape detection in analyses of extracts by the ethylenediamine method. The estimated concentration of the acidic catechol compound was about the same in the first human brain and in the sample from the second, but there was no evidence for the presence of 3,4-dihydroxyphenylalanine in the latter. Since there were (unidentified) non-basic catechol compounds in rabbit brains, which were extracted immediately after death, it seems unlikely that their presence in human brain is due to post-mortem change.

SUMMARY

1. 3-Hydroxytyramine constituted between 50 and 80% of the total catecholamine concentration of the whole brains of male rats, guinea pigs,

rabbits, cockerels and man. In human brain 3,4-dihydroxyphenylalanine and an acidic catechol compound were also present.

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α -Glucosidase Deficiency in Generalized Glycogen-Storage Disease (Pompe's Disease)

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The term 'glycogen-storage disease' covers a group of congenital hereditary diseases characterized by an abnormal accumulation of glycogen in tissues. Several forms can be recognized from their clinical manifestations and a greater variety is revealed by the biochemical analysis of tissues. As was shown in the review by Hers (1961), deficiencies of glucose 6-phosphatase, amylo-(1 $\rightarrow$ 6)-glucosidase or phosphorylase have been described and can be

taken as responsible for the abnormal glycogen deposition. Up to now, no abnormality of the enzymes involved in the classical scheme of glycogen degradation has been observed in the generalized cardiomegalic form of glycogen disease. This form, also known as Pompe's disease or as type 2 in Cori's classification (Cori, 1957), is the most severe type of glycogenosis, as it causes the death of the patient during the first years of life.