Discussion. The decrease in the noradrenaline content of the submaxillary gland following an infusion of tyramine supports the hypothesis that tyramine causes a release of noradrenaline. It may therefore well be that the effects of tyramine are exerted via a release of noradrenaline. The total disappearence of noradrenaline from the gland, as well as of the secretory response to tyramine (STRÖMBLAD[4,5]) after removal of the sympathetic supply, is also consistent with such a mode of action of tyramine.

At the end of the infusion of tyramine, no secretory response was obtained, but the gland was found to retain some 50% of its noradrenaline. No explanation can be given; but it could be pointed out that the sympathetic innervates also the vessels of the gland and therefore the remaining noradrenaline could be associated with terminals around the vessels. On electrical stimulation of the sympathetic, the nerve terminals in contact with the secretory cells seem to be more easily depleted of transmitter than those in contact with the vessels (EMMELIN and ENGSTRÖM[6]).

The figures for the adrenaline content of the glands were low and varied much more than those for the noradrenaline content. It is, however, of interest to note that adrenaline was present in apparently normal quantities after excision of the superior cervical ganglion. It could be that adrenaline is located outside the nerve terminals in the submaxillary gland.

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Zusammenfassung

Der Noradrenalingehalt in der Submaxillarisdrüse der Katze nahm nach der Infusion von Tyramin ab, wodurch die Auffassung unterstützt wird, dass diese Substanz durch Freisetzung von Noradrenalin wirkt. Sympathische Ganglionektomie verursachte das vollständige Verschwinden von Noradrenalin in der Drüse.

In Vivo Uptake of Dopamine and 5-Hydroxytryptamine by Adrenal Medullary Granules

In 1953 the adrenaline and noradrenaline of the adrenal medulla were shown to be localized within intracellular granules of about the same size as mitochondria but not identical with these[1,2]. Subsequent observations[3,4] indicate that also catechol amines in other tissues are kept in similar particles. In studies of this storage mechanism, the adrenal medulla is often the tissue of choice, because it is easy to isolate and prepare. However, it has the drawback of a comparably slow turnover, which makes investigations of the kinetics of the storage mechanism difficult.

In an attempt to overcome the difficulty, we have studied the uptake of catechol amines of the catechol amine containing granules after administration of 3,4-dihydroxyphenylalanine (DOPA). This amino acid penetrates into the cell, where it is decarboxylated to dopamine by means of DOPA-decarboxylase. This enzyme is located in the cytoplasmic sap[5]. It was observed that most of the newformed dopamine was present in the granular fraction after differential centrifugation. The procedure is briefly described below.

L-DOPA in a dose of 100 mg/kg was administered i. v. into rabbits weighing about 2 kg. The animals were killed at varying intervals of time after the injection. The adrenals were rapidly removed, and the adrenal medulla was dissected free from cortical tissue. The medulla was homogenized in ice-cooled 0.3 *M* sucrose in a Potter-Elvehjem homogenizer. From the homogenate three fractions were prepared by means of differential centrifugation according to HILLARP *et al.*[1]: (1) the low speed sediment, containing cell nuclei and cell debris, (2) the high speed sediment, containing the mitochondria and catechol amine storing granules, (3) the high speed supernatant, containing the cytoplasmic sap. The catechol amine contents of the fractions were determined as previously described[6]. As will be seen in the Table, the granular fraction contained 3.7 µg dopamine 30 min after the administration of DOPA, whereas less than 1 µg was localized in the cytoplasmic sap. The newly-formed dopamine is thus rapidly taken up by the granules. In the kidney cortex, however, which is rich in DOPA-decarboxylase but lacks catechol amine storing granules, most of the dopamine formed was found in the cytoplasmic sap. Pretreatment of the animals with reserpine impaired the uptake of dopamine by adrenal medullary granules after DOPA administration. Thus the primary effect of reserpine which is known to deplete the body stores of catechol amines[7–9] may be its interaction with the uptake of catechol amines by the granules.

In-vivo uptake of dopamine and 5-hydroxytryptamine by adrenal medullary granules after administration of DOPA and 5-hydroxytryptophan respectively (µg per pair of adrenals. Each value represents the mean of two determinations. DA: dopamine; NA: noradrenaline; A: adrenaline; 5-HT: 5-hydroxytryptamine; 5-HTP: 5-hydroxytryptophan).

