tors, however, attribute the non-staining of interband regions to the dispersal of the protein covering the DNA phosphate groups[7].

The precise role of SSC is not known. SSC, however, is an effective chelating agent in the presence of the divalent cation calcium at pH 7.0[8], which is also the optimum pH for good Giemsa band production. Cohn[9] has shown that the metal chelator 2.2′ bipyridine (which chelates iron at biological pH without affecting ions higher up in the Mellor-Maley series[10]) causes breaks in heterochromatic regions in *V. faba* chromosomes (around the M centromere and in the mid-regions of the S chromosomes) and he emphasises the possible role of iron in maintaining structural stability of the chromosome. Certainly, in the author's experience, EDTA causes total degradation of chromosomes. If specific chromosome regions were susceptible to selective chelation by SSC this could explain the production of Giemsa bands by the SSC-Giemsa banding technique.

The trypsin banding technique reveals bands in *V. faba* chromosomes by the removal of chromosomal material with Giemsa bands representing regions resistant to trypsin digestion. The chromosome 'ghosts' are probably peripheral pools of digestion products. An interesting comparison is made between the 2 banding regimes. Although band distribution is identical with the SSC-Giemsa and trypsin-Giemsa banding techniques, it is clear that band production is achieved by the 2 methods with opposite effects. Sequential analysis of the SSC-Giemsa method shows that specific chromosomal sites are exposed by SSC and these subsequently stain positively with Giemsa, whereas in marked contrast, trypsin-Giemsa bands are manifest as a result of selective removal of chromosome material from interband regions by enzymatic digestion. These results define further the nature of the 2 banding regimes and provide a more precise basis upon which to interpret chromosome structure from cytochemical studies.

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Inhibition of Y-chromosome loop formation in primary spermatocyte nuclei of *Drosophila hydei* by trimethylphosphate

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Summary. Primary spermatocyte nuclei of either late 3 instar larvae or newly eclosed adult males of *D. hydei* contain lampbrush loops which are associated with Y-chromosome fertility factors. Formation of the loops is inhibited by continual larval development on food medium containing 0.008 M, or more, trimethylphosphate (TMP) and is accompanied by disruption to spermiogenesis.

The spermatocyte nuclei of *Drosophila hydei* contain distinctive pairs of lampbrush loops which are formed by the Y-chromosome during the growth phase of the spermatocyte[1,2]. 7 sites on the Y-chromosome which develop lampbrush loops have been recorded and deficiencies of one or more sites, aneuploidy as in X/O's or suppression of loop unfolding in one or more sites, invariably cause sterility of males[3–5]. Autoradiography using tritiated uridine shows that spermatocyte loops are active in RNA synthesis and subsequent studies have shown that male germ line cells of *D. hydei* contain specific RNA molecular types which are not found in somatic cells[6,7]. These data suggest that the loop formation together with RNA synthesis allows protein synthesis that is mandatory for completion of spermatogenesis. When the synthesis of testicular RNA is inhibited by irradiation with X-rays, the loops completely disintegrate within about 24 h; the process is usually reversible, although irreversible disintegration does occur[4].

A recent review of the industrial uses and the toxicological, carcinogenic, chemosterilizing and mutagenic properties of TMP has been published[8]. In mammals, TMP appears to produce a temporary chemosterilizing effect in the form of a 'functional' type of sterility involving postmeiotic cells, in which sperm, although mobile, are rendered incompetent[9]. A later study indicates that TMP, as well as other tri-alkyl phosphates, may inhibit choline acetyltransferase of spermatozoa and produce a functional sterility[10]. In *D. melanogaster*, prolonged sterility can be induced by TMP and it would seem that sperm, and then other cells, probably of premeiotic stages, are affected[11].

This paper shows that in *D. hydei*, TMP affects the unfolding of lampbrush loops in spermatocytes and is accompanied by disruption to spermiogenesis.

Materials and methods. Wild-type stocks of *D. hydei* were raised at 25 °C on standard food medium supplemented with live yeast. Food medium containing TMP (obtained from Albright and Wilson Ltd., Warley, U.K.) was prepared by adding TMP to freshly prepared liquid medium prior to setting during cooling. Newly eclosed adult flies and late 3rd instar larvae were collected after allowing groups of 10 pairs of wild-type flies to produce F_1 generations on either uncontaminated or TMP-contaminated medium. Strains used to produce X/O flies were obtained from Dr W. Hennig (Dept. of Genetics, Katholieke Universiteit, Nijmegen).

Testes were then dissected, either in Ringer's solution for phase contrast and Nomarski interference examination of living material, or in the fixative glutaraldehyde (2% in 0.1 M cacodylate buffer at pH 7.4) for preparation of sections. Fixed testes were rinsed in buffer then postfixed in 1% cacodylate buffered osmium tetroxide for subsequent electron microscopy. After rinsing and dehydration in ethanol, they were embedded in Spurr's resin[12]. Sections of 0.5 μm were cut on a LKB-Huxley ultramicrotome and

