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Subcellular Localisation of Noradrenaline in Sympathetic Neurons

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I. Introduction

In 1956, 3 years after the discovery that the catecholamines of the adrenal medulla were stored in a cell particle (11, 42), Euler and Hillarp (27) reported that some of the noradrenaline in homogenates of ox splenic nerve, ox spleen and rat spleen was recovered in a particulate fraction after centrifugation. This finding opened up the possibility of applying biochemical methods to study the nature of the particle which contains the neurotransmitter. In this review an attempt has been made to summarise most of the centrifugation experiments carried out since 1956 and to see what progress has been made in answering the question: What is a noradrenergic vesicle and what is it made of?

II. Central Nervous System (Table 1a)

As much as 60 % to 80 % of the noradrenaline in brain homogenates is recovered in a particulate form and most of the amine appears to be present in large particles, *i.e.*, those which sediment with mitochondria. These large particles are recovered after density gradient centrifugation in layers ranging from 0.8 M to 1.2 M of sucrose and, since these layers contain lactate dehydrogenase and pinched-off nerve endings, it has been concluded that the noradrenaline is present in synaptosomes (see De Robertis, 22; Whittaker, 101). Hypo-osmotic shock of the synaptosome-containing fraction releases up to half the noradrenaline into the supernatant, but it is not possible to conclude that all this soluble noradrenaline was originally free in the cytosol within the synaptosome; some of it may have been released from vesicles damaged by hypo-osmotic shock.

Biochemical evidence concerning the nature of the noradrenaline-containing particles within the synaptosome is meager. Two methods have been used: in the first, the lysed suspension of synaptosomes is fractionated by differential

centrifugation (23, 66) and, in the second, the suspension is fractionated by sucrose density gradient centrifugation (68). Differential centrifugation of lysed synaptosomes from the hypothalamus showed that a greater proportion of the noradrenaline is in "large" particles compared with a similar fractionation of synaptosomes isolated from the whole brain (23). The interpretation of the gradient experiments is not straight forward because parallel assays of marker-enzymes for other particles and for the cytosol have not been performed. In the experiments of Michaelson *et al.* (68) on the midbrain the distribution of noradrenaline was bimodal: one peak was at the top of the gradient, and was mainly due to non-particulate noradrenaline; the other peak was in the layer between 0.8 M and 1.2 M of sucrose. It is possible that the noradrenaline in the denser layers was present in undisrupted synaptosomes, as has been suggested in order to account for the acetylcholine content of similar fractions from the whole brain (102); if this is so, it is surprising that the lactate dehydrogenase content of these layers was no higher than that of adjacent layers. Another possibility is that the noradrenaline-containing particle recovered in the layers of 0.8 M to 1.2 M of sucrose is a kind of vesicle, similar to the noradrenergic vesicles of sympathetic ganglia and splenic nerve which also sediment to this region in a density gradient (see sections IV and V).

III. Sympathetic Ganglia (Table 1b)

There have been very few studies on the subcellular distribution of noradrenaline in sympathetic ganglia, and those that have been reported are not in good agreement. Whereas Fischer and Snyder (33) found that 84% of the noradrenaline in homogenates of cat superior cervical ganglia remained in the supernatant after high speed centrifugation, Schümann *et al.* (81) found that only 21% of the noradrenaline in homogenates of bovine stellate ganglion could not be sedimented. These discrepancies may be related not only to differences between the tissues studied; they could also be the result of the different conditions of homogenisation. In the experiments of Schümann *et al.* (81) as much as 66% of the noradrenaline was recovered in the low-speed sediment, perhaps because of incomplete homogenisation of the tissue. Under these milder conditions of homogenisation, 40% of the noradrenaline in the low-speed supernatant of stellate ganglia was recovered in particles. These particles were recovered in a layer between 1.1 M and 1.5 M of sucrose after density gradient centrifugation, and were distinguished from fumarase-containing particles (71).

It should not be assumed, in studies on sympathetic ganglia, that the cell bodies of postganglionic neurons are the only sites of origin of the catecholamine-containing particles in homogenates. In many ganglia there are catecholamine-containing nerve endings (69, 97) which, in the cat superior cervical ganglion, appear to be the terminals of axon collaterals from postganglionic processes (49). Furthermore, small intensely-fluorescent cells are also found in sympathetic ganglia (25, 49, 69). Electronmicroscopic studies on these small cells have shown that they contain a dense-cored granule very similar to the adrenal chromaffin granule (24, 65, 83, 100). It is not yet certain what catecholamine they contain, although there have been suggestions that it might be dopamine (10, 63).

Tentatively, it can be concluded that the particle storing noradrenaline in sympathetic ganglia is similar to the heavy type of noradrenergic vesicle found in nerve axons and terminals (see sections IV and V), but further biochemical studies are clearly necessary.

Note added in proof: An important biochemical study on the noradrenergic vesicles present in homogenates of bovine stellate ganglion has recently been published by Chubb, I. W., De Potter, W. P. & De Schaepdryver, A. F. in Life Sciences **11** (part 1), 323–333, 1972.

IV. Sympathetic Nerve Axons (Table 1c)

All the published studies on the subcellular distribution of noradrenaline in the preterminal axons of sympathetic nerves have been done on the bovine splenic nerve. As in the studies on the stellate ganglion, a large part of the noradrenaline is often recovered in the low-speed sediment (46, 80, 81): this must be due to incomplete homogenisation of the tissue. When the homogenate is filtered before centrifugation, only a small proportion of the noradrenaline is recovered in the low-speed sediment (21). Between 30% and 50% of the noradrenaline in the low-speed supernatant is recovered in particles after high-speed centrifugation (21, 46, 81).

Recent biochemical studies on the subcellular fractions obtained from bovine splenic nerve have distinguished the noradrenaline-containing particle (noradrenergic vesicle) from other cell particles and have also provided some information about the composition of the noradrenergic vesicle. The noradrenergic vesicles can be distinguished from large and small lysosomes, from mitochondria, and from microsomal elements probably derived from the endoplasmic reticulum and the cell membrane, either by differential or by density gradient centrifugation (21, 46, 59, 60). In these experiments there was no evidence for more than one population of particles containing noradrenaline, in confirmation of the sucrose gradient experiments of Roth *et al.* (79). Four groups of workers have now shown that the noradrenergic vesicles of splenic nerve equilibrate in a density gradient at about the layer of 1.2 M of sucrose (12, 21, 59, 79). This type of vesicle has been defined as a "heavy" noradrenergic vesicle (79).