		DA	NA	A	5-HT
DOPA, 100 mg/kg	Cytoplasmic sap	0.8	0.9	8.2	—
(30 min)	Granules	3.7	4.8	72	—
DOPA, 100 mg/kg	Cytoplasmic sap	1.4	2.7	14.5	—
(1 h)	Granules	5.4	15.5	127	—
Reserpine, 5 mg/kg	Cytoplasmic sap	1.5	2.0	11.6	—
(3 h) + DOPA,	Granules	2.0	–0.5	69	—
100 mg/kg (30 min)					
Reserpine, 5 mg/kg	Cytoplasmic sap	4.0	1.3	1.1	—
(20 h) + DOPA,	Granules	0.8	1.0	1.9	—
(30 min)					
5-HTP, 100 mg/kg	Cytoplasmic sap	—	—	—	0.45
(1 h)	Granules	—	—	—	1.40
Control	Cytoplasmic sap	0.0	0.2	6.7	0.0
	Granules	0.3	1.2	105.3	0.0

[1] N.-Å. HILLARP, S. LAGERSTEDT, and B. NILSSON, Acta physiol. scand. *29*, 251 (1953).

[2] H. BLASCHKO and A. D. WELCH, Arch. exp. Path. Pharmak. *219*, 17 (1953).

[3] U. S. v. EULER and N.-Å. HILLARP, Nature *177*, 44 (1956).

[4] Å. BERTLER, N.-Å. HILLARP, and E. ROSENGREN, Acta physiol. scand., in press.

[5] H. BLASCHKO, P. HAGEN, and A. D. WELCH, J. Physiol. *129*, 27 (1955).

[6] Å. BERTLER, A. CARLSSON, E. ROSENGREN, and B. WALDECK, Kgl. Fysiograf. Sällskap. Lund Förh. *28*, no. 12 (1958).

[7] A. CARLSSON and N.-Å. HILLARP, Kgl. Fysiograf. Sällskap. Lund Förh. *26*, no. 8 (1956).

[8] Å. BERTLER, A. CARLSSON, and E. ROSENGREN, Naturwissenschaften *22*, 521 (1956).

[9] A. CARLSSON, E. ROSENGREN, Å. BERTLER, and J. NILSSON, *Psychotropic Drugs* (S. Garattini and V. Ghetti, Eds. Elsevier Publishing Company, Amsterdam 1957), p. 363.

[10] Å. BERTLER and E. ROSENGREEN, Exper. *15*, 382 (1959).

[11] E. ROSENGREN, Acta physiol. scand., in press.

The mechanism by which catechol amines are taken up and stored does not seem to be specific for catechol amines, 5-hydroxytryptamine (5-HT) being found in the catechol amine containing granules after administration of DL-5-hydroxytryptophan (5-HTP) (Table).

From these experiments it thus may be concluded that 5-HT and catechol amines are stored in the cells in similar particles, the identity of which is under further investigation. Which of the two types of amines a cell containing these granules possesses, seems to be due to its contents of DOPA and 5-HTP synthezising enzymes, as 5-HTP decarboxylase appears to be identical with DOPA-decarboxylase[10,11].

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Zusammenfassung

Die Einlagerung von neugebildetem Dopamin in den Katecholamin-haltigen Granula des Nebennierenmarks nach Injektion von DOPA ist untersucht worden. Dopamin wird von den Granula aufgenommen, und nur wenig davon wird im Cytoplasma gefunden. Vorbehandlung der Tiere mit Reserpin verhindert diese Einlagerung. Der Mechanismus der Einlagerung ist nicht spezifisch, weil 5-Hydroxytryptamin nach Injektion von 5-Hydroxytryptophan von den Granula auch aufgenommen wird.

Storage of New-Formed Catecholamines in the Adrenal Medulla

In previous papers[1,2] it has been shown that large amounts of dopamine (DA) and noradrenaline (NA) are rapidly formed in the adrenaline (A) cells of the rabbit suprarenal medulla after an intravenous injection of L-3,4-dihydroxyphenylalanine (dopa). It was also found that the new-formed amines rapidly become 'particle-bound'. In the present study, evidence is presented in support of the view that DA and NA are in fact incorporated in the amine granules which normally store only A.

