

needed before definite conclusions can be drawn with regard to the processes involved in the permeation of these solutes. In view of the low sodium content of the milk the apparently high permeability of the udder with regard to sodium ions may, however, indicate participation of both diffusion and active transport of this ion.

P.-G. KNOTSSON

Department of Animal Physiology,
Royal Agricultural College,
Uppsala 7.

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Particle-bound and Free Catecholamines in Dog Hearts and the Uptake of Injected Norepinephrine

SUBMICROSCOPIC structures containing catecholamines have been found by high-speed centrifugation of homogenates or pressed juice of adrenal medulla¹⁻³, splenic nerves⁴⁻⁶ and brain⁷. A granular fraction containing catecholamines has been isolated from the pressed juice of cyclostome hearts⁸. It was the purpose of this investigation to see whether also in mammalian hearts the catecholamines are stored in a particle-bound state; the question was also examined whether injected norepinephrine is taken up by sub-microscopic cell elements. This is of interest since Raab and Gige⁹ reported a specific avidity of the heart muscle to absorb and store catecholamines.

Six puppy hearts, which have a high catecholamine content (Wegmann *et al.*, unpublished data), were submitted to successive low- and high-speed centrifugation after homogenization. The catecholamine content in the different fractions was analysed fluorimetrically. In another series of 7 animals an identical procedure was applied 20 min. after the intraperitoneal administration of 10 mgm. norepinephrine/kgm. The hearts were dissected into very small pieces in ice-cold physiological sodium chloride solution. After washing three times and re-suspending in ice-cold saline solution the hearts were homogenized in a Potter-Elvehjem glass homogenizer with a loose-fitting pestle. The homogenates were centrifuged at 600-1,000g for 10 min. The homogeneous supernatant was again centrifuged at 50,000-100,000g for 60 min. The sediment from this centrifugation was taken up in a few ml. of *N* hydrochloric acid. Both sediment and supernatant were then deproteinized by trichloroacetic acid for fluorimetric estimation according to Euler and Lishajko¹⁰ after adsorption on alumina and elution with acetic acid. Only a set of two filters (excitation at 412 m μ ; emission at 508 m μ) was used; thus norepinephrine and epinephrine were determined together.

The catecholamine content in the different fractions of the normal hearts is shown in Table 1. The supernatant layer obtained by low-speed separation did not contain microscopically visible cell structures. After high-speed centrifugation 67-85 per cent (average 74.5 per cent) of the catecholamines were located in the small sediment.

The catecholamine content in the different fractions 20 min. after injection of 10 mgm./kgm. norepinephrine is shown in Table 2. In these experiments only 9-31 per cent (average 19.6 per cent) of the catecholamines were found in the sediment after high-speed

Table 1. DISTRIBUTION OF CATECHOLAMINES IN HEART HOMOGENATES OF PUPPIES

Weight of heart (gm.)	Total homogenate (μ gm./gm.)	Supernatant (800g)		Sediment (75,000g)	
		Total homogenate (μ gm./gm.)	Total homogenate (per cent)	Total homogenate (μ gm./gm.)	Super-natant (per cent)
13	1.00	0.54	54	0.36	67
14	0.64	0.34	50	0.26	76
16	1.20	0.56	47	0.38	68
10.5	1.10	0.41	37	0.35	85
12.5	1.04	0.58	56	0.41	71
13.5	0.71	0.34	48	0.27	80
Mean	0.95	0.46	49.0	0.34	74.5

Table 2. DISTRIBUTION OF CATECHOLAMINES IN HEART HOMOGENATES OF PUPPIES 20 MIN. AFTER INTRAPERITONEAL INJECTION OF 10 MGm. NOREPINEPHRINE/KGM.

Weight of heart (gm.)	Total homogenate (μ gm./gm.)	Supernatant (800g)		Sediment (75,000g)	
		Total homogenate (μ gm./gm.)	Total homogenate (per cent)	Total homogenate (μ gm./gm.)	Super-natant (per cent)
12	2.15	1.16	54	0.36	31
11.5	12.2	10.33	85	0.93	9
10	4.00	2.64	66	0.78	30
7	3.76	1.83	49	0.39	21
11	2.75	1.44	52	0.41	29
12	3.90	2.59	67	0.34	13
18	13.3	10.92	82	0.42	4
Mean	6.01	4.42	65.0	0.52	19.6

centrifugation, the greater part being recovered in the supernatant. The difference between the results in the two series is highly significant ($P < 0.001$).

This investigation demonstrates the presence of particle-bound catecholamines in dog hearts. More than 95 per cent of injected norepinephrine found in the supernatant after low-speed centrifugation were not particle-bound. Whether or not these large amounts recovered in the soluble fraction were intra- or extra-cellular cannot be stated at the present time.

A. WEGMANN
K. KAKO

Department of Medicine,
Wayne State University,
Detroit.

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Response of the Reticulo-Endothelial System to Stimulation with Oestrogens

WE have previously shown that diethylstilbœstrol and tri-*p*-anisylchloroethylene are strong stimulants of the reticulo-endothelial system¹. We have also found that when the reticulo-endothelial system is stimulated by diethylstilbœstrol the body defence is raised, as indicated by increased phagocytic activity, elevation of the serum γ -globulin to about three times the normal level, increased antibody formation, and the protection of mice and guinea pigs against lethal doses of virulent bacteria. In previous experiments it was found that the minimum dose of diethylstilbœstrol required to stimulate the reticulo-endothelial phagocytes is about a hundred times greater than that necessary to induce œstrus² and that after treatment with 0.5 mgm. diethylstilbœstrol daily for 6 days the