resuspended by gentle hand homogenization in the sucrose solution and layered on top of a 1.4/1.2/0.8 *M* discontinuous sucrose gradient and centrifuged for 1 h at 80,000 *g*. The layer between 1.2 and 1.4 *M* sucrose contained the bulk of the material which was a virtually pure glomerular fraction (Figure 1). Cells and nuclei were found in the pellet and finely granular material seen in the 1.2/0.8 *M* interphase. The final glomerular fraction was diluted with 0.32 *M* sucrose and pelleted as described above.

The ultrastructural examination was made after fixation of either the suspensions or the pellets with Karnovsky's fixative, followed by dehydration in ethanol, embedding and sectioning as previously described[10].

E.M. examination showed that approximately 70% of the 'crude glomerular fraction' consisted of glomerular profiles. The glomeruli were well preserved, i.e. the central mossy fibre rosette contained neurotubuli, filaments, endoplasmic reticulum, numerous mitochondria and clusters of synaptic vesicles. They were surrounded by a sheath of pocesses from granule cells and Golgi cells. Delicate lamellar astrocytic pocesses outlined some of the glomeruli, Most of the other material in the crude glomerular fraction consisted of small cells, mostly granule cells and astrocytes, cell nuclei, with or without a rim of cytoplasm, and blood capillaries. The final glomerular fraction consisted of well-preserved glomeruli as described above (Figures 2 and 3). Occasionally, scattered debris could be observed. The purity of the fraction was determined from electron micrographs, from which the structures were cut out and weighed. The percentage of the fraction which consisted of glomeruli was estimated as 93.6 $\pm$ 4.0 (S.D.) from 5 different preparations.

The average yield of glomerular protein from a purified fraction of rabbit cerebellum (wet weight ~1.1 g) was 0.1 mg as determined with the method of LOWRY et al.[11]. Between 800,000 and 900,000 glomeruli per cerebellum were isolated which gives a protein content of 12×10^{-11} g per glomerulus.

The present method offers the possibility to isolate a highly purified fraction of morphologically well preserved glomerular complexes from rabbit cerebellum. The fraction has particular advantages for biochemical and neuropharmacological studies, as glomeruli are of known origin and function[12]. However, the complex composition of the glomerulus, containing several pre- and postsynaptic compounds, as well as glial cell processes makes them rather unique as synaptosomes, but may also limit the usefulness of this fraction for functional studies, until further subfractionation methods have been developed. Since the disruption of the tissue is carried out without homogenization, the possibility to preserve larger intact particles is improved. Previous procedures for cerebellar tissue subfractionation employed homogenization of the fresh tissue. ISRAEL and WHITTAKER[13] isolated the central core of the glomerular complex, i.e. the mossy fibre endings, by sucrose gradient separation of a crude nuclear fraction. HAJOES et al.[14] and TAPIA et al.[15] have described in a careful study of subcellular fractionation of rat cerebellum homogenates, the isolation of a fraction which consisted of glomerular complexes to a purity of at least 50%. The higher purity of the fraction obtained by our method is partly outweighed by the more time-consuming isolation procedure. However, a preliminary biochemical characterization showed good retention of lactic dehydrogenase and active accumulation of amino acids by the isolated glomeruli which indicates their usefulness for functional studies[16].

Zusammenfassung. Es wird eine neue Methode zur Präparation der sogenannten «glomerulären Komplexe» aus Kaninchen-Kleinhirn beschrieben, deren komplexe innere Struktur weitgehend intakt bleibt. Mit Hilfe der quantitativen elektronenmikroskopischen Methode wurden über 90% intakter Glomeruli errechnet.

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Acid Phosphatase in Golgi Vesicles of Osteoblasts

The collagen producing cells have been studied in detail by various workers[1-3]. These cells contain very large ribosomal complexes[4] related to the assembly of long α-chains of protocollagen. The latter have been shown to be present in the cisternae of the endoplasmic reticulum (ER) and it has been shown that this material is then transferred to the Golgi complex[2,4-6]. In addition to collagen these cells also synthesize and secrete other components of the matrix and Golgi complex of odontoblasts has been shown to be involved in the synthesis and secretion of glycosaminoglycans[7]. The Golgi complex in osteoblasts is quite large and consists of many flat saccules with distended ends (Figure 1). As shown previously[3,4] a substructure can be made out in these distentions. Most often it is in the form of randomly coiled filaments (*). In the Golgi region, there are also present many oblong or elongated vesicular structures. The filaments present in these vesicles are so aligned that longitudinal striations can be clearly made out (→). These filaments do not show any banding pattern.