In three experiments, the intracellular distribution of DA, NA, and A was examined by means of density gradient centrifugation. In each experiment, the adrenal medullas from two rabbits (1.5 to 2 kg), to which dopa (100 mg/kg body weight) had been given 1 h previously, were homogenized in 0.3 *M* sucrose and then fractionated into a low-speed sediment (800 × g, 5 min), a 'large granule' fraction and a high-speed supernatant (20000 × g, 20 min; details are found in a previous paper[2]). The 'large granule' fraction, i. a. containing the mitochondria and the amine storage granules, was resuspended in sucrose and subjected to density gradient centrifugation (gradient: 0.4 to 1.8 *M* sucrose) in the way described previously[3]). The content of the tube was divided into three subfractions: a top fraction (G1) containing microsomal material, mitochondria, and amine granules of low density, a bottom fraction of non-sedimented amine granules of high density (G2), and a small sediment of high density amine granules which seem to be practically free from other cell particles[3]. The amine content in all the fractions was determined spectrophotofluorimetrically[4,5].

It was found (Table I) that the 'particle-bound' DA and NA showed practically the same distribution as that of A. Most noteworthy is the fact that 50 to 60% of all three amines were recovered in the sediment (G3) containing the high density amine granules. These findings strongly support the view that the new-formed DA and NA must have been taken up in the specific amine storage granules. It seems quite unlikely that the medullary cell contains any other 'particles' that - besides being able to bind the large amounts of DA and NA – have the same sedimentation characteristics as those of the storage granules.

The newly formed DA and NA were not taken up preferentially by the amine granules of low density. This is interesting since these granules which – in contrast to the high density granules – seem to store amines largely without adenosinetriphosphate might be thought to represent granules in an early storage phase[6]. The present findings do not support such a view.

Another approach to the storage problem was also attempted. There are several findings suggesting that reserpine destroys the amine storage mechanism[7,8]. It is thus of interest to see whether the DA and NA formed after a dopa injection become 'particle-bound' also in animals treated with reserpine.

Reserpine (5 mg/kg body weight) was administered to rabbits and dopa was injected after varying periods of time (Table II). After homogenization of the medullas

Tab. I

Exp. Nr.	Amine	Low-speed sediment µg	High-speed supernatant µg	Granule-bound amines: Total µg	Distrib. in % of total: G1	G2	G3
I	A	13	15	255	11	34	55
	NA	3.2	3.1	42	9	31	59
	DA	0.8	0.8	10	11	32	57
II	A	26	15	260	10	31	60
	NA	−0.1	1.6	55	10	36	54
	DA	1.0	0.5	9.2	12	28	60
III	A	15	23	175	6	45	49
	NA	2.1	0.6	18	13	35	52
	DA	0.7	1.9	4.3	7	42	51

Tab. II

	Adrenaline Total µg	Adrenaline 'Free' %	Noradrenaline Total µg	Noradrenaline 'Free' %	Dopamine Total µg	Dopamine 'Free' %
Reserpine 3 h Dopa 30 min	91 83	12 17	1.8 1.0	100 100	3.8 2.9	42 44
Reserpine 5 h Dopa 30 min	37 24	24 18	4.4 1.9	54 65	4.0 2.3	49 57
Reserpine 20 h Dopa 30 min	3.6 3.3	21 57	1.9 3.3	53 57	5.0 5.7	82 80

[1] Å. Bertler, N.-Å. Hillarp, and E. Rosengren, Ciba Found. Symp. Adrenergic Mechanisms. J. & A. Churchill, London, in press (1960).

[2] Å. Bertler, N.-Å. Hillarp, and E. Rosengren, Acta physiol. scand., in press (1960).

[3] N.-Å. Hillarp, Acta physiol. scand. *43*, 82 (1958).

[4] A. Carlsson and B. Waldeck, Acta physiol. scand. *44*, 293 (1958).

[5] Å. Bertler, A. Carlsson, and E. Rosengren, Acta physiol. scand. *44*, 273 (1958).

[6] N.-Å. Hillarp, Acta physiol. scand., in press (1960).

[7] A. Carlsson, E. Rosengren, Å. Bertler, and J. Nilsson, in *Psychotropic Drugs* (Ed.: S. Garattini and V. Ghetti, Elsevier Publ. Comp., Amsterdam 1957), p. 363.

[8] F. B. Hughes and B. B. Brodie, J. Pharmacol. exp. Therap. *127*, 96 (1959).