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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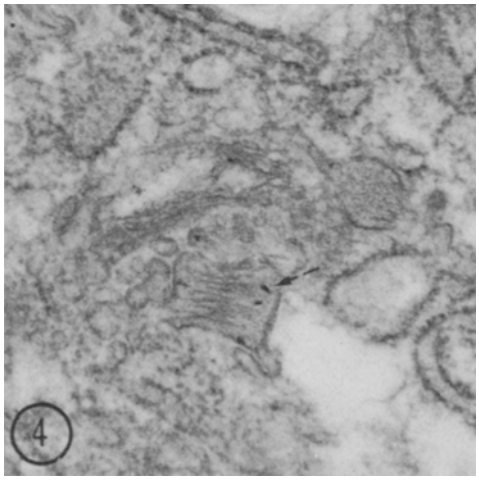

Fig. 4. At higher magnification reaction product is seen together with discrete filaments in the elongated vesicles. × 51,790.

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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

with the techniques of light microscopy, we investigate the possible occurrence of such hypothetical synaptic structure beyond the limit of the light microscope.

The specimens of *Tisbe holothuriae* examined were the offspring of several ovigerous females collected in the Lagoon of Venice near Chioggia. Employing the usual methods (BATTAGLIA[8]), populations of *Tisbe holothuriae* have been reared in our laboratory. 10 ripe virgin females supplied material for squash preparations of chromosomes, as described by COLOMBERA and LAZZARETTO-COLOMBERA[6] in 1972. Observations and photos were made with the aid of a Zeiss phase-contrast microscope. In addition electron microscope (EM) preparations were made as follows: 10 ripe virgin females were fixed in 1.5% glutaraldehyde in sea-water pH 7.6 at 0 °C for 2 h and post-fixed in 1% OsO_4. The material was then washed in sea-water, dehydrated with ethanol and embedded in Dew Epoxy Resin (LOCKWOOD[9]). This sections were counterstained with lead citrate (REYNOLDS[10]).

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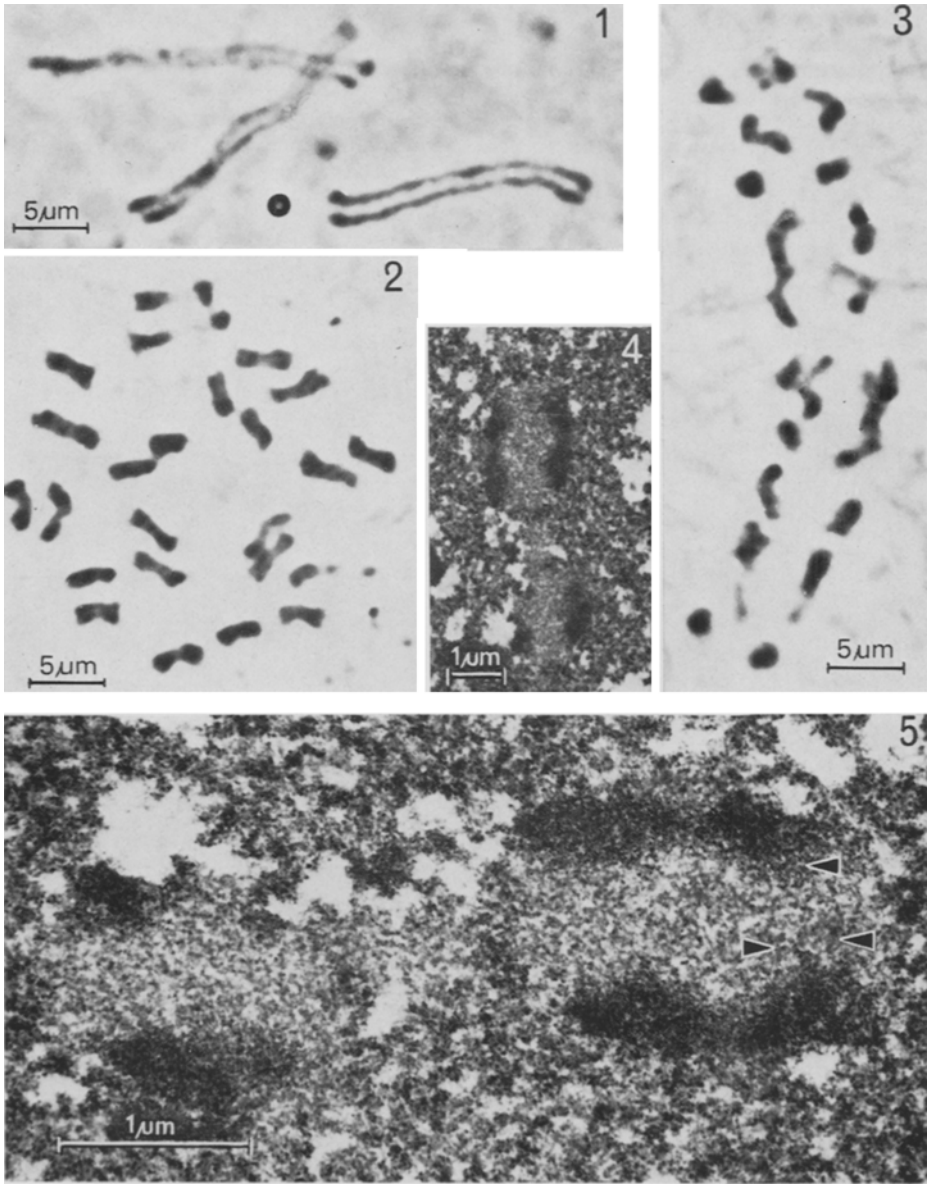