Although the noradrenergic vesicle of the splenic nerve could be distinguished from other cell particles, the biochemical studies (21, 46) showed that the separation of the different types of particle was by no means complete. For this reason it was not possible to determine the composition of the noradrenergic vesicle by analysis of a single fraction; it was necessary to compare the distribution of a substance with that of noradrenaline between all the fractions obtained by centrifugation. An example of this approach is given by the work of De Potter *et al.* (21) in which the distribution of ATP between different fractions of the splenic nerve is compared with those of markers for different cell particles. Particulate fractions of splenic nerve have previously been found to contain adenosine triphosphate (ATP) (4, 29, 80, 88, 90) and the molar ratio of noradrenaline to ATP found by these workers ranged from 3.0 to 5.2. Stjärne (88) and Stjärne and Lishajko (90) pointed out that the fractions analysed were unlikely to be free of contamination by other cell particles and that these particles might also contain

TABLE 1

Biochemical studies on the subcellular localization of endogenous noradrenaline (NA) in neurons

Tissue	Homogenisation (Potter-Elvehjem unless otherwise stated	Centrifugation	Proportion of NA in Particles	Notes	Reference
			%		
a) Central nervous system					
Rabbit brain stem	Sucrose (10%); EDTA (0.1%)	10,000 × *g* for 15 min	47		99
Rabbit hypothalamus	0.3 M sucrose	44,000 × *g* for 60 min	60-70		5
Rat brain	0.3 M sucrose	137,000 × *g* for 35 min	51	Total NA 15 min after reserpine unchanged, but only 34% in particles.	39
Dog hypothalamus	0.3 M sucrose	15,000 × *g* for 30 min, sediment put on sucrose gradient (0.7 M-1.6 M): 10,400 × *g* for 60 min		NA recovered in layer of 0.8 M sucrose, above mitochondria.	18
Dog hypothalamus	0.32 M sucrose	1,000 × *g* for 11 min	7		61
		17,300 × *g* for 60 min	43		
		100,000 × *g* for 60 min	18		
Rat brain	0.25 M sucrose	Homogenate on gradient 0.25 M-1.8 M sucrose; 125,000 × *g* for 90 min		NA in intermediate layer of gradient, above mitochondria. Some NA also in pellet. Exogenous ^{3}H-NA had similar distribution.	77
Guinea-pig diencephalon and midbrain	0.32 M sucrose	100,000 × *g* for 60 min, sediment homogenised in water, put on sucrose gradient (0.4 M-1.2 M): 53,000 × *g* for 2 hr		Bimodal distribution of particulate NA: most in denser region (0.8 M-1.2 M sucrose) but some in lighter fraction (0.4 M sucrose).	68
Rat brain stem	0.32 M sucrose	900 × *g* for 10 min	3.6	Layer between 0.8 and 1.2 M sucrose contained 29% of total NA in homogenate.	62
		15,000 × *g* for 30 min	61.2		
		110,000 × *g* for 30 min	6.7		
		15,000 × *g* sediment on sucrose gradient (0.8-1.2 M): 100,000 × *g* for 60 min			
Rat brain stem	0.32 M sucrose	11,500 × *g* for 20 min, sediment (i) exposed to ultrasound, resedimented and supernatant spun 100,000 × *g* for 30 min; (ii) exposed to water, and then centrifuged as in (i)		Up to 27% of NA in 11,500 × *g* sediment recovered in sediment at 100, 00 × *g* after either method (i) or (ii). This sediment contained 0.06 nmoles NA/mg protein by (i) and 0.14 nmoles NA/mg protein by (ii).	66
Rat brain	0.32 M or 0.8 M sucrose; 0.3 mM tranylcypromine	"Mitochondrial" fraction exposed to osmotic shock, sedimented at 20,000 × *g* for 30 min, 100,000 × *g* for 30 min	26 27	100,000 × *g* sediment contained 0.04 nmoles NA/mg protein.	23

Rat anterior hypothalamus	As above for rat brain	As above		100,000 × *g* sediment contained 0.22 nmoles NA/mg protein. Electronmicroscopy showed large dense core vesicles and smaller vesicles.	23
		20,000 × *g* for 30 min	47		
		100,000 × *g* for 30 min	19		
Rat cerebellum	0.25 M sucrose	70,000 × *g* for 60 min	60		38
Rat cortex			72		
Rat medulla			77		
Rat hypothalamus			80		
Pig hypothalamus	0.3 M sucrose containing Pargylin (0.75 μg/ml)	600 × *g* for 30 min	6	30,000 × *g* sediment had highest concentration of NA, 0.06 nmoles/mg protein. Molar ratio of NA/ATP was 0.56.	72
		15,000 × *g* for 20 min	53		
		30,000 × *g* for 20 min	11		
		60,000 × *g* for 20 min	4		
		100,000 × *g* for 20 min	2		
Rat hypothalamus	0.32 M sucrose	Homogenate on linear density gradient (0.32 M–1.46 M sucrose): 130,000 × *g* for 90 min		NA in particles (synaptosomes) approx. at level of 1.1 M sucrose (data not given). ^{3}H-NA had same distribution. With ^{3}H-NA they showed that synaptosomes from different areas of brain had different sedimentation properties. Lactate dehydrogenase approx. paralleled NA.	40
b) Sympathetic ganglia					
Cat superior cervical ganglion	0.9% KCl	125,000 × *g* for 60 min	16		33
Bovine stellate ganglion	0.3 M sucrose, Ultra-Turrax	2,000 × *g* for 30 min	66	60,000 × *g* sediment contained 0.65 nmoles NA, mg protein. Molar ratio NA/ATP was 3.12.	81
		15,000 × *g* for 20 min	6.6		
		30,000 × *g* for 20 min	2.9		
		60,000 × *g* for 20 min	2.1		
		100,000 × *g* for 20 min	1.6		
Bovine stellate ganglion	0.3 M sucrose, Ultra-Turrax	Supernatant from 15,000 × *g* for 20 min spun at 60,000 × *g* for 30 min; sediment on gradient (0.9 M–1.5 M sucrose): 125,000 × *g* for 2 hr		Fraction between 1.1 M and 1.5 M sucrose contained 51% of NA in gradient and 0.48 nmoles NA/mg protein. This fraction contained 4% of fumarase and 10% of ATPase activity. Electronmicroscopy showed irregular membrane-limited vesicles, mainly without dense cores.	71
c) Sympathetic nerve axons					
Bovine splenic nerve	0.3 M sucrose, Waring Blendor	Supernatant from 600 × *g* for 8 min spun at 6,800 × *g* for 60 min	15–19		27
Bovine splenic nerve	0.3 M sucrose, ground with sand	Homogenate filtered through gauze and paper; supernatant from 600 × *g* for 5 min spun at 6,000–10,000 × *g* for 0.5–2 hr	11	Homogenisation in salt solutions decreased the yield of NA in particles.	26
	0.3 M sucrose, Potter-Elvehjem;	As above	13		
	0.44 M sucrose, nylon rollers;	As above	32		
	0.88 M sucrose, nylon rollers	As above	20		

