Subcellular Distribution of Catecholamines and Enzymes in Human Neuroblastoma

Cell fractionation studies of neuroblastoma are scarse. PAGE and JACOBY[1] observed that a 'granule fraction', obtained from a neuroblastoma, contained noradrenaline (NA); however, these authors did not mention the distribution of noradrenaline in the various subcellular fractions. HÖRTNAGL et al.[2], studying one neuroblastoma by means of density gradient centrifugation methods, reported that noradrenaline was stored in dense cell particles.

Material and methods. 18 human tumors with the histological diagnosis of neuroblastoma were cut into small pieces immediately after surgical removal and kept in an ice-cold 0.25 *M* sucrose solution. Upon arrival in the laboratory, i.e. after time intervals from 30 min to 3 h, the pieces were finely chopped, suspended in 5 volumes of 0.25 *M* sucrose and homogenized in a Potter-Elvehjem glass homogenizer fitted with a teflon pestle.

After filtration through surgical gauze, the homogenate was repeatedly subjected to differential centrifugation in which 5 sediments and a final supernatant were obtained (rotor A 40 of Spinco L Ultracentrifuge), as previously described[3] for the study of the noradrenergic nervous system. In short, the filtrate was firstly centrifuged at 5,000 rpm (3,020 g_{av}) for 10 min to give sediment 1. The supernatant was centrifuged at 17,500 rpm (20,203 g_{av}) for 15 min to give a sediment which was resuspended gently by hand in 10 ml of 0.25 *M* sucrose. The suspension was recentrifuged at 17,500 rpm for 15 min to yield sediment 2 and a supernatant which was combined with the previous supernatant. The combined supernatants were centrifuged at 17,500 rpm for 30 min to give sediment 3. The supernatant was centrifuged at 30,000 rpm (59,364 g_{av}) for 22 min to give sediment 4. The supernatant was centrifuged at 30,000 rpm for 35 min to give sediment 5 and a final supernatant.

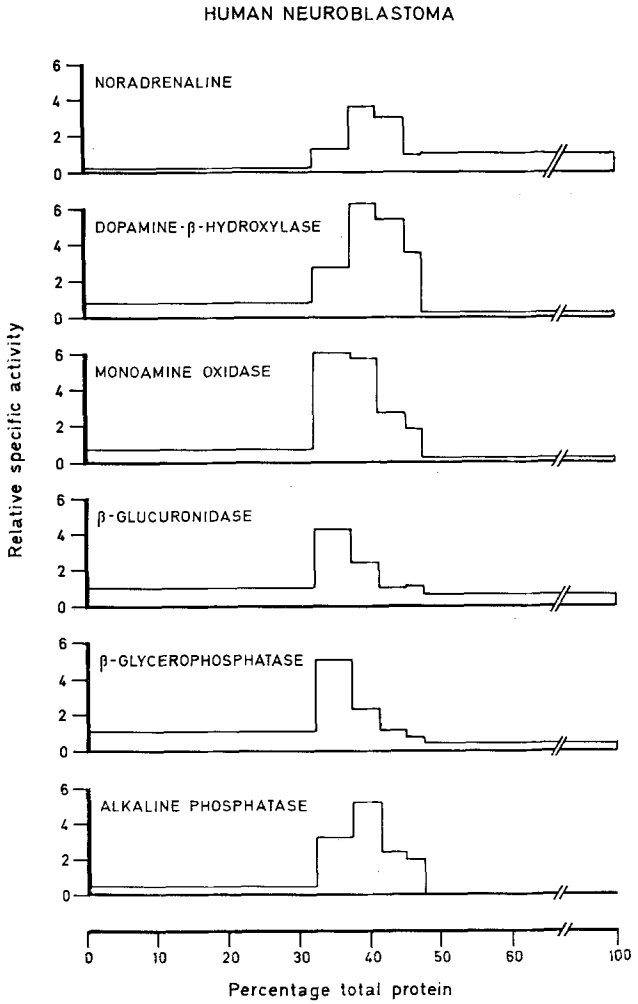
For density gradient centrifugation, a sediment corresponding to the particulate fractions 2 to 5 was resuspended in 0.25 *M* sucrose (0.2 ml/g of original tissue) and applied to a density gradient. Gradients ranging from 0.3 *M* to 1.7 *M* sucrose were used. The bottom of each tube contained 0.5 ml of 2.0 *M* sucrose. The tubes containing the density gradients were centrifuged for 150 min in the SW 40 head of the Spinco ultracentrifuge at 40,000 rpm (200,704 g_{av}). After centrifugation the tubes were punctured at the bottom and fractions of 1 ml collected for analysis. Estimations of noradrenaline (NA), protein and enzymes were carried out as previously described[3].

