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.

D. COLOMBERA, IVANA LAZZARETTO-COLOMBERA and LAURA ONGARO[16,17]

Istituto di Biologia Animale dell'Università, Via Loredan 10, I-35100 Padova (Italy), 1 August 1974.

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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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into 4 groups: Group 1 served as control. Group 2 was treated with 0.01 µg actinomycin-D/ml culture medium on day 1 in vitro. Group 3 and 4 was treated the same as group 2 except that they received the actinomycin-D on days 1+2 (group 3) resp. 1.2+3 (Group 4). The limb buds were harvested on cultivation day 6, stained with methylene blue, and cleared with Xylene.

Results. In vitro and without treatment (group 1), the cartilaginous anlagen of the scapula and the humerus developed on day 2, the radius and the ulna anlagen on day 3, and the hand skeleton on day 4. Of course, this cartilage skeleton in vitro did not grow undisturbed. By surface anchoring[5] compression and bending did occur. Since, however, the basic forms, relative size and localization of the cartilage remained intact, identification of individual components was easy.

Treatment with actinomycin-D within the first 24 h (group 2) already lead to a change in the cartilage-skeleton observable after 6 days in culture. The anlagen of the scapula and of the larger part of the humerus were missing in all cases. Only a small distal section of the humerus near the elbow was often still present. Both lower arm bones were often shortened. The hand skeleton was, however, always enlarged. This was due to an increased growth of the metacarpalia which, in 75% of cases, were twice as long as the metacarpalia of the control group. After treatment on days 1 and 2 (group 3) and even on days 1,2 and 3 (group 4), conditions were the same.

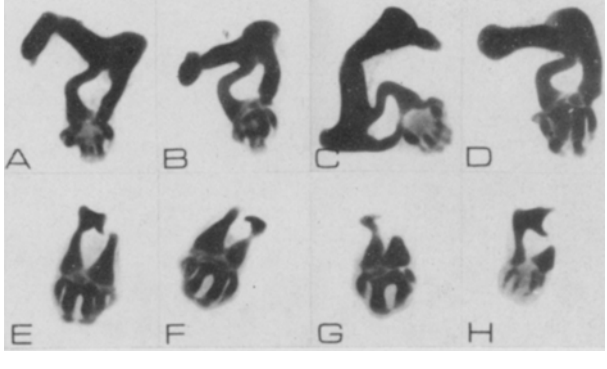
Discussion. From these results, we conclude that actinomycin-D prevents the formation of scapula and humerus cartilage within the first 24 h. The development of cartilage depends on the synthesis of special collagens and proteoglycans[13–15]. With the methods we used (methyleneblue-clearing), we determine mainly these proteoglycans, which must, however, be stabilized by the collagen in the intercellular space[16]. Actinomycin-D inhibits the synthesis of DNA-dependent RNA[17,18]. Therefore we must assume that RNA necessary for cartilage differentiation of scapula and humerus is formed during the first day in vitro. The conditions in the area of the cartilage of the lower arm seem to be less critical. Although this cartilage is visible during day 3 in untreated cultures, it still formed even after a 3-day treatment by a recover mechanism.

It must be assumed that, apart from differentiation of cells already present in the proximal area of the limb in the distal hand area, proliferation takes place in vitro also[19]. It is however theoretically also possible that all cells necessary for the formation of the hand skeleton are already present. Since, however, the metacarpalia after treatment with actinomycin-D are approximately doubled in length, proliferation despite actinomycin-D must be postulated, given a constant cell intercellular space ration in the cartilages. Hand cartilage was present however in our experiments on day 4. The proliferation time after a 3-day treatment would therefore be a bit too short. Another possible explanation would be a difference in sensitivity to actinomycin-D between different cell types[20–23]. This difference is at least partially due to the varying lifetime of RNA. It can be assumed that such differences do exist between differentiating cells in the proximal area and proliferating cells in the distal area. The phenomenon of increased growth of the metacarpalia could also be explained by this behaviour. While actinomycin represses differentiation, proliferation continues for a longer period. Here, however, the function of the apical ridge must be considered. This epithelial layer is supposed to stimulate the proliferation at the tip of the limb bud[19]. Nothing, however, is known about the effect of actinomycin-D on this process.

Zusammenfassung. In einer Organkultur von Extremitätenknospen (Mäuse-Embryonen Tag 11) gelingt es bereits nach Gaben von 0,01 µg/ml Actinomycin-D in den ersten 24 h die Bildung der Knorpelanlagen von Scapula und Humerus zu unterdrücken, also Dysmelien oder Phokomelien in vitro zu erzeugen. Dieses Modell scheint zur in vitro-Testung von teratogenen Substanzen geeignet zu sein.

B. ZIMMERMANN, D. NEUBERT, D. BACHMANN and H.-J. MERKER

Anatomisches Institut der Freien Universität Berlin, Königin-Luise-Strasse 15, D-1 Berlin 33 (German Federal Republic, BRD), 28 August 1974.



A–D) Mouse upper limb buds (day 11) after 6 days in vitro. Stained with Methylene-blue and cleared in Xylene, untreated.

E–H) As A), but treated on day 1 (E), days 1 and 2 (F) or days 1–3 (G) in vitro with actinomycin-D. Scapula and the proximal part of the humerus is missing. The Metacarpalia are lengthened (except H).

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