TABLE 1—*Continued*

Tissue	Homogenisation (Potter-Elvehjem, unless otherwise stated)	Centrifugation	Proportion of NA in Particles	Notes	Reference
			%		
Bovine splenic nerve	0.9% NaCl, Latapiemühle	800 × *g* for 15 min	38	12,000 × *g* sediment contained ATP: molar ratio NA/ATP was 5.2.	8
		12,000 × *g* for 15 min	17		
Bovine splenic nerve	0.3 M sucrose, Ultra-Turrax	2,000 × *g* for 30 min	38	60,000 × *g* sediment contained 2.9 nmoles NA/mg protein.	81
		15,000 × *g* for 20 min	9.6		
		30,000 × *g* for 20 min	7		
		60,000 × *g* for 20 min	3.9		
		100,000 × *g* for 20 min	2.2		
Bovine splenic nerve	0.3 M sucrose, Ultra-Turrax	Sediment from 60,000 × *g* for 30 min on gradient (0.9 M–1.5 M sucrose): 64,000 × *g* for 4 hr		Fraction at 1.3 M sucrose level contained 4.9 nmoles NA/mg protein. This fraction contained an ATPase, inhibited by reserpine, but no detectable fumarase activity.	12
Bovine splenic nerve	0.25 M sucrose, Ultra-Turrax	Supernatant from 9,000 × *g* for 10 min on sucrose gradients: (a) 0.25 M–2.0 M, 154,000 × *g* for 30 min, (b) 0.5 M–1.0 M, 169,000 × *g* for 60 min		NA-containing particles at about 1.0 M sucrose level in (a) and at interface 1.0 M and 2.0 M sucrose in (b). Electronmicrograph showed a few dense-cored vesicles.	79
Bovine splenic nerve	0.17 M NaCl (press juice)	600 × *g* for 10 min, supernatant spun at 20,000 × *g* for 20 min		Sediment contained 6% of the protein present in the 600 × *g* supernatant. Electronmicroscopy showed numerous vesicles (800–900 Å diam.), some with dense cores. Sediment contained 5 nmoles NA/mg protein, and molar ratio NA/ATP was 5.2. Sediment lysed with deoxycholate cross-reacted with antiserum to adrenal chromogranin A.	4
Bovine splenic nerve	0.3 M sucrose	800 × *g* for 20 min, supernatant filtered	49	Gradient (a) distinguished mitochondria and lysosomes from NA-containing particles. Gradient (b) distinguished NA-containing particles from microsomes which contained glucose 6-phosphatase and ATPase. In gradient (b) the NA-containing particles equilibrated at layer of 1.2 M sucrose: this fraction contained 2.9 nmoles NA/mg protein. Dopamine β-hydroxylase had the same distribution as NA in both gradients.	46
		12,000 × *g* for 20 min	4		
		135,000 × *g* for 60 min	13		
		12,000 × *g* sediment on gradient (a) 0.6 M–1.2 M sucrose: 127,000 × *g* for 45 min, 135,000 × *g* sediment on gradient (b) 0.8 M–1.4 M sucrose: 127,000 × *g* for 2.5 hr.			
Bovine splenic nerve	0.25 M sucrose, 1 mM tris-HCl pH 7.4, Ultra-Turrax	600 × *g* for 10 min, supernatant filtered and centrifuged at 10,500 × *g* for 10 min, supernatant on sucrose D_2O density gradient (0.25 M–1.5 M) 100,000 × *g* for 90 min, gradient fractions diluted with 0.25 M sucrose and centrifuged to give sediments	13.3	Fraction containing 1.2 M sucrose had 19.9 nmoles NA/mg protein. Distribution of NA between gradient fractions was different from that of markers for lysosomes, Golgi elements and of glucose 6-phosphatase.	60

Bovine splenic nerve	0.25 M sucrose, homogenate filtered	6,600 × *g* for 8 min	2.4	Differential centrifugation distinguished NA-containing particles, from large and small lysosomes, mitochondria and microsomal elements. Distribution of dopamine β-hydroxylase and chromogranin A paralleled that of NA, but distribution of ATP showed that it was in both mitochondria and noradrenergic vesicles. 20,200 × *g* for 30 min sediment contained 9.1 nmoles NA/mg protein. Gradient centrifugation confirmed distinction between noradrenergic vesicles and the 4 other types of particle: the noradrenergic vesicles equilibrated at layer of 1.2 M sucrose.	21
		20,200 × *g* for 15 min (sediment recentrifuged)	7.8		
		20,200 × *g* for 30 min	22.7		
		59,400 × *g* for 22 min	9.1		
		59,400 × *g* for 35 min	1.9		
		A total particle fraction obtained from 6,600 × *g* supernatant at 59,400 × *g* for 35 min put on gradient (0.3 M–1.7 M sucrose): 125,000 × *g* for 2.5 hr			
d) Sympathetically innervated tissues					
Bovine spleen	0.3 M sucrose	800 × *g* for 6 min, supernatant spun at 6,800 × *g* for 60 min	16–44		27
Rat spleen	0.3 M sucrose	As above	14–29		27
Dog heart	0.9% NaCl	600 × *g* for 10 min, supernatant spun at 75,000 × *g* for 60 min	74		98
Dog heart: right atrium	0.075 M potassium phosphate buffer as above	2,000 × *g* for 5 min	21.5	After reserpine, NA content of particulate fraction fell more rapidly than that of soluble fraction.	
		10,500 × *g* for 60 min	52.5 (atrium)		
left ventricle		2,000 × *g* for 5 min	28		
		10,500 × *g* for 60 min	53		
Rat heart	0.25 M sucrose	Homogenate on gradient (0.25–2.2 M sucrose): 125,000 × *g* for 30 min		NA recovered in a particulate layer close to top of gradient, above mitochondria. Exogenous ^{3}H-NA had similar distribution.	76
Rat vas deferens	0.25 M sucrose	As above (Potter and Axelrod, 1962)		NA recovered in particulate layer close to top of gradient and also in the pellet. Exogenous ^{3}H-NA had a similar distribution.	77
Rat submaxillary gland	0.25 M sucrose	As above (Potter and Axelrod, 1962)		NA recovered in particulate layer close to top of gradient and also in the pellet.	77
Cat heart: atria	0.075 M potassium phosphate buffer pH 7.5 as above	2,000 × *g* for 5 min	24	During perfusion with reserpine, NA level in soluble fraction fell first, followed by depletion of particulate NA and rise in level of NA in soluble fraction.	15, 16
		105,000 × *g* for 60 min	21		
left ventricle		2,000 × *g* for 5 min	32		
		105,000 × *g* for 60 min	36.5		
Rabbit heart	Phosphate buffer, Ultra-Turrax	9,000 × *g* for 10 min	25		28
		100,000 × *g* for 45 min	39		
Rat heart	0.25 M sucrose	Supernatant from 750 × *g* for 5 min spun at 110,000 × *g* for 60 min and sediment put on gradient (0.25 M–0.73 M sucrose): 125,000 × *g* for 60 min		NA recovered in layer of 0.45 M–0.5 M sucrose. Electronmicroscopy showed a few dense-cored vesicles (500 Å) and many larger membrane fragments.	68

