observed chromosomal aberrations with slight variations in mitotic index or cellular enlargement. Nevertheless, according to the designated parameters of this investigation, radio waves at 27.120 MHz and 10 W of power (ERP) apparently cause an increased incidence of chromosomal breaks compared to controls without significantly altering cellular response in culture.[20]

Zusammenfassung. Hypotonische Streuungen, die in radiobestrahlten Kulturen menschlicher Lymphozyten Chromosomenaberrationen erkennen liessen, traten dort siebenmal häufiger auf als in unbestrahlten Kulturen; offenbar das Ergebnis einer wärmefreien Bestrahlung. Trotz des Fehlens bedeutender Unterschiede in der DNA-Synthese, der Zellvergrösserung und im mitotischen Index zwischen bestrahlten und unbestrahlten Kulturen kam es zu Aberrationen.

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Highly Purified Noradrenaline Storage Vesicles from Bovine Splenic Nerve Trunk: Preliminary Electron Microscopy

An improved method for the purification of noradrenaline (NA) storage vesicles from bovine splenic nerve trunk was recently described[1]. Sucrose-heavy water density gradients are used to take advantage of a differential increase in NA vesicle density relative to that of microsomal contaminants. The average concentration of sedimentable NA in the purest gradient fraction, representing $^1/_3$ of the total sedimentable NA in the original nerve trunk, is about 3.5 µg/mg protein. This is a 4–7 fold improvement in purification over values previously reported in the literature[2–6].

From quantitative analyses of marker enzymes to estimate contamination by other subcellular components relative to NA content, it could be calculated that in the purest fraction a minimum of 20% of the total sedimentable protein is associated with the NA storage vesicles. This improvement in vesicle purity enhanced the chances for meaningful electron microscopic examination of the fraction.

Materials and methods. Fraction FIII of purified NA storage vesicles from bovine splenic nerve trunk was prepared as previously described[1]. Aliquots were diluted to isotonicity in 0.25 *M* sucrose containing 20 m*M* potassium phosphate buffer at pH 7.2–7.4. Samples were incubated for 15 min at 37 °C in a medium containing 0.5 µg/ml L-NA, 3 m*M* $MgCl_2$ and 3 m*M* *tris*$_4$-ATP (adenosine triphosphate). This medium is known to prevent loss of NA from isolated vesicles and also to promote uptake of NA into partially depleted vesicles[7]. The incubated samples were chilled immediately to 0–4 °C and layered over an equal volume of 6% glutaraldehyde containing 0.154 *M* potassium phosphate buffer at pH 7.2 to 7.4. The suspension was then centrifuged at 226,000 g_{max}-30 min in a refrigerated Beckman Ultracentrifuge L2-65B. Thus, the vesicles are fixed in suspension while being sedimented into a pellet. The pellets were post-fixed for 90 min in 2% OsO_4 containing the same buffer. Grey to silver-grey sections were cut through the entire pellet from top to bottom, both vertically and parallel to the surface, with a diamond knife on an LKB Ultrotome. The pellets were thin enough to allow the entire depth to be examined in a single section. Sections were stained for 15 min in 4% uranyl acetate and 5–10 sec in lead citrate. Micrographs were taken with a Zeiss EM9A.

Results and discussion. When the vesicle fraction is fixed with glutaraldehyde (or OsO_4) in suspension during centrifugation, the result is superior to fixation of the pellet after sedimentation, particularly in terms of vesicle distribution in the pellet. In addition, incubation of the vesicle suspension with Mg^{++} and ATP before fixation yields a more homogeneous appearing population of vesicles.

A layer of essentially pure vesicles accounting for about 25% of the total pellet depth occurs at the upper surface

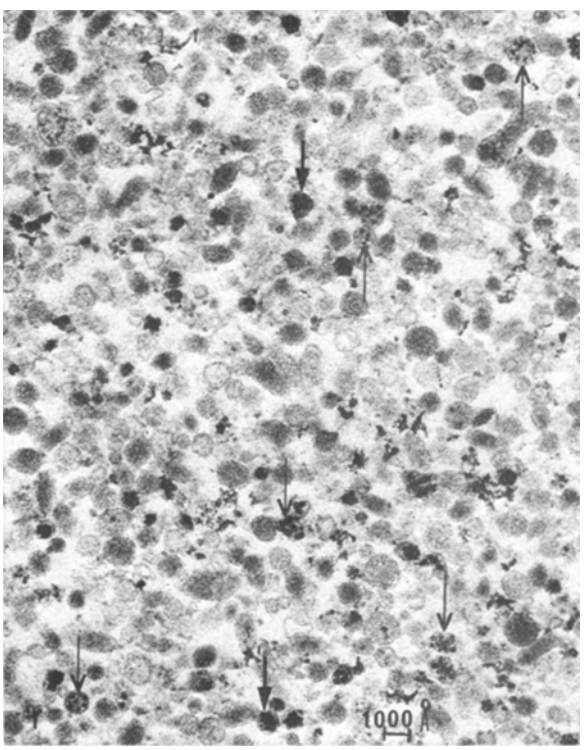

Fig. 1. Purified NA storage vesicles from fraction FIII of sucrose-heavy water density gradient. Thin arrows indicate 200 Å intravesicular granules; thick arrows indicate clusters of these granules giving the appearance of a dense 'core'. × 35,000.

of the pellet (Figure 1). The average diameter of the vesicles is between 750 and 800 Å. A higher magnification of the vesicles is shown in Figure 2. It demonstrates their single unit membranes about 70 Å in width (arrowheads) and the fine granular matrix in most of the vesicles.

