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

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(one animal in each experiment), the low-speed sediment and the high-speed supernatant and sediment were examined. The experiments clearly showed that to a high extent reserpine may prevent the newly formed DA and NA from becoming 'particle-bound'. This result thus further supports the conclusion that these amines – after dopa administration to normal animals – are incorporated in the specific storage granules.

The findings in the present study, together with those in a previous work[2], seem to admit of the following general conclusions.

(1) The incorporation of newly formed amines in the storage granules is a rapid process.

(2) The storage mechanism is highly efficient since the newly formed amines are rapidly taken up by the granules, even at very high rates of synthesis.

(3) The storage mechanism is non-specific in the sense that it cannot choose between DA, NA, and A.

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Zusammenfassung

Die intrazellulare Lokalisation des Dopamins und Noradrenalins, die schnell nach einer intravenösen Injektion von L-DOPA in die Adrenalinzellen des Nebennierenmarks gebildet werden, ist bei normalen und reserpinbehandelten Kaninchen untersucht worden. Die Versuche sprechen dafür, dass die neugebildeten Amine sehr schnell den spezifischen Granula, die gewöhnlich nur Adrenalin enthalten, einverleibt werden. Der Einlagerungsmechanismus der Amine scheint sehr wirkungsvoll zu sein, aber unspezifisch in dem Sinn, dass er nicht zwischen Dopamin, Noradrenalin und Adrenalin unterscheidet.

Immunological Tolerance in Chickens Induced by Blood Group Substance

Since the classical work of BILLINGHAM *et al.*[1], numerous investigators have found that the treatment of embryos, and newborn or newly hatched animals, with foreign red blood cells or proteins may be followed by immunological tolerance. Some workers[2] have succeeded in producing in chickens an immunological tolerance to erythrocytes. On the other hand, BAER *et al.*[3] have shown that chickens injected with hog blood group substance are able to produce the agglutinins which react with human red cells. However, there are no data in the literature concerning the induction in animals of immunological tolerance to human erythrocytes by means of blood group substances. This is the subject of this preliminary communication, which represents only a part of a larger work.

The 12-day old Rhode Island Red embryos and infant chickens from the 2nd to the 32nd day of age were injected with hog 0(H) blood group substance (kindly provided by Dr. E. A. KABAT, Department of Microbiology, Columbia University, N. Y). The embryos received intravenously 0·08 mg of blood group substance, and the treatment was followed during posthatched life until the 32nd day of age (subcutaneous injections of 0·08 mg of substance each in three-day intervals). The challenging injections of human group 0 red cells were performed on the 42nd and 62nd day of postembryonic life. The control group of chickens was not preliminary treated with hog 0(H) substance, and received two provocative injections of 0 red cells at the age of 42 and 62 days. The treated and control chickens were bled on the 50th and 70th day of age. The haemagglutination reaction was performed in small 7 × 45 mm tubes, using serial doubling dilutions of chicken sera starting from 1:10, and 1% suspension of human 0 erythrocytes. The incubation lasted 2 h at room temperature and the reactions were read microscopically. The hemagglutinin titers were expressed in the term of logarithms to the base 2.

The first and second antibody responses of chickens to the injected human group 0 red cells are presented in the Table. The data clearly show that hog 0(H) blood group substance, used for the treatment of chickens in early life, may elicit the depression in the formation of agglutinins reacting with human 0 red cells. It is of interest to note that an antigen which is chemically a muco-polysaccharide, mainly composed of non-reducing and reducing carbohydrates, represents an active substance able to produce the immunological tolerance in chickens to human erythrocytes. Furthermore, the foregoing data indicate that an antigen from one animal species (hog), when injected into another species (chicken), may cause immunological tolerance of red cells belonging to a third animal species (man). This phenomenon is based upon the same serological specificity of hog 0(H) substance and human group 0 red cells.

Chicken	Normal haemagglutinins 10 chickens Age: 42 days MPT ± SD	First response 10 chickens Age: 50 days MPT ± SD	Second response 10 chickens Age: 70 days MPT ± SD
Treated with hog 0 (H) substance	4·02 ± 0·69	6·52 ± 1·11	7·98 ± 0·81
Control	3·62 ± 0·45	8·42 ± 0·94	10·72 ± 0·49

MPT: geometric mean peak agglutinin titer ($\log_2$).
SD: standard deviation.

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Résumé

L'injection de substance 0(H) du porc à des embryons et à des poussins récemment éclos a comme résultat un abaissement de leur capacité de produire dorénavant l'immunisation active d'agglutinines spécifiques pour les globules rouges humains du groupe 0.

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