TABLE 1—*Continued*

Tissue	Homogenisation (Potter-Elvehjem, unless otherwise stated)	Centrifugtion	Proportion of NA in Particles	Notes	Reference
			%		
Guinea-pig heart	0.3 M sucrose, Ultra-Turrax	2,000 × *g* for 20 min	61	100,000 × *g* sediment contained 0.4 nmoles NA/mg protein. Adrenaline mainly in supernatant. After reserpine all fractions were depleted in NA.	82
		15,000 × *g* for 20 min	5		
		100,000 × *g* for 20 min	26		
Dog heart	Locke's solution, ground with silica in mortar	800 × *g* for 30 min (twice), 5,000 × *g* for 2 hr	4.5	Coarse particles (800 × *g* sediments) contained 70% of total NA. Adrenaline was only found in the 800 × *g* sediment and final supernatant. In the density gradient 30% of the NA was concentrated in the layer of 1.0 M–1.2 M sucrose, and 54% remained at the top of the gradient.	37
		10,000 × *g* for 2 hr	8		
		20,000 × *g* for 2 hr	10.7		
		40,000 × *g* for 2 hr	7.2		
		100,000 × *g* for 2 hr; 800 × *g* supernatant on sucrose density gradient of Potter and Axelrod (1963a): 100,000 × *g* for 2 hr	6.5		
Rat vas deferens	0.25 M sucrose; 1 mM $MgCl_2$	1,000 × *g* for 5 min; supernatant on gradient (0.35 M–1.75 M sucrose): 125,000 × *g* for 45 min	42	NA entered layer of 0.8 M sucrose on gradient. Coaxial stimulation for 1 hr caused a 27% decrease in total NA content: the fall in NA was confined to the particulate fraction.	16
Rat heart (atrium)	0.25 M sucrose	1,500 × *g* for 5 min; supernatant layered on 0.4 M sucrose: 125,000 × *g* for 45 min	38	After cold stress, there was a 34% fall in the NA content of the atrium; the particulate fraction lost 59% of its amine and the soluble fraction 25%.	17
Cat heart	0.25 M sucrose, 5 mM phosphate buffer, pH 7.4, 1 mM $MgCl_2$	1,000 × *g* for 10 min	68	Gradient centrifugation (0.3 M–1.4 M sucrose) revealed two peaks of exogenous ^{3}H-NA.	92
		10,000 × *g* for 10 min	4		
		25,000 × *g* for 10 min	2		
		100,000 × *g* for 60 min	15		
Rat heart	0.3 M sucrose, 0.1% EDTA; 0.01% ascorbic acid; Ultra-Turrax	10,000 × *g* for 60 min	72.5	β-Phenylethylamine decreased NA in particulate fraction, not in supernatant.	53
Rabbit heart	0.3 M sucrose, Ultra-Turrax	1,000 × *g* for 20 min	3		73
		14,000 × *g* for 20 min	11		
		66,000 × *g* for 75 min	74		
Rat vas deferens	Liquid nitrogen, powdered, homogenised 0.3 M sucrose	1,000 × *g* for 11 min	40	Electronmicroscopy showed that 1,000 × *g* sediment contained intact nerve endings and occasional synaptosomes.	101
		17,000 × *g* for 60 min	10		
		100,000 × *g* for 60 min	10		
Rat vas deferens	0.32 M sucrose, 0.01 mM Ca^{2+}, 1 mM Mg^{2+}, ascorbic acid (1 μg/ml), homogenate filtered	800 × *g* for 10 min	11	NA concentrated in intermediate layer of gradient, which contained electron dense particles *ca.* 350 Å in diameter.	2
		11,500 × *g* for 20 min	33		
		100,000 × *g* for 60 min, 100,000 × *g* sediment on gradient (0.32 M–1.4 M sucrose): 56,000 × *g* for 2 hr	26		

Rat heart	0.25 M sucrose	Supernatant from 9,000 × *g* for 10 min diluted with potassium phosphate, spun 105,000 × *g* for 30–60 min and sediment on gradient (0.5 M–1.0 M sucrose): 169,000 × *g* for 60 min		Distribution of NA was bimodal: first peak at layer of 0.47 M sucrose, second peak at interface of 1.0 and 2.0 M. Similar result with exogenous ^{3}H-NA.	79
Rat vas deferens	0.25 M sucrose	1,000 × *g* for 2 min, 12,000 × *g* for 10 min	19	Mitochondrial enzymes were mainly recovered in the 12,000 × *g* sediment. 100,000 × *g* sediment contained 1.5 nmoles NA/mg protein, NA on gradient was recovered in layer of 0.46 M sucrose, distinct from peaks of monoamine oxidase and NADH$_2$-cytochrome *c* reductase activities. Peak fraction on gradient contained 0.4 nmoles NA/mg protein.	52
		100,000 × *g* for 60 min, 12,000 × *g* supernatant on gradient (0.4 M–0.75 M sucrose): 120,000 × *g* for 60 min	52		
Rat vas deferens	0.3 M sucrose	600 × *g* for 10 min, 10,000 × *g* for 60 min	7–27	10,000 × *g* pellet contained fluorescent particles; in the electron microscope occasional synaptosomes were seen.	7, 8
Rat iris	0.15 M sucrose, 0.05 M Na phosphate pH 7.2, 0.01 M $MgCl_2$	Homogenate on gradient (0.4 M–1.6 M sucrose): 63,580 × *g* for 3 hr		81% of endogenous NA in fraction recovered in layer of 0.4 M sucrose.	47
Rat heart	0.25 M sucrose	650 × *g* for 10 min, supernatant on gradient (0.9 M–1.5 M sucrose): 17,500 × *g* for 12 hr		NA in ventricles was recovered mainly at top of gradient (0.3 M–0.8 M sucrose): 0.81 pmoles/mg protein. NA in atria was also at the top (0.3 M–0.8 M sucrose), which contained 1.27 pmoles/mg protein, but 30% was in an intermediate layer (0.8 M–1.02 M sucrose), which contained 1.62 pmoles/mg protein. Electronmicroscopy showed small vesicles in top fraction, larger vesicles in middle fraction. The intermediate fraction also contained the specific dense granules of atrial muscle cells.	87
Dog spleen	0.25 M sucrose	Particulate fraction on three types of sucrose density gradient		Bimodal distribution of NA in gradient, peaks at densities of 1.126 and 1.178. Dopamine β-hydroxylase activity also gave bimodal distribution: one peak at density of 1.178 but the second peak was less dense than that given by the "light" noradrenergic vesicles.	19
Rat vas deferens	0.3 M sucrose	600 × *g* for 10 min, 10,000 × *g* for 60 min, 100,000 × *g* for 2 hr		100,000 × *g* sediment on sucrose density gradient: 53,000 × *g* for 3 hr. NA was recovered in a peak at layer of 0.7 M sucrose: this fraction contained 3.5–14.5 nmoles NA/mg protein. Fractions at 0.4 M–0.7 M and at 0.8 M–1.3 M sucrose layers were examined by electronmicroscopy: small dense-cored vesicles were found in the former and occasional large dense-cored vesicles in the latter.	8
Cat spleen	0.3 M sucrose	600 × *g* for 10 min, 10,000 × *g* for 60 min, 100,000 × *g* for 2 hr		100,000 × *g* sediment on sucrose density gradient: 53,000 × *g* for 3 hr. NA was recovered in two regions: 0.7 M sucrose layer and 1.0 M–1.1 M sucrose layer. Peak at 0.7 M sucrose layer contained 0.3–1.45 nmoles NA/mg protein, and that at 1.0 M–1.1 M sucrose layer contained 0.6–2.0 nmoles NA/mg protein.	8

ATP. De Potter *et al.* (21) concluded from their results that ATP had a distribution consistent with its presence in both mitochondria and noradrenergic vesicles. Since the fractions analysed in the earlier studies (quoted above) all contained mitochondria, the values for the molar ratio of noradrenaline to ATP do not represent the molar ratio of the substances actually present in the noradrenergic vesicles. By allowing for the ATP present in the mitochondria, De Potter *et al.* (21) estimated that the molar ratio of noradrenaline to ATP in the noradrenergic vesicles fell within the range 7.5 to 12. This is up to three times the value of the ratio found in adrenal chromaffin granules (84), and is also higher than the value of "close to 4" given in a recent preliminary report on purified splenic nerve vesicles (41).