For the present study in addition to routine preparations described above, some of the material (calvaria of 15-day-old chick embryos, White Leghorn) was incubated for acid phosphatase[8] after fixation (30 min) in 1% glutaraldehyde in 0.1 *M* cacodylate, pH 7.4 and prepared for electron microscopy. The material incubated for the shortest period (20 min) was found to be most useful as the reaction product did not obscure the morphological

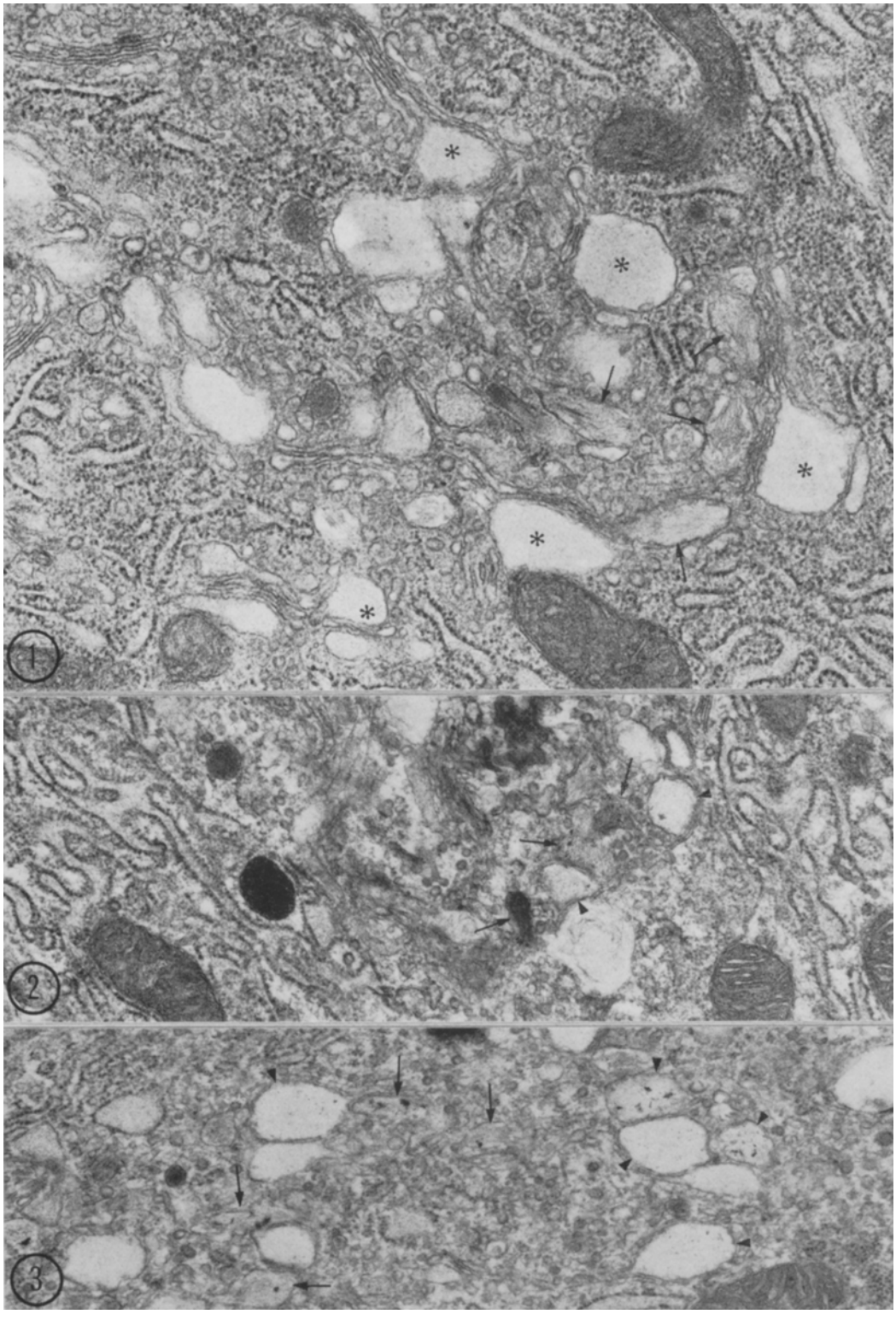

Fig. 1. Golgi region of an osteoblast shows many units. The distended ends of saccules (*) show a faintly visible randomly coiled, filamentous material. The elongated vesicles (arrows) show striations running in the direction of their long axes. An assortment of coated and non-coated vesicles are also seen. ×38,000.

Fig. 2. Same as in Figure 1 but from material incubated for acid phosphatase. A typical lysosome showing finely granular reaction product is seen. The Golgi saccules are cut tangentially and small amounts of reaction product are present in distended ends of its saccules (arrow heads) as well as in vesicles showing filamentous contents (arrows). ×19,000.