The pure upper layer merges into a less pure zone amounting to about 70% of the pellet depth. It contains large numbers of vesicles mixed with a few small mitochondria and other membrane profiles. Only in the bottom zone amounting to 5–10% of the pellet are the vesicles relatively sparsely distributed compared with the contaminants. Of the vesicles present here, many have a larger range in diameter, 1000–2000 Å. The bottom zone usually appears compressed and is composed of amorphous material scattered between mitochondria, of which many are empty or broken. Also numerous large empty membrane profiles are present and a few Golgi membranes may be identified.

Various sized dense osmiophilic granules can be seen within some of the vesicle matrices and in areas between the vesicles. The most prominent dark granules are about 200 Å in diameter (Figure 1, fine arrows). Smaller granules of 30–60 Å diameter are also common in many vesicles. The 200 Å granules often cluster together and give the appearance of a single large black granule or 'core' (Figures 1 and 2, thick arrows). Identical dark granules occur free, both singly and in various sized clusters. In general, the clusters are distributed according to size, the larger occur nearer to the bottom of the pellet. The free granules are believed to originate from ruptured vesicles. It is not uncommon to find discontinuities in vesicle membranes and their granular content emerging. We interpret the dark granules to represent polymerized and/or contracted vesicle matrix material, which probably results from loss of membrane integrity leading to degenerative changes in the vesicle and to the strong osmiophilic reaction. The electron density of the granules is not dependent on uranyl acetate or lead citrate staining, as it is obvious also in unstained material.

On the basis of electron microscopic examination, we feel that the original estimate of about 20% purity based on biochemical data is probably too low and conservatively could be doubled. A more comprehensive study of vesicle appearance after various treatments is in progress.

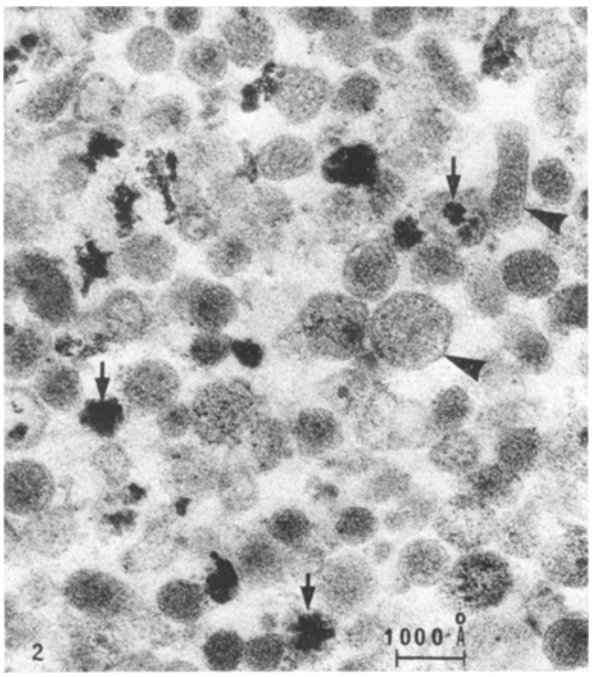

Fig. 2. Same as in Figure 1. Arrowheads indicate 70 Å unit membranes of the NA storage vesicles. Thick arrows indicate clusters of 200 Å granules giving appearance of a dense 'core'. × 100,000.

Résumé. Les vésicules de la NA du nerf splénique de Bœuf ont été obtenues par gradient de centrifugation («sucrose-heavy water»). Le rapport NA/protéine observé est de 4 à 7 fois plus élevé que celui qui a été mentionné précédemment. L'examen préliminaire de cette fraction, par microscopie électronique, révèle une couche importante et pratiquement pure de vésicules à la surface du sédiment.

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Heterogeneity in the Fibre Composition in the Flight Muscles of *Periplaneta americana* and *Belostoma* sp.[1]

The structural and functional significance of heterogeneity in the fibre composition of vertebrate muscles is well known[2]. Heterogeneity in fibre composition in insects was first observed by Bhat[3] in the flight muscles of the dragonfly *Pantala flavescens*. Recently Kallapur[4] has also reported the prescence of some specilized large fibres in the leg muscles of 2 species of cockroaches, *Blatella germanica* and *Periplaneta australasiae*. He further reported that neither the leg nor the flight muscles of *Periplaneta americana*, *Cybister confusus*, *Ranatra elongata*, *Atractomorpha crenulata* and *Cyrtacanthacris ranacea* showed such specilized fibres. This communication