A similar approach has been used to identify specific protein components of the noradrenergic vesicle. The distribution of dopamine β-hydroxylase in both differential (21) and sucrose density gradient centrifugation (21, 46) was very similar to that of noradrenaline. Material which cross-reacted with antiserum to bovine adrenal chromogranin A also had a distribution, in two types of differential centrifugation, similar to that of noradrenaline (21). These observations provide unequivocal evidence that dopamine β-hydroxylase and a protein like chromogranin A are components of the noradrenergic vesicle. Earlier work had shown that dopamine β-hydroxylase (91) and chromogranin A (4) were present in a particulate fraction from a splenic nerve homogenate, but although this fraction contained the noradrenergic vesicles it also contained other cell particles.

Dopamine β-hydroxylase and chromogranin A were present in the particle-free supernatant of splenic nerve as well as in the vesicles (21). The occurrence of both these proteins, as well as noradrenaline, in the supernatant can be accounted for in two ways. Either the proteins and noradrenaline exist both in particles and in the soluble axoplasm, or they are normally confined to the vesicles but are released from them during homogenisation and centrifugation. In the experiments of De Potter *et al.* (21), the proportion of the dopamine β-hydroxylase activity in the supernatant was more than could be accounted for by the lysis of noradrenergic vesicles, just as was found by Viveros *et al.* (95) for the adrenal medulla. It is possible, therefore, that in both splenic nerve and adrenal medulla there is a proportion of the total dopamine β-hydroxylase normally in the cytosol. However, the distribution of chromogranin A among fractions of the adrenal medulla is consistent with the localisation of most, if not all, of it in the chromaffin granules (57). The work of De Potter *et al.* (21) suggests that the chromogranin of splenic nerve is present only in the noradrenergic vesicles, because the amount of chromogranin A found in the final supernatant is close to that which would be released upon lysis of the vesicles damaged during homogenisation.

The experiments of Hörtnagl *et al.* (46) and De Potter *et al.* (21) were based on the assumption that it was not possible to isolate noradrenergic vesicles sufficiently free from other cell particles to permit direct analysis of their composition. However, experiments by Lagercrantz *et al.* (60) have shown that the use of D_2O instead of H_2O in the sucrose density gradients improves the separation of noradrenergic vesicles from other membranous material. Lagercrantz (59) has

made detailed studies on the composition of a noradrenaline-rich fraction obtained by this method, and has estimated that at least 20% of the protein in the fraction is present in noradrenergic vesicles. Although the fraction is richer in noradrenergic vesicles than any isolated previously, caution should still be exercised in drawing conclusions about the composition of noradrenergic vesicles from analysis of this fraction alone.

Provisionally, it can be concluded that most of the noradrenaline in the splenic nerve is stored within a population of noradrenergic vesicles of the heavy type and that these vesicles contain ATP, dopamine β-hydroxylase and chromogranin A.

V. Sympathetically Innervated Tissues (Table 1d)

A problem the biochemist has to consider when studying homogenates of sympathetically innervated tissues is whether all the particle-bound catecholamine was derived from the neurons. Two other possible sites of origin have to be considered: first, extra-adrenal "chromaffin cells" or similar small, intensely-fluorescent cells, which have been found in the heart (34, 48, 54, but see 1); second, the specific secretory granules of atrial muscle cells (70, 87, but see 51). The simplest way of showing that the catecholamines present in particulate fractions of an homogenate are derived from the nerve is to examine the same fractions prepared from the tissue after chronic sympathetic denervation. This approach may not be easily applicable to tissues containing short adrenergic neurons, but a surgical method of sympathectomy has recently been described for the vas deferens (6). The catecholamine content of denervated tissues is usually considerably less than 10% of that of the normal tissue.

It can be seen from table 1d that in many studies on tissues innervated by sympathetic nerves, a large proportion of the noradrenaline was recovered in the low-speed sediment, just as in the studies described above on ganglia and on splenic nerve. It is, of course, likely that this was due to incomplete homogenisation of the tissue and to trapping of small particles in the large sediment of non-nervous tissue fragments. However, there are two reports that sediments obtained from homogenates of vas deferens contain intact, pinched-off nerve endings (F. Clementi, see 101; 8); these are similar to the synaptosomes obtained from the central nervous system. The formation of synaptosomes from peripheral nerves could complicate the interpretation of centrifugation studies, but it seems unlikely that more than a trace of the noradrenaline in the homogenate will be present in such particles, because the homogenisation conditions are usually far from gentle. Nevertheless, in future studies the possible presence of synaptosomes will have to be monitored by fluorescence and electronmicroscopy, and by biochemical methods such as analysis for lactate dehydrogenase and dopa decarboxylase.

The proportion of the noradrenaline in the low-speed supernatant of homogenates of sympathetically innervated tissues that is recovered in particles varies widely (16%–74%), as can be seen from table 1d. The low values reported in some of the early experiments (27) may be explained, in part, by the low cen-

trifugal force used, which was not sufficient to sediment all the particles of microsomal size (diameters of 1000 Å or less). It is, in fact, significant that in many studies as much as 50% to 75% of the noradrenaline in the low-speed supernatant is recovered in particles. Since a certain proportion of the noradrenaline in the final supernatant will have come from particles damaged during homogenisation and centrifugation, it can be concluded that the particle-free pool of noradrenaline may not represent more than about 20% of the total.