Results. The Table shows enormous variations in the activities of the biosynthetic enzymes and of monoamine oxidase (MAO), and in the NA content of the human neuroblastomas studied. In general, tumors with a high dopamine-β-hydroxylase (DβH) activity also showed a high content of NA, but exceptions did occur.

Figure 1 illustrates the result of a differential centrifugation of a tumor homogenate. The homogenate of human neuroblastoma contains several enzymes which in other tissues have been shown to be characteristic for different types of subcellular particles or structures, e.g. MAO (mitochondria), β-glycerophosphatase and β-glucuronidase (lysosomes) and alkaline phosphatase (microsomal elements). It can be seen that a large part of both NA and DβH is associated with particles, the distribution of which differs from the enzyme markers already mentioned.

Figure 2 shows that these subcellular particles can also be distinguished on the basis of their equilibrium density. Thus the distribution of NA is different from that of the marker enzymes for other particles but resembles very closely the distribution of DβH, except for a relatively larger fraction of the latter in lower density regions. However, some neuroblastomas also showed distributions in which NA and DβH did not completely coincide.

From the values of NA (ng) and DβH (units) calculated from gradients from those parts of the curve on the more dense side of the peaks – where only intact NA particles occur[3] – a ratio can be obtained, which gives the relative NA content of the storage particles, since DβH, which is bound for 80–85% to the membrane of the particle,



← Fig. 1. Distribution patterns of NA and enzyme markers between fractions obtained by differential centrifugation of human neuroblastoma homogenates. The abscissa gives the proportion of the total protein in each fraction, beginning with the low speed sediment on the left; the ordinate gives the relative specific activity of each substance, which is the proportion of the constituent in a fraction divided by the proportion of the total protein in the fraction.

[1] L. B. PAGE and G. A. JACOBY, Medicine, Baltimore *43*, 379 (1964).
[2] H. HÖRTNAGL, H. HÖRTNAGL, H. WINKLER, H. ASAMER, H. J. FÖDISCH and J. KLIMA, Lab. Invest. *27*, 613 (1972).
[3] W. P. DE POTTER, A. D. SMITH and A. F. DE SCHAEPDRYVER, Tiss. Cell *2*, 529 (1970).

can be taken as an index for the number of NA particles. This ratio ranged from 0.30 to 1.50 in the 18 neuroblastomas studied.

Discussion. The very large variations in enzyme activities and NA content observed in these neuroblastomas are not surprising, in view of the large functional and evolutional differences displayed by human neuroblastomas, as reflected in the different patterns of adrenergic substances and metabolites excreted in the urine.

The observations presented in this paper provide evidence that in neuroblastoma NA is stored in a particle just as it is in adrenergic nerves. The particle contains DβH and its sedimentation and density characteristics are very similar to those of bovine splenic nerve particles[3]. Comparison of the NA/DβH ratios in neuroblastoma with the same in splenic nerve[3] (0.38 ± 0.09) suggests that the NA content is not lower in human neuroblastoma than in splenic nerve. Accordingly, assuming that the activity of DβH is similar in both cases, it can be concluded that the amount of NA per particle is at least as high in neuroblastoma as in bovine splenic nerve. These results confirm and extend those of HÖRTNAGL et al.[2] and permit us to conclude that there is no reason to believe that the continuous release of amines, precursors and metabolites in human neuroblastoma is due to a NA storage defect.

In sympathetically innervated tissues, 2 types of storage particles have been found. The more dense of these particles – at least for the dog spleen[4] – contain DβH, the others contain only very little if any DβH activity. From the results of the present gradient centrifugation experiments it would appear that there is at present no evidence for the occurrence of 2 types of storage particles in human neuroblastoma. It is obvious that if the less dense particle is indeed lacking, this difference between adrenergically innervated tissue and human neuroblastoma may be an important one.

Another point which is worth mentioning is the occurrence, in some cases, of DβH in lower density regions, where no NA has been found. If the occurrence of DβH in low density regions is real and not the result of an artefact, we are dealing with a similar situation as has been found in stellate ganglia[5]. Whether the presence of membranous DβH is the result of immature vesicles or produced by exocytosis[6] which may be coupled with an increased threshold value for its removal is difficult to decide at present but the presence of these structures could play a role in determining the quantity and nature of the products which are released.

