^{3}H in inducing the cytogenetic effects. Kuzin et al.[15] showed that the ^{14}C incorporated in plant seedlings was 10 times more effective in producing chromosomal aberrations than that of an equal dose of external gamma irradiation. ^{14}C was also found to induce a very high frequency of sex-linked recessive lethal mutations in *Drosophila*[10-13]. The mutagenic potential of ^{14}C is both due to the emission of beta particles and the transmutation of ^{14}C into ^{14}N[6,15].

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Sequential analysis of Giemsa banded chromosomes in *Vicia faba*

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Summary. *Vicia faba* (broad bean) root-tip chromosomes were subjected to the SSC-Giemsa and trypsin-Giemsa regimes. Phase-lucent cross-bands seen after fixation/SSC treatment subsequently stain positively with Giemsa. Sequential analysis of the trypsin-Giemsa regime shows, in contrast, that bands are manifest through selective removal of material from interband regions.

Several investigators have shown that Giemsa banding involves a removal or rearrangement of chromosomal material[2-4]. Most evidence indicates that this is a removal, or conformational change, of nucleoprotein. The question of the biochemical basis of Giemsa banding is still the subject of much research and is partially equated with the questions of the difference between euchromatin and heterochromatin. This study is a sequential cytological analysis of *Vicia faba* chromosomes subjected to the SSC-Giemsa and trypsin-Giemsa regimes. Band formation by the former method is primarily determined at the SSC step by the selective removal of chromosomal material. Giemsa bands are seen at specifically exposed sites by subsequent staining with Giemsa. On the other hand trypsin-Giemsa bands represent stained chromosomal regions resistant to trypsin digestion.

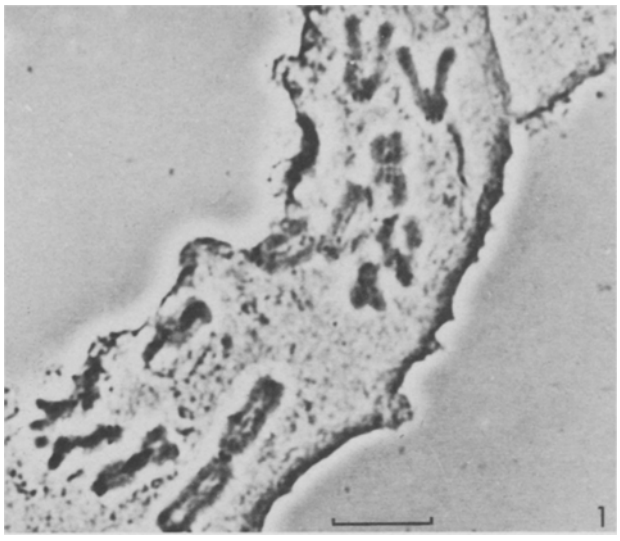
Materials and methods. *Vicia faba* var. Coles Early Dwarf beans were germinated in vermiculite. Excised lateral meristems from 10-day-old seedlings were pretreated with 0.05% colchicine for 3.5 h, fixed in freshly prepared 1/3 glacial acetic acid/methanol overnight, softened in 10% aqueous pectinase for 5 h at 37 °C, and then squash preparations were made in 45% acetic acid. a) Slides were immersed in 2 × SSC (pH 7.0) at 65 °C for 20 h, rinsed in 3 changes of deionized water, air dried, then placed in Giemsa stain (2 ml of Gurr's improved R66 diluted with 2 ml of 0.1 M Sørensen's buffer, pH 6.9) for 2 h. b) Slides were twice washed in 100% ethanol, dipped briefly into 1 N saline, then treated with a buffered trypsin solution in an ice bath for 1–7 min. The buffer composition was 1.6 g NaCl, 0.04 KCl, 0.4 KH_2PO_4, 0.23 g Na_2HPO_4, 0.02 g $CaCl_2$, 0.02 g $MgCl_2 \cdot 6\ H_2O$, 0.5 g trypsin (from beef pancreas · BDH), dissolved in deionized water and made up to 200 ml[5]. The slides were then washed in 70%, 95% and 100% ethanol, air dried, then stained with Giemsa as in a) for 10 min.

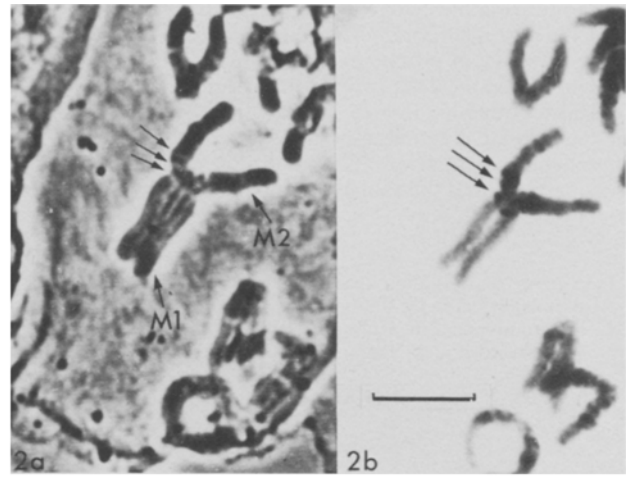
Results. Directly after fixation, c-metaphase chromosomes show no differentiation of light or dense regions along the chromosome arms. After fixation/SSC treatment most chromosomes show 2 positive dots at the centromere, 1 on each sister chromatid (fig. 1). Different techniques applied to other organisms also reveal these C-dots[6]. Phase-lucent cross-bands appear in certain positions along many chromosome arms. For example, the large M chromosome in figure 2a shows 1 phase-lucent band on the M1 arm close to the centromere and 2 similar bands on the M2 arm. The phase-lucent bands evident after SSC treatment are stained positively by Giemsa (fig. 2b). The centromeric regions, presumably the centromeric dots, can stain positively with Giemsa (fig. 3) but this is not a consistent feature.