What kind of particles store the noradrenaline in sympathetically innervated tissues? Potter and Axelrod (76, 77) found that when tissue homogenates were centrifuged for a short time on a sucrose density gradient, the noradrenaline-containing particles sedimented very slowly, remaining with the microsomes at the top of the gradient tube. By this means they could be distinguished from mitochondria, which sedimented more rapidly and were recovered nearer the bottom of the tube. Several subsequent studies (see table 1d) on the distribution of noradrenaline between fractions obtained by differential centrifugation have confirmed that much of the noradrenaline is present in particles which sediment in the microsomal fraction: the proportion of noradrenaline in this fraction is usually equal to, or greater than, that recovered in the crude mitochondrial fraction. The noradrenaline-containing particles of these tissues are, therefore, distinct from the bulk of the mitochondria in the tissue. However, most of the mitochondria do not come from the sympathetic nerve terminals. It has been found that mitochondria in axons of the splenic nerve sediment more slowly than do the mitochondria in non-nervous tissues (21) and so some of the neuronal mitochondria will be recovered in the microsomal fraction of homogenates of innervated tissues. This is one of several possible explanations of why the microsomal fraction of vas deferens contained a greater proportion of mitochondrial enzymes than the microsomal fraction of liver did in the experiments of Jarrott and Iversen (52), since the vas deferens is a richly innervated tissue. In the density gradient experiments of Jarrott and Iversen (52) it was possible to distinguish the noradrenaline-containing particles of vas deferens both from monoamine oxidase present in intact mitochondria, recovered in denser layers in the gradient, and from the microsomal monoamine oxidase which was recovered in a less dense layer of the gradient. It can be concluded that the noradrenaline-containing particles of sympathetically innervated tissues are mainly of microsomal size, and that, at least in the vas deferens, it is possible to distinguish them from other components of the microsomal fraction. Is the noradrenaline of these tissues, which is derived mainly from the terminal varicosities of the neurons, stored in one type of particle, or is there more than one kind of noradrenergic vesicle in nerve terminals?

The heterogeneity of the paticulate store of noradrenaline in sympathetically innervated tissues is suggested by a number of observations with density gradients which, at first sight, appear somewhat contradictory (see table 1d). Some of the apparent contradictions are accounted for by different experimental conditions. Two points in particular have to be borne in mind when interpreting the results of gradient centrifugation experiments: 1) the nature of the material applied to

the gradient, which varies from whole homogenates to a resuspended microsomal pellet, and 2) the time of centrifugation and the centrifugal force applied; thus, some experiments were designed to separate particles solely by virtue of their different rates of sedimentation (76, 77) whereas in others the aim was to approach the equilibrium density of the particles. In the original density gradient experiments of Potter and Axelrod (76, 77) on rat heart homogenates a considerable part of the total noradrenaline was found at the bottom of the gradient, in addition to that in the "microsomal" layer at the top; the authors concluded that the noradrenaline in the pellet was in incompletely homogenised tissue. However, density gradient experiments on the *low-speed supernatant* of dog heart homogenates also showed a bimodal distribution of noradrenaline: 54% remained near the top of the gradient (0.25 M–0.4 M of sucrose) and 30% was recovered lower down in the layer of 1.0 M to 1.2 M of sucrose (37). The latter authors used the same type of density gradient as Potter and Axelrod (76) but centrifuged for 2 hr instead of 30 min. A further indication that, in the heart, there may be more than one type of noradrenaline-storing particle was the finding by Taylor *et al.* (92) of two peaks of radioactivity in a gradient after infusion of [^{3}H] noradrenaline into the cat heart. This observation was confirmed by Roth *et al.* (79) in experiments on the rat heart and these authors also determined the distribution of endogenous noradrenaline which likewise had a bimodal distribution: one peak was at the layer of 0.47 M of sucrose, the other at the interface of 1.0 M and 2.0 M of sucrose; these authors introduced the terminology "light" and "heavy," respectively for the two kinds of particle. Accumulation of material above a layer of 2.0 M of sucrose is difficult to interpret since it may be due to a second population of particles, or simply to a concentration of the "tail" of a peak of noradrenaline given by the lighter particles. A bimodal distribution of endogenous noradrenaline in sucrose gradients from rat atria, but not from the ventricles has also been reported by Sosa-Lucero *et al.* (87).

The tissue where a bimodal distribution of noradrenaline after gradient centrifugation is most easily demonstrated is the spleen. In the dog spleen, the second peak of noradrenaline, at about the layer of 1.2 M-sucrose, comprises about half the noradrenaline applied to the density gradient (19, 20). In similar experiments on the cat spleen Bisby and Fillenz (8) found a bimodal distribution of noradrenaline (peaks at layers of 0.7 M and 1.0 M of sucrose), whereas homogenates of rat vas deferens treated in the same way only gave a single peak of noradrenaline in the region of 0.7 M of sucrose.

Before accepting these biochemical findings as evidence for two populations of noradrenergic vesicles in the nerve terminals, several other possibilities have to be considered. First, it must be shown that both noradrenaline-containing particles come from the nerve. This has so far only been demonstrated by Chubb *et al.* (19) who found that both types of particle were absent in a dog spleen that had been denervated and that the particles did not form artifactually upon addition of noradrenaline to the homogenate before fractionation. The suggestion by Sosa-Lucero *et al.* (87) that, in the heart, some noradrenaline is stored in the "secretory granules" of atrial muscle cells could possibly be tested by

carrying out experiments after sympathetic denervation. However, since Potter *et al.* (78a) found that denervation of the dog heart led to a fall in noradrenaline content to 1.2% of normal, although the "secretory granules" were still present in muscle cells, the amount of noradrenaline in these particles must be very small. (Of course, the possibility remains that the granules in muscle cells take up noradrenaline released from the nerves.) The morphological criteria used by Sosa-Lucero *et al.* (87) to support their claim are insufficient since their micrographs show that, in addition to secretory granules from the muscle, the noradrenaline-rich fraction from atria also contained much smaller particles, some of which look like the large dense-cored vesicles of sympathetic nerves. Nevertheless, the observation that the second population of noradrenaline-containing particles is characteristic of the atrium rather than the ventricle is interesting and deserves further study. Finally, the possibility that any noradrenaline-containing particles which enter the denser layers of a gradient (1.0 M to 1.2 M of sucrose) might be synaptosomes must be considered. Experiments in which the entire homogenate is placed on a density gradient are suspect, since not only will the homogenate contain large tissue fragments of non-nervous origin, which might trap the noradrenergic vesicles, it will also contain any synaptosomes which may have been formed. It is important to sediment the large particles first, as was done in the studies of Chubb *et al.* (19) and of Bisby and Fillenz (8), even if this means losing a proportion of the noradrenaline-containing particles. The loss of noradrenergic vesicles by trapping in low- to medium-speed sediments can be minimised by resuspending these and recentrifuging (21). There have, so far, been no biochemical studies on density gradient fractions to test for the presence of entrapped soluble axoplasm which will be present in synaptosomes.

VI. Correlation of Biochemical and Morphological Observations

Two types of dense-cored vesicle have been distinguished in electron micrographs of sympathetic nerves (for reviews see 32, 35, 45). It is now considered likely, from electron histochemical studies, that not only the small dense-cored vesicle but also the large dense-cored vesicle (43, 50, 94) contains a primary catecholamine. Whereas nerve terminals contain both types of vesicle, preterminal axons contain mainly (44, 56) or exclusively (31, 36, 58) one type of vesicle, which is probably identical with the large dense-cored vesicle of the nerve terminals. The biochemical studies described above have shown that axons of the splenic nerve contain only one type of vesicle, which equilibrates at the density of 1.2 M of sucrose. It is possible, therefore, that the particle in sympathetically innervated tissues, which also equilibrates in a gradient close to the level of the 1.2 M of sucrose layer, may be the large dense-cored vesicle present in terminals and in the preterminal axons within the organ. The second type of vesicle, found only in sympathetically innervated tissues, which is recovered in less concentrated layers of sucrose after gradient centrifugation, may be the small dense-cored vesicle which the microscopists find is specifically located in nerve terminals.