Fig. 3. Same as Figure 2. The reaction product is seen in distended ends of the Golgi saccules (arrow heads) as well as in vesicles showing filamentous material (arrows). ×30,000.

details. In addition to typical lysosomes, the reaction product was observed in the distended ends of the Golgi saccules (Figures 2 and 3, arrow heads). Also it was found in the elongated vesicles which showed well aligned filaments (Figures 2–4, arrows).

The collagen synthesis occurs by sequential steps[9]. The α-chains of protocollagen undergo hydroxylation of certain proline and lysine residues in the cisternae of the ER[6]. From thence they are transferred to the Golgi complex[2–6]. Before this material is secreted, a substitution of some of the hydroxylysine residues takes place with galactose or glucosylgalactose[9]. It has been shown that carbohydrate material in association with filamentous material containing labelled proline, occurs for the first time in these Golgi vesicles[3]. On this basis it has been suggested that the substitution occurs in the Golgi complex. It has also been shown that the synthesis of carbohydrate components of the glycosaminoglycans, a part of the extracellular matrix, occurs in the Golgi complex, where it is combined with the proteinous components and then secreted[7]. The acid phosphatase, seen presently, occurs in vesicles containing filamentous material and is present concomitant with the alignments of the filaments. The function of the hydrolytic enzyme is not at all apparent. It may be related to the substitution, the synthesis of carbohydrate components or even to the combination of the carbohydrate components with the proteinous material, mentioned above. It may even have something to do with the alignment of the filamentous material.

Acid phosphatase is generally used as a marker for other hydrolytic enzymes[10]. The procollagen has an amino-terminal extention which is cleaved off before it can polymerize into native collagen fibrils. The cleavage has been shown to be an enzymatic process and involves an aminopeptidase[9,11]. On the basis of lack of conversion of procollagen into tropocollagen in matrix free fibroblast suspensions, it has been suggested that the cleavage occurs extracellularly. If however, the presence of acid phosphatase in the Golgi vesicles concomitant with the alignment of the filaments is suggestive of the presence of an aminopeptidase, then it seems reasonable to suggest that the cleavage is initiated in the Golgi vesicles. It may be pointed out that aminopeptidase has to be of lysosomal origin and these organelles are produced in the Golgi complex[12].

Resumen. El complejo de Golgi de los osteablostos presenta en la proximidad de los extremos distendidos de los sáculos filamentos enrollados. En las vesículas elongadas del complejo de Golgi se ven filamentos ordenados en paralelo. El producto de reacción de la fosfatasa ácida ha sido observado en los extremos distendidos asi como en las vesículas elongadas. El significado funcional de esta enzima no es claro. Puede estar relacionada con la síntesis y secreción del colágeno o de otros componentes de la matriz extracelular.

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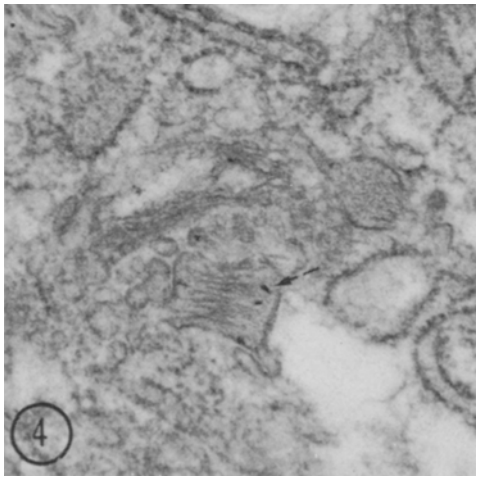

Fig. 4. At higher magnification reaction product is seen together with discrete filaments in the elongated vesicles. × 51,790.

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Ultrastructure of Distant Paired Homologues in Oocyte of *Tisbe holothuriae* (Copepoda)[1]

The distant parallel pairing of homologous chromosomes at late prophase and metaphase-I, regularly present in oocytes of harpacticoid copepods, has received attention due to its possible significance in relation to sex-determination and formation of chiasmata (Matschek[2], Heberer[3,4], Ar-Rushdi[5], Colombera and Lazzaretto-Colombera[6]). However, nothing is known nor has there been speculation concerning the mechanism responsible for maintaining the pairing of the homologous chromosomes in copepod oocyte at metaphase-I.

On the basis of Östergren's theory[7], it seems necessary to postulate the presence of some cohesive forces between distant parallel homologues, since these chromosomes could not otherwise attain and then keep a metaphase configuration. Since no structure which could account for the presence of such cohesive forces have been individuated