

Fig. 1. Sepharose 6B gel filtration of LAP and CAP in non-pregnancy serum (N), pregnancy serum (P), lysosomal extract (L), microsomal extract (M) and fetal serum (F). Column:  $2.5 \times 100$  cm; eluant:  $0.1 M$  sodium phosphate buffer (pH 7.0); fraction volume: 4 ml. For the sake of reference, the electrophoretic pattern of LAP in each sample<sup>6</sup> is presented to the left side of the elution diagram. Disc electrophoresis of pregnancy serum (P) exhibits three bands with LAP activity. In the present electrophoretic experiment of 2 separated LAP peaks of pregnancy serum, Peak I showed CAP<sub>1</sub> and CAP<sub>2</sub> bands; Peak II LAP band.

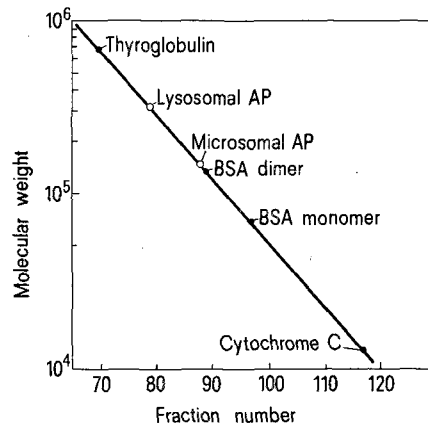


Fig. 2. Molecular weight estimation of AP by gel filtration. The molecular weight was calculated by plotting the fraction numbers of marker proteins against the logarithmic values of their molecular weights. The markers used were cytochrome C (mol. wt. 12,400), bovine serum albumin (BSA, mol. wt. 67,000) and thyroglobulin (mol. wt. 680,000). Cytochrome C was measured by reading the absorbance at 410 nm; BSA and thyroglobulin at 280 nm.

*Zusammenfassung.* Mit Hilfe von Gelfiltration wurden 2 verschiedene Molekularformen von Aminopeptidase (AP)-Isozymen in der menschlichen Placenta charakterisiert: die Lysosomen-AP (Molekulargewicht ca. 320,000) und die Mikrosomen-AP (Molekulargewicht ca. 145,000).

M. OYA, M. YOSHINO<sup>11</sup> and  
S. MIZUTANI<sup>12</sup>

*Department of Legal Medicine,  
Nagoya City University School of Medicine,  
Mizuho-ku Kawasumi, Nagoya (Japan), 11 April 1975.*

<sup>11</sup> Department of Biochemistry, Yokohama City University School of Medicine, Yokohama (Japan).

<sup>12</sup> Department of Obstetrics and Gynecology, Shizuoka Saiseikai Hospital, Shizuoka (Japan).

## Chromatin-Free Synaptonemal Complex in Whole Mount Preparations

The synaptonemal complex in meiosis has been largely studied in thin section and recently in whole mount preparations. In general the aspects and dimensions of the tripartite structure are very regular in the eukaryote species investigated (see review of WESTERGAARD and VON WETTSTEIN<sup>1</sup>).

In mammals, the reconstitution of the synaptonemal complex (SC) from serial sections of the mouse has been elegantly shown by SOLARI<sup>2</sup>. COMINGS and OKADA<sup>3</sup> described the SC in whole mount preparations of mice. By enzymatic treatment they demonstrated the proteic nature of this structure.

Recently COUNCE and MEYER<sup>4</sup> in testing several saline hypophases as spreading media obtained chromatin-free SC, in *Locusta migratoria*. The authors described the lateral elements, their terminal attachment points to the nuclear membrane and the morphological evolution of the kinetochore during meiotic prophase.

MOSES et al.<sup>5</sup>, applying the same technique, described in human chromosome preparations the SC, the kinetochore, the attachment points to the nuclear membrane

and the XY sex bivalent, paired by the short arm of the X.

In this paper we describe aspects of the chromatin-free SC in the male Swiss albino mouse, obtained by the water spreading technique. These aspects are similar to those obtained by COUNCE and MEYER<sup>4</sup> with the use of diluted saline solutions as spreading medium.

Dissected seminiferous tubules are immediately immersed in Ringer solution (0.9%). Fragments of about 2 mm are rapidly dipped in 10 successive baths of Ringer for 1 min each. The fragments are then mechanically pulverized on slides which are then dipped in distilled water pH 6.4, in a plastic tray with Teflon bars.