These suggested correlations could be tested by electronmicroscopic studies on isolated subcellular fractions, but most of the microscopic studies so far reported have simply confirmed the gross contamination of the fractions by other cell particles, which is indicated by the low concentration of noradrenaline per weight of protein (see table 2 and below). However, there are some qualitative studies which are consistent with the above correlation. Thus, small dense-cored vesicles were found in the fractions containing the light noradrenergic vesicles of rat heart (74) and of rat vas deferens (2, 8), whereas large dense-cored vesicles were seen in the fraction containing the heavy noradrenergic vesicles of splenic nerve (58, 79, 93) and of rat vas deferens (8). Further support for the proposed correlation was provided by Bisby and Fillenz (8), who found that nerve terminals in the cat spleen contain far more (about 20% of the total vesicles) large dense-cored vesicles than do the terminals in the vas deferens, where the proportion is only 4%. In the biochemical experiments, a definite peak of noradrenaline in the denser layers of the sucrose gradient was found only in experiments on homogenates of the spleen (8). As the biochemical methods for purifying the vesicles improve it will be interesting to see whether this correlation, at present only tentative, is substantiated by quantitative morphological analyses of the fractions.

VII. Composition of Noradrenergic Vesicles

The biochemical studies described above have shown how hazardous it is to conclude that the noradrenergic vesicle contains a substance just because that substance was present in a noradrenaline-rich subcellular fraction. One indication of the degree of contamination of the noradrenaline-rich fractions by other cell particles can be obtained by comparing the ratio of the amount of noradrenaline to that of protein in each fraction, with the ratio found for purified noradrenaline-containing adrenal chromaffin granules: this has been done in table 2. The fractions with the highest concentrations of noradrenaline, those from the splenic nerve, vas deferens and heart, contain 10 to 100 nmoles of noradrenaline per milligram of protein, whereas the noradrenaline-containing chromaffin granules of bovine adrenal medulla contain 2500 nmoles of noradrenaline per milligram of protein (3). It can also be seen, from table 3, that the noradrenaline-rich fraction from splenic nerve contains several hundred times as much lipids per mole of catecholamines as do purified chromaffin granules.

A comparison of the amount of noradrenaline per unit of protein or lipid in a fraction isolated from nerves with these ratios in chromaffin granules should only be used as a rough indication of the purity of the fraction. There are many unknown factors, *e.g.*, we do not know whether the small noradrenergic vesicles contain soluble proteins, or whether the concentration of noradrenaline in the interior of a noradrenergic vesicle is necessarily the same as that inside a chromaffin granule. Allowance should also be made for the different sizes of the particles. The importance of this can be seen from table 4, where the proportion of the total volume of each type of particle that is occupied by the membrane is given. It is clear that a considerable part of the protein in a small noradrenergic

TABLE 2

Noradrenaline and protein content of noradrenaline-rich subcellular fractions from different tissues

Tissue	Noradrenaline (nmoles)/ Protein (mg)*	Method for Protein	Reference
Ox adrenal medulla	2500	Nitrogen	3
Rat brain stem	0.14	Folin	66
Rat brain	0.04	Folin	23
Rat anterior hypothalamus	0.22	Folin	23
Rat heart	.101	Folin	75
Pig hypothalamus	0.06	Nitrogen	72
Bovine stellate ganglion	0.65	Nitrogen	81
Bovine stellate ganglion	0.48	Folin	71
Bovine splenic nerve	2.9	Nitrogen	81
Bovine splenic nerve	4.9	Folin	12
Bovine splenic nerve	2.9	Biuret	46
Bovine splenic nerve	5.0	Folin	4
Bovine splenic nerve	19.9	Folin	60
Bovine splenic nerve	9.1	Folin	21
Guinea-pig heart	0.4	Nitrogen	82
Rat vas deferens	1.5	Folin	52
Rat heart	0.0016	Biuret	87
Rat vas deferens	up to 14.5	Folin	8
Cat spleen	up to 2.4	Folin	8

* Only a few authors have given the amount of noradrenaline per milligram protein in the homogenate before fractionation. Potter (75) found a value of 0.07 nmoles/mg protein for rat atria. M. Fillenz (personal communication) has provided the following figures: rat heart, 0.029 nmoles/mg protein; cat spleen, 0.073; rat hypothalamus, 0.053; rat vas deferens, 0.98. De Potter *et al.* (21) found a value of 0.92 for bovine splenic nerve.

TABLE 3

*Composition of two noradrenaline-rich particulate fractions obtained from homogenates of bovine splenic nerve**

Constituent	Units	Splenic Nerve Fraction		Adrenal Chromaffin Granules (Purified)
		Fraction 3 from ref. 21	Fraction III from ref. 59	
Catecholamine	nmole	1	1	1
ATP	nmole	0.132	ca. 0.25	0.22
Dopamine β-hydroxylase	pmole/20 min	443	900	7.25
Chromogranin A	μg	0.26	1.28	0.10
Protein	μg	110	50	0.29
Lipid-phosphorus	nmole	161	31	0.18
Cholesterol	nmole	52	14	0.10

* The composition of each fraction is expressed per nmole of catecholamine. The data for bovine adrenal chromaffin granules were calculated from figures in reference 84, except for the activity of dopamine β-hydroxylase (21).

TABLE 4

Size of catecholamine-containing particles and the proportion of their volume occupied by the membrane

Particle	Diameter (Å)*	Proportion of Volume Occupied by Membrane of Thickness 70 Å	Volume of Interior Relative to Interior of a Chromaffin Granule	Volume of Membrane Relative to Membrane of a Chromaffin Granule
Chromaffin granule	3000	13.4%	1	1
Large noradrenergic vesicle	700	48.8%	1/133	1/22
Small noradrenergic vesicle	443	67.9%	1/833	1/61

* The diameters of the noradrenergic vesicles are those reported by Geffen and Ostberg (36) for vesicles in the non-terminal axons and terminal varicosities of the cat splenic nerve. The membrane thickness is of the membrane of large noradrenergic vesicles in the ox splenic nerve (58).

vesicle will be present in its membrane. Furthermore, the volume of the interior of the vesicle (that part of the vesicle which presumably contains the noradrenaline) relative to that of the whole particle is less for the noradrenergic vesicles of nerves than for adrenal chromaffin granules. One would not, therefore, expect to find the same ratio of noradrenaline to membrane components (lipids and proteins) in the three types of particle. Let us assume, for the sake of argument, that the concentration of noradrenaline in the interior of each type of particle is the same; the large noradrenergic vesicle will, then, only contain about 1/6, and the small noradrenergic vesicle will only contain about 1/13, of the amount of noradrenaline per unit volume of membrane found in the adrenal chromaffin granule (see table 4).