Fig. 1. Late prophase-I Chromosomes. Homologues are distant parallel paired.
Fig. 2. Parallel paired homologues at metaphase-I. from polar view.
Fig. 3. Distant paired homologues at metaphase-I from lateral view. The chromosomes are all V-shaped.
Fig. 4. By E. M. a differentiated zone is clearly visible between the pairs of homologues. Lateral view of two couples of chromosomes.
Fig. 5. Lateral view of chromosomes at metaphase-I at higher magnification. Some chromosomal extrusions are probably present (see arrows).

Results and discussion. At late prophase-I, the homologous chromosomes are already distant parallel paired (Figure 1). The chromosomes appear as long single threads of slightly irregular cross section. The ends are differentiated from the rest of the chromosomal body by virtue of their thicker roundish form and dark aspect. The distances between homologues are about 0.5 µm and are minimal at the chromosomal ends which often bend toward each other; there is occasional contact between chromosomes by means of lateral extrusions. These chromosomes later become fully separated before they reach the equatorial plane of the spindle. In polar view (Figure 2), the metaphase-I chromosomes appear as double rod-shaped bodies with darker roundish extremities and a thinner paler medial zone. Some chromosomes are slightly bent in the medial zone, so that they exibit a V-shaped outline. There is an optical void between homologues so that they are distantly paired. In lateral view (Figure 3), the chromosomes at metaphase-I are clearly separated into two homologous groups, one 'below', the other 'above' the equator of the spindle. The average distance between two homologues is about 2–2.5 µm. Again some chromosomes appear V-shaped and there is no indication of any structure between homologues in all the 25 metaphase-I plates examined. In lateral view, metaphase-I chromosomes (Figures 4 and 5) observed by EM appear as 2 roundish masses connected by a thinner medial zone. In some cases, extrusions of chromosomal bodies seem to occur from the distal part of homologues (Figure 5). Oocyte nuclei of *T. holothuriae* at metaphase-I are characteristic for the presence, between and only between the homologues, of a zone (Figures 4 and 5) about 0.6 µm wide, well differentiated from the other nuclear component because it appears to be homogeneously packed by convoluted and randomly arranged fibrils; it shows a lower affinity for stain and it does not undergo losses of material during the fixation of the material.

With this research we have confirmed once more the distant parallel pairing of homologues at metaphase-I in the genus *Tisbe* (COLOMBERA and LAZZARETTO-COLOMBERA[6]; LAZZARETTO-COLOMBERA[11]) and in other copepods (MATSCHECK[2]; HEBERER[3,4]; AR-RUSHDI[5]).

DARLINGTON[12] and BAUER[13] held the view that association of meiotic homologues in Diptera, usually distantly paired, is due to the forces of somatic pairing. Unfortunately this hypothesis is not explanatory since the phenomenon of somatic pairing appears still to be an open question (COLOMBERA[14]). COOPER[15] suggests that the members of a bivalent are hold together at metaphase-I by residual chromosomes extrusions or spindle elements, despite the optical void present between homologues.

In our opinion, the peculiar ultrastructure individuated between the 2 partners of a metaphase-I bivalents in oocyte of *Tisbe holothuriae*, so far ignored for other species, is strongly suspect of acting as 'synaptic' body between homologues, as far as Copepods are concerned.

Riassunto. Abbiamo esaminato l'appaiamento a distanza dei 12 cromosomi omologhi nella tarda profase e metafase ovocitaria in *Tisbe holothuriae* evidenziando una ultrastruttura non prima descritta in letteratura.

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Induction of Skeletal Malformations in Organ Cultures of Mouse Limb Buds

The determination of the effects of teratogenic substances is complicated by the existence of three compartments in the mother animal: a) the mother, b) the placenta, and c) the embryo. They possess different distribution spaces, different pharmaco-kinetic conditions, and are separated by membrane barriers. For this reason, tissue cultures, and in particular organ cultures with a high degree of differentiation, can be extremely useful for determining teratogenic effects and modes of action as well as dosage-effect relationships. In vitro techniques also make it possible to work with human material[1]. Until now, however, only ADELOTTE and KOCHHAR[2]; KOCHHAR[3,4] and NEUBERT et al.[5] succeeded in producing in vitro malformations comparable to typical skeletal malformations.

In our investigations, using this technique, we were able, after treatment with actinomycin-D, to produce in vitro skeletal malformation which could be described as phocomelias or dysmelias. This substance does produce malformations when administered to test animals early in pregnancy[6–10]. After the beginning of placenta functioning, however, it cannot pass the placental barrier[11].

Material and methods. On day 11, the upper limb buds of NMRI-mice embryos were removed and put into a modified Trowell-culture[12]. The limb buds were divided

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