<sup>1</sup> M. WESTERGAARD and D. VON WETTSTEIN, *A. Rev. Genet.* 6, 71 (1972).

<sup>2</sup> A. J. SOLARI, *Chromosoma* 34, 99 (1971).

<sup>3</sup> D. E. COMINGS and T. A. OKADA, *Chromosoma* 30, 269 (1970).

<sup>4</sup> S. J. COUNCE and G. F. MEYER, *Chromosoma* 44, 231 (1973).

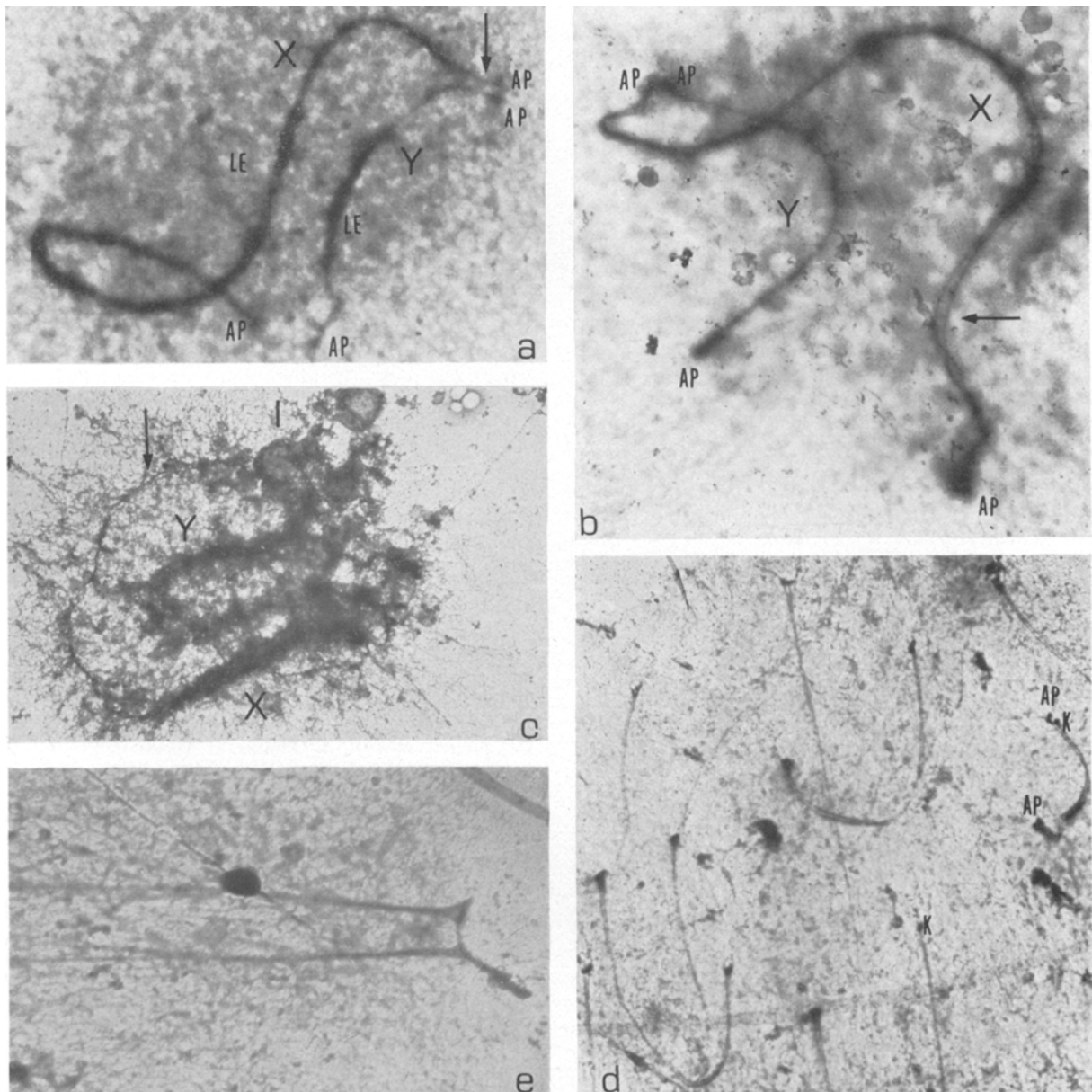
<sup>5</sup> M. J. MOSES, S. J. COUNCE and D. F. PAULSON, *Science* 187, 363 (1975).