Enzyme activities and noradrenaline content of human neuroblastomas (n = 18)

Constituent	Neuroblastoma
Tyrosine hydroxylase	0–0.46
Dopa decarboxylase	0–6.34
Dopamine-β-hydroxylase	366–38,365
Monoamine oxidase	0.05–5.23
Noradrenaline	4.2–190

The concentration of each constituent is expressed per g wet weight of original tissue; NA in nmoles; enzyme activities in enzyme units.

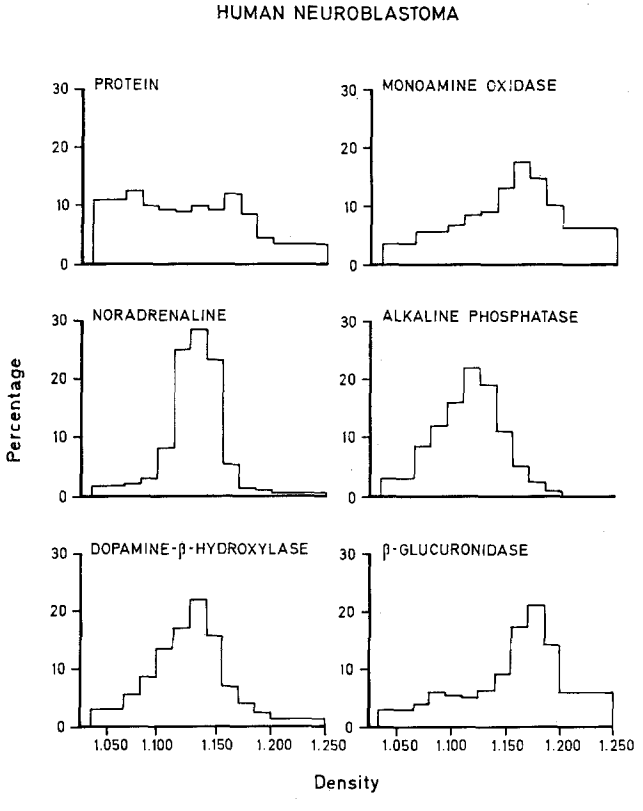


Fig. 2. Sucrose density gradient centrifugation of particles from human neuroblastoma. A particulate fraction equivalent to fractions 2 to 5 (see methods) was suspended in 0.25 M sucrose and centrifuged on a linear gradient (0.3–1.7 M sucrose). For normalization methods see BOWERS and DE DUVE[7].

Résumé. Dans 18 cas de neuroblastome humain la noradrénaline (NA) et la dopamine-β-hydroxylase présentent des profils de distribution intracellulaire identiques, suggérant un stockage dans la même particule. Le contenu en NA de ces particules n'est pas inférieur à celui des particules du nerf splénique; on peut en conclure qu'un stockage défectueux de NA dans les cas de neuroblastome est peu probable.

W. P. DE POTTER, A. F. DE SCHAEPDRYVER, F. DE SMET, M. J. DELBEKE and C. HOOFT[8,9]

Heymans Institute of Pharmacology and Paediatric Clinic, University of Ghent Medical School, De Pintelaan 135, B–9000 Gent (Belgium), 13 May 1974.

[4] I. W. CHUBB, W. P. DE POTTER and A. F. DE SCHAEPDRYVER, Nature, Lond. *228*, 1203 (1970).

[5] I. W. CHUBB, W. P. DE POTTER and A. F. DE SCHAEPDRYVER, Life Sci. *11*, 323 (1972).

[6] W. P. DE POTTER, I. W. CHUBB and A. F. DE SCHAEPDRYVER, Archs int. Pharmacodyn. *196, Suppl.*, 258 (1972).

[7] W. BOWERS and C. DE DUVE, J. Cell Biol. *32*, 339 (1967).

[8] Authors' addresses: W. P. D. P., A. F. D. S. and F. D. S.: J. F. and C. Heymans Institute of Pharmacology, M. J. D. and C. H.: Paediatric Clinic, University of Ghent Medical School, De Pintelaan 135, B–9000 Ghent, Belgium.

[9] The authors are grateful to Prof. F. DEROM (Surgical Clinic, University of Ghent Medical School), Prof. R. EECKELS and Prof. E. EGGERMONT (Paediatric Clinic, University of Leuven Medical School), Prof. A. LACQUET and Prof. J. GRUWEZ (Surgical Clinic, University of Leuven Medical School), Dr. G. DELALIEUX, Dr. F. DELANGE, Dr. P. DE MOL, Dr. R. DENIS, Dr. R. MAURUS and Dr. J. OTTEN (Paediatric Clinic, University of Brussels Medical School) and Dr. W. MATTHEIEM (Surgical Clinic, Jules Bordet Institute, Brussels) for making the tumors available to them.