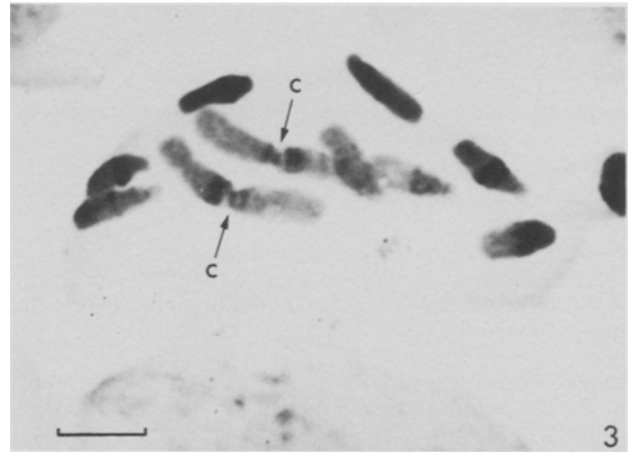
Chromosomes stained after 1–2 min trypsin treatment appear swollen and slightly understained with suggestions of bands (fig. 4). After 4–7 min trypsin treatment there is a decrease in chromosome stainability with a corresponding differentiation of bands (fig. 5 and 6). These bands show very little increase in stain intensity. Concomitant with decrease in chromosome stainability is the appearance of an amorphous 'ghost' around the boundary of each chromosome which is not seen around chromosomes which retain their stain. Ghosting is weak or absent in banded regions (sub-telocentric S chromosome in fig. 7). Monitoring of the above procedure with phase contrast microscopy endorses these results.

In advanced stages of trypsin digestion (> 7 min) individual chromosomes, along with their bands, are faint and barely discernible.

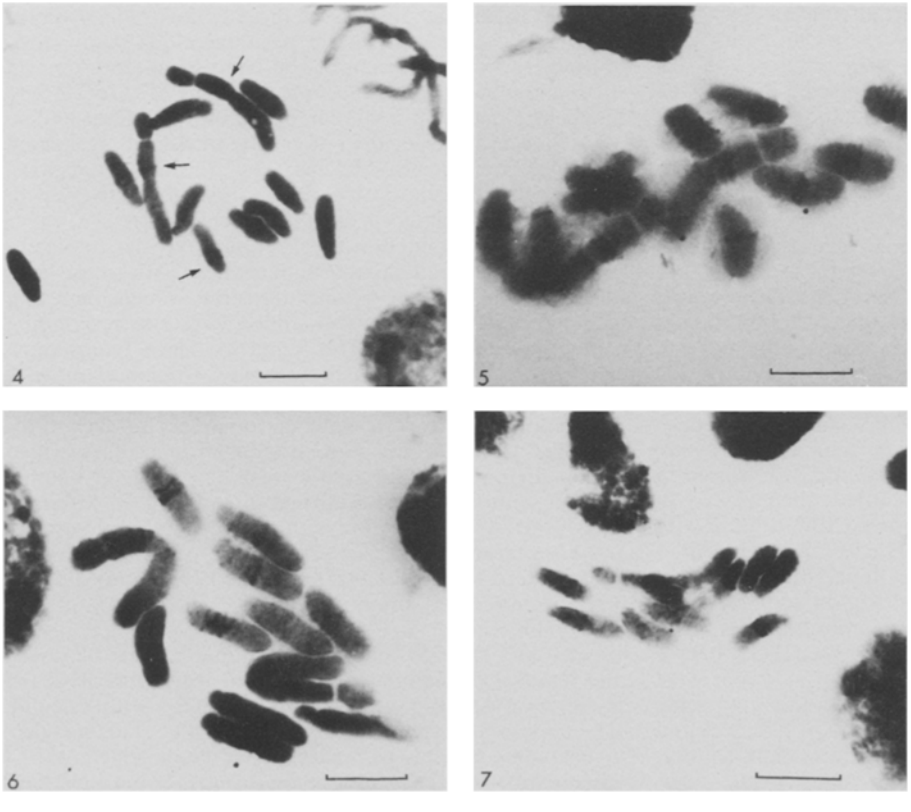
Discussion. The SSC-Giemsa technique reveals bands by the removal of material from banded regions by SSC and these regions subsequently stain positively with Giemsa. The exposure of specific chromosomal sites leads to selective banding possibly by the linking of the thiazine group of Giemsa to free DNA phosphate groups. Some investiga-







Figures 1–3. SSC-Giemsa banded squashes. *1* Unstained SSC-treated squash; C-dots on chromatids. *2a* Unstained SSC-treated squash; phase-lucent cross-bands (arrows). *2b* Giemsa-stained complement of 2a; Giemsa bands coincide with arrowed phase-lucent bands. *3* Giemsa-banded squash; positively-stained M centromeres (c). Bar = 10 µm.



Figures 4–7. Trypsin-Giemsa banded squashes. *4* Stained after 1–2 min trypsin digestion; partial appearance of bands (arrows). *5*, *6* and *7* Stained after 4–7 min trypsin digestion; prominent bands with peripheral chromosome ghosting. Note absence of ghosting in mid-band region of the subtelocentric S chromosome in figure 7. Bar = 10 µm.

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