The spreading material is collected by gently laying 1% collodium covered grids on the water surface. In general, 6 grids were prepared with an interval of about 2-3 min between the first and the last. The grids were quickly air-dried, stained in 2% uranyl acetate for 10 min and again air-dried. Some grids were dehydrated in the alcohol series, before staining. The grids were examined and photographed in a Siemens Elmiskop I at 60 Kw.

In typical preparations the SC were preserved and always denuded of chromatin. The entire length of the lateral elements of the autosomes and XY pair could be traced. The lateral element terminals at the points of attachment to the nuclear membrane are larger and more dense. Frequently nuclear membrane fragments are associated with the lateral element terminals. The

kinetochore was preserved in some autosomes and no central element was observed. The small dense spherical structure found next to the lateral elements and below one of the terminal points was interpreted as the proteic part of kinetochores (Figure a-e).

The XY sex bivalent is paired in a short distal region (0.5  $\mu$ m) where attachment points to the nuclear membrane were also observed. After this short pairing region, the lateral elements keep apart near each other (Figure a). The lateral element (core or axial element) of the X is double in the non-synaptic portion (Figure b-c), as shown by SOLARI<sup>2</sup>, and larger than the Y. In preparations not completely free of chromatin, the presence of a double core in the non-paired regions of the X could also be observed (Figure c). In



Water spread synaptonemal complexes of male mice preparations. a) b) c) pairing of the XY sex bivalent. The short core represents the Y chromosome, and the long core, the X. a) shows the region of pairing (arrow); b) c) shows the double core of the X (arrow); c) the chromatin was partially removed; membrane fragments are present (bar); d) shows autosome synaptonemal complexes; e) autosome synaptonemal complex showing a dense body attached to the upper lateral element. LE, lateral element; AP, attachment point; K, kinetochore. The dense shadow seen in the background is constituted of residual chromatin. a)  $\times 12,500$ ; b)  $\times 16,000$ ; c)  $\times 7,750$ ; d)  $\times 4,000$ ; e)  $\times 12,800$ .

pachytene configurations, completely free of chromatin, the SC of the autosomes preserve the morphology of the complement (Figure d). Some chromosomes show a dense body attached to the lateral element (Figure e). SOLARI<sup>2</sup> in analyzing thin sections, using as a marker the quadrivalent produced by the Searle's *X*-autosome translocations, concluded that the *X* chromosome pairing region belongs to the long arm. This agrees with the conclusions of FORD and EVANS<sup>6</sup> but not with those of OHNO and LYON's<sup>7</sup> who assume a pairing by the short arm of the *X*. But HSU et al.<sup>8</sup>, observing the centromeric heterochromatin of the *X* chromosome in the diplotene *XY* bivalent, confirm that the pairing region is the long arm of the mouse *X* chromosome.

According to this last hypothesis, the small pairing region of the *XY* bivalent shown in our preparations would correspond to the distal region of the long arm of the *X* (Figure a-b).

The aspects of the *XY* cores in normal meiosis and in Searle's translocations, led SOLARI<sup>2</sup> to suggest that the lateral elements are part of the chromosome structure.

Such association may be inferred from the fact that the lateral elements which remain in the treated preparations maintain the topographical disposition of the autosomes

and the *XY* pair, even at the non-pairing region. However the implication that these protein axes are part of the chromosome structure is difficult to reconcile with the actual knowledge of chromosome ultrastructure.

**Résumé.** Des complexes synaptonématiques de souris, exempts de chromatine, ont été obtenus par une méthode d'étalement aqueux après traitement par une solution de NaCl 0,9%. Les aspects d'appariement de la paire sexuelle *XY* et des autosomes sont présentés.

MARIA LUIZA BEÇAK<sup>9</sup>, W. BEÇAK and SYLVIA M. CARNEIRO

*Serviço de Genética, Instituto Butantan, Caixa Postal 65, São Paulo (Brasil), 28 April 1975.*

<sup>6</sup> C. E. FORD and E. P. EVANS, *Cytogenetics* 3, 295 (1964).

<sup>7</sup> S. OHNO and M. F. LYON, *Chromosoma* 16, 90 (1965).