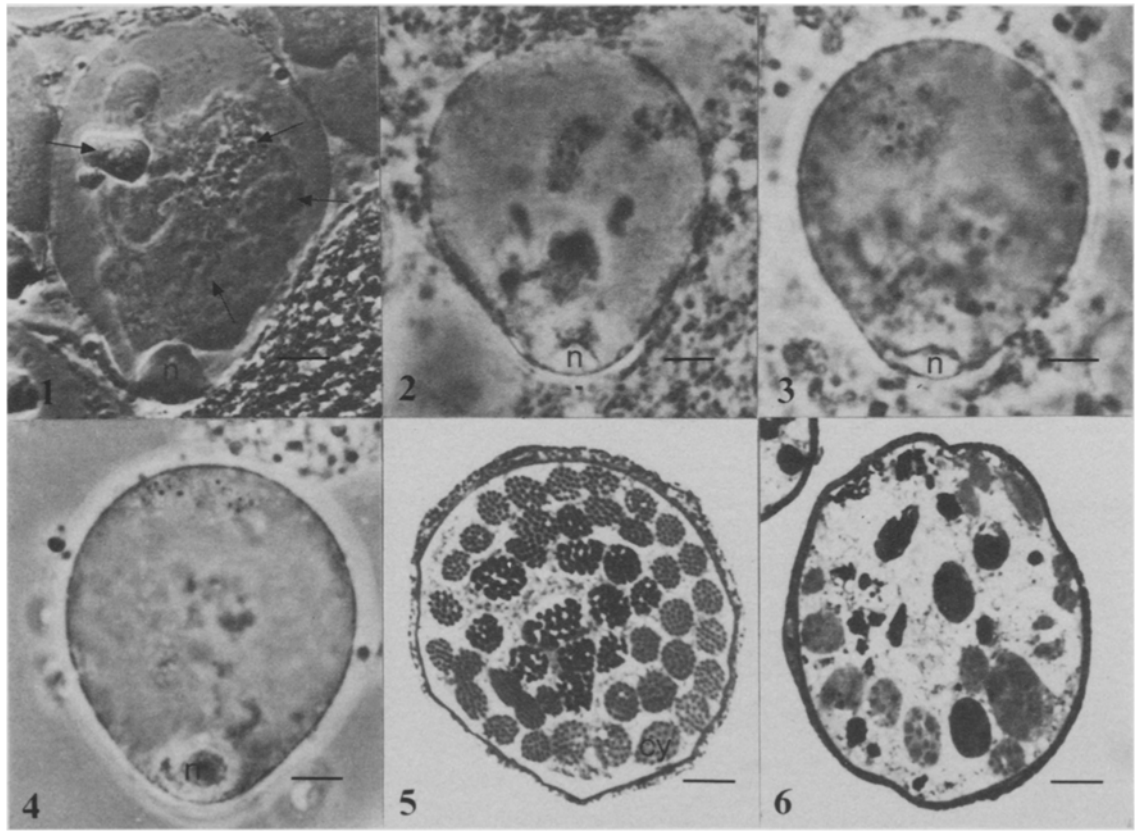

Figure 1. Control *D. hydei* adult primary spermatocyte showing Y-chromosomal loops. Nomarski interference. n, nucleolus; arrows, Y-chromosomal loops.
Figure 2. As per figure 1, but treated throughout larval development with 0.008 M TMP in the food medium. Loops are not unfolded. Phase contrast.
Figure 3. As per figure 2, but treated throughout larval development with 0.01 M TMP in the food medium. Loops are absent.
Figure 4. *D. hydei* X/O adult primary spermatocyte. Absence of the Y-chromosome results in absence of loops. Phase contrast.
Figure 5. T.S. of testis from a control adult *D. hydei*. cy, undifferentiated spermatid cyst. Compare to inner and darker differentiating spermatid cysts.
Figure 6. As per figure 5, but testis from an adult which has been treated throughout larval development with 0.008 M TMP. Degenerating cysts are observed. In all figures the bar represents 5 μm.

stained for light microscopy by heating in toluidine blue (0.05% w/v in benzoate buffer at pH 9.0)[13]. Squash preparations of newly eclosed adult testes were selectively stained with Giemsa or eosin in order to determine the presence or absence of Y-chromosomal loops in spermatocyte nuclei[14].

Results and discussion. The primary spermatocyte nuclei of adult males with normal fertility contain well defined Y-chromosomal lampbrush loops (fig. 1). However, in the primary spermatocyte nuclei of adults treated throughout larval development with 0.008 M TMP the loops are noticeably uncoiled (fig. 2) and in particular the pseudonucleolus always remains close to the nucleolus. Furthermore, when near lethal doses of about 0.01 M TMP are administered throughout larval development, an absence of all loops is found in the spermatocyte nuclei of newly eclosed adults (fig. 3) and in this regard is similar to X/O nuclei (fig. 4). Testicular sections of untreated late 3rd instar larvae also show components of Y-chromosome loops in the primary spermatocyte nuclei whereas in the sectioned nuclei of larvae which have developed on medium containing 0.008 M TMP, normal unfolding of loops is not observed.

The absence of loops in X/O flies or the uncoiling of loops in Y-translocation flies has been shown to produce sterile males[5]. Sections of testes from newly eclosed control flies contain well defined bundles of elongating spermatids (fig. 5), whereas in similar sections of adult testes after continuous larval treatment with 0.008 M TMP, a disruption of normal structural development is observed (fig. 6). These data show that the disruptive effects of TMP on spermatogenesis in *D. hydei* is premeiotic and possibly results from inhibition of Y-chromosome loop formation. A similar sterilizing action of TMP could occur in *D. melanogaster*[11].

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