Large dense-cored vesicles. In order to identify a component of the noradrenergic vesicle it is necessary to show that it has a distribution between subcellular fractions very similar to that of noradrenaline. For the noradrenergic vesicles of splenic nerve axons this has so far only been done for ATP, chromogranin A and dopamine β-hydroxylase (see above). The amount of each of these substances in particulate fractions isolated from bovine splenic nerve by two groups of workers (21, 59) is given in table 3 relative to the amount of noradrenaline. Although the amounts of ATP, chromogranin A and dopamine β-hydroxylase are not identical in the two preparations, it is gratifying that they are at least of the same orders of magnitude. When the composition of the splenic nerve particles is compared with that of adrenal chromaffin granules, which is also given in table 3, the most striking differences are found in the amounts of proteins, lipids and dopamine β-hydroxylase activity relative to noradrenaline. As discussed above, the higher content of protein and lipid in the nerve particles could reflect not only contamination of the fraction but also the difference in size between noradrenergic vesicles and chromaffin granules. A third possibility, pointed out by Stjärne (89) and Lagercrantz (59), is that the noradrenergic vesicles might contain a lower concentration of noradrenaline than the adrenal chromaffin granules. The much higher level of dopamine β-hydroxylase activity found in

the fractions from splenic nerve could possibly be because the membrane of noradrenergic vesicles contains a higher concentration of this enzyme than the membrane of chromaffin granules does. However, it is equally likely that this large difference in the ratio of dopamine β-hydroxylase activity to the amount of catecholamine is due to a much lower concentration of catecholamine within the nerve vesicles.

Much less is known about the composition of large dense-cored vesicles in the terminal varicosities of sympathetic neurons. The only component, in addition to noradrenaline, that has been identified is dopamine β-hydroxylase: this enzyme has the same distribution as the heavy peak of noradrenaline in density gradient fractions after centrifugation of particles from dog spleen (19). A peak of dopamine β-hydroxylase activity, but not of noradrenaline, has been found in density gradient fractions that contain large dense-cored vesicles after centrifugation of particles from rat vas deferens (9). It is possible that in studies on tissues, such as the vas deferens, where the proportion of large dense-cored vesicles in terminals is low (8, 30) the presence of this type of vesicle in fractions will be more easily revealed by its content of dopamine β-hydroxylase than by its content of noradrenaline. The terminals of the splenic nerve contain a considerable proportion of large dense-cored vesicles (8) and these can be distinguished from the light vesicles by density gradient centrifugation (8, 19, 20). It is particularly interesting that the ratio of the amount of noradrenaline to the activity of dopamine β-hydroxylase in the heavy vesicles of dog splenic nerve *terminals* is about 10 times that in the noradrenergic vesicles of the dog splenic nerve *axons* (see 20). This finding supports suggestions (59, 89) that the large vesicles in preterminal axons are relatively deficient in noradrenaline but can take up and store more noradrenaline when they reach the terminals.

Small dense-cored vesicles. Very little is known about the composition of the light noradrenergic vesicles of nerve terminals (see review by Potter, 74) because most of the analyses have been done on a single fraction. Analysis of such a fraction from rat heart showed that it contained ATP and dopamine β-hydroxylase (75, 78), but no studies have yet been published showing that the distribution of ATP and dopamine β-hydroxylase parallels that of noradrenaline in fractions from the heart. In the microsomes of the rat vas deferens, the distribution of ATP in a density gradient (0.32 M to 1.4 M of sucrose) paralleled that of noradrenaline: the molar ratio (noradrenaline/ATP) in fractions containing the "light" vesicles ranged from 2.2 to 3.7 (64). However, a large amount of ATP was also found in the pellet at the bottom of the density gradient and this was only associated with a trace of noradrenaline.

Chubb *et al.* (19) have carefully analysed the distribution of dopamine β-hydroxylase in density gradients of a microsomal fraction from dog spleen. Two peaks of dopamine β-hydroxylase activity were obtained: one peak corresponded closely to that given by the heavy noradrenergic vesicle, but the other peak was in a less dense region of the gradient than the peak of noradrenaline in the light noradrenergic vesicle. The authors suggested, therefore, that the light noradrenergic vesicle does not contain dopamine β-hydroxylase. However, it should not

be forgotten that a similar, but less marked, difference occurs in the density gradients of fractions from adrenal medulla where the peak of dopamine β-hydroxylase activity is found in a less dense layer than that of the catecholamines (96). The explanation offered by Viveros *et al.* (96) for the situation in the adrenal medulla may, perhaps, apply *a fortiori* to the nerve terminal: the dopamine β-hydroxylase-containing particles may become denser as they fill up with noradrenaline. (Alternatively, the noradrenaline may leak out of the vesicles during isolation more easily than the dopamine β-hydroxylase does.) Preliminary studies on the rat heart and vas deferens indicate a much closer, but not quite identical distribution of dopamine β-hydroxylase and noradrenaline in density gradients of the light noradrenergic vesicles (9).

VIII. Conclusions

What has been learned about the noradrenergic vesicle in the 15 years since its discovery by Euler and Hillarp (27)? No reviewer likes to draw hard and fast conclusions from evidence which is at times contradictory and which is always incomplete. However, an attempt has been made to summarise below some of the most important findings.

Central nervous system. Biochemical evidence concerning the nature of the noradrenaline-containing particles in the central nervous system is meagre. Although it has been shown that noradrenaline is present in synaptosomes and that some of this synaptosomal noradrenaline is in particles within the nerve ending, there is no evidence whether or not the nerve endings contain more than one type of noradrenergic vesicle.

Peripheral nervous system. Much more is known about the noradrenergic vesicles of peripheral sympathetic neurons. Our present knowledge can be summarised as follows:

1) Most of the noradrenaline is stored in particles.

2) The noradrenaline-containing particles of non-terminal axons of the splenic nerve can be distinguished from other cell particles, such as mitochondria, lysosomes and membrane fragments.

3) The particulate store is heterogeneous: noradrenaline is found in the light noradrenergic vesicle (possibly identical with the small dense-cored vesicle described by electron microscopists) and in the heavy noradrenergic vesicle (possibly identical with the large dense-cored vesicle).

4) In the cell body and non-terminal axons, the heavy vesicles are the predominant type.

5) In the terminal varicosities both heavy and light noradrenergic vesicles occur, but the latter store most of the noradrenaline.

6) The heavy noradrenergic vesicles of non-terminal axons contain ATP, chromogranin A and dopamine β-hydroxylase. The heavy vesicles in the terminals also contain dopamine β-hydroxylase, but it is not yet certain whether the light vesicles contain this enzyme. There is some evidence that the light vesicles contain ATP.

7) Preliminary observations suggest that the heavy vesicles in the nerve

terminals contain a higher concentration of noradrenaline than the heavy vesicles in the non-terminal axons.

This knowledge, gained mainly by the application of biochemical methods, is not only of interest for what it tells us about the nature of the storage particles that contain noradrenaline; it has already been useful in studies on the mechanism of release of noradrenaline from peripheral neurons. These studies have shown that vesicle proteins (dopamine β-hydroxylase and chromogranin A) are released upon electrical stimulation of the nerve (for reviews see 35, 85, 86). Now that it is possible to identify the different sites of storage of noradrenaline in neurons it is likely that much new information will be obtained about the mechanism of action of those drugs that modify the amount of noradrenaline in nerve terminals and that influence the release of the neurotransmitter.

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