<sup>8</sup> T. C. HSU, J. E. K. COOPER, M. L. MACE JR. and B. R. BRINKLEY, *Chromosoma* 34, 73 (1971).

<sup>9</sup> Supported by the Conselho Nacional de Pesquisas and Fundo Especial de Despesas do Instituto Butantan.

### A Gross Abnormality in a Common Invertebrate: 'twinning' in *Chthamalus depressus* (Poli)

Although there is vast literature on vertebrate teratology and numerous experimentally induced abnormalities have been described by invertebrate biologists, records of 'fully developed', naturally occurring abnormalities in invertebrates are rare. The existence of abnormal development in the living adult of a common cirripede leading to a 'twinned' body is, therefore, of considerable interest.

The animal concerned was found in a collection of *Chthamalus depressus* (Poli) from high in the littoral on Isola Rossa, near Rovinj (Yugoslavia) in May 1973. It was living and apparently quite viable. The size of the animal was 10 mm along its rostrocarinal axis. The shell itself was eroded, thus reflecting the conditions of its habitat (KLEPAL<sup>1</sup>; KLEPAL and BARNES<sup>2</sup>) and the animal

was probably at least 5 years old. The appearance, as seen after the animal had been removed from the substratum, is shown in Figure 1; the membranous basis was left on the rock. The abnormality appears only to have affected the body of the animal since the wall plates and the opercular valves were completely normal (Figure 2).

The abnormality consisted of the development of an additional and smaller prosoma and thorax, with a second set of cirri present on the latter. This smaller prosoma, projecting somewhat laterally from the larger and facing the carina, had a series of cirri arising at an angle and projecting somewhat basally; in life, it would therefore lie closer to the basis when the animal was withdrawn into its shell. The first set of cirri had normal pedicels and rami and the penis was also normal in position and morphology. The second set of cirri was incompletely developed, only the pedicels being well defined; the rami and distal parts of the cirri were incomplete and their pedicels set more closely together at their attachment to the smaller thorax. So placed, it would seem that this smaller 'twin' would be carried, at least partly, out of the mantle cavity when the larger 'twin' was extruded but it seems doubtful whether, with such an incomplete cirral net, it could take part in any feeding activity.

Abnormalities of various kinds have been recorded in the Cirripedia but they largely refer to the nauplius stages. Thus NORRIS and CRISP<sup>3</sup>, and NORRIS, JONES, LOVEGROVE and CRISP<sup>4</sup>, have described intermediates in the normal sequence of 6 nauplius stages in the form of larvae with characters intermediate between 2 normal stages. They suggest that this is due to the rate of morphological development getting 'out of step' with physio-

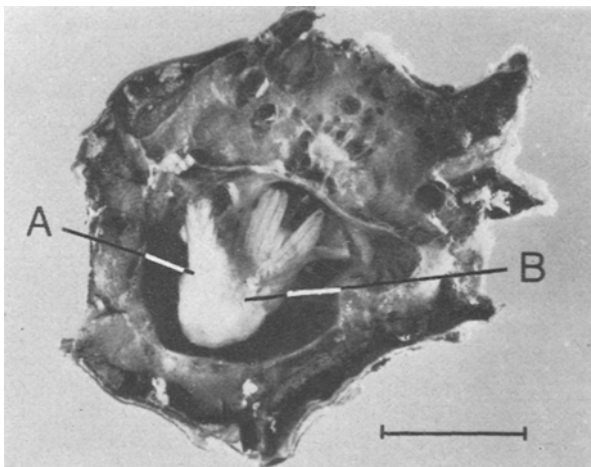


Fig. 1. *Chthamalus depressus* (Poli): animal removed from the substratum and viewed from underside. A) smaller 'twin' with incompletely developed cirri; B) larger prosoma and thorax with normal cirri; scale bar: 3 mm.

<sup>1</sup> W. KLEPAL, *J. exp. mar. Biol. Ecol.* 7, 1 (1971).

<sup>2</sup> W. KLEPAL and H. BARNES, *J. exp. mar. Biol. Ecol.* 17, 269 (1975).

<sup>3</sup> E. NORRIS and D. J. CRISP, *Proc. zool. Soc. Lond.* 123, 393 (1953).

<sup>4</sup> E. NORRIS, L. W. G. JONES, T. LOVEGROVE and D. J. CRISP, *Nature, Lond.* 167, 444 (1951).