the tip. The gels were removed from the tubes and a small wire was inserted to note the position of the marker dye. The gels were incubated in a reaction mixture containing Tris-HCl (pH 8.0), 100 mM; $MgCl_2$, 10 mM; $CaCl_2$, 200 mM; $NaHCO_3$, 10 mM and phosphoenol pyruvate, 5 mM for 30 min at 40 °C. During the incubation, a white ring of precipitate developed, indicating the region where the enzyme had migrated as a band (figure). The white precipitate thus developed remains for a long period without fading or diffusing on the gels.

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Arrangement of nucleosomes in condensed chromatin fibres[1]

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Summary. Condensed chromatin shows globules of 300 Å formed by ~ 8 to 10 nucleosomes. Each globule might be an uncoiled turn of a supercoil. This supercoil forms major coils along the fibre.

Eukaryotic chromatin is organized in subunits, known as 'nucleosomes', which are repeated along the fibre. Each one of these subunits measures ~ 80 Å and contains a core constituted by 8 histones surrounded by ~ 200 base pairs of DNA. The estimate of the packing ratio to the DNA in the nucleosomes is 7:1. The beaded aspect of the chromatin is revealed in experimental conditions of low salinity or with the addition of trypsin, which removes histones H1[3-6].

Concerning the relation of the beads-on-a-string fibre (80 Å) with the 200–250 Å fibre evidenced by other procedures[7], it has been assumed[8] that the removal of histone H1 disturbs the native organization of the chromatin. In the presence of Mg^{++} ions, the fibre shows a supercoil or solenoidal aspect. Thus, histone H1 would be acting in the folding of the beaded chain to form a 300 Å native fibre. Each turn of the solenoid model would contain an average of 6–7 nucleosomes with a DNA packing ratio of ~ 40:1. Condensed chromatin fibres organized in globules of 170 Å by the assembly of nucleosomes were also described[9]. It has been suggested that native chromatin may be composed of knobs or superbeads (200 Å) which are agglomerates of about 8 nucleosomes[10]. In this paper, we show the occurrence of globules of 300 Å in condensed chromatin as obtained by treatment with Ca^{++} ions, and its transition to the beaded configuration (80 Å) by NaCl.

Material and methods. We used spermatocyte and oocyte nuclei of *Odontophrynus americanus* (Amphibia Anura, Ceratophrydidae). Chromatin fibres with nucleosomes of 80 Å were obtained by squashing small pieces (0.5 cm) of seminiferous tubules in 0.6–0.7% NaCl. A drop of this suspension was spread on bidistilled water (adjusted to pH 9.0 with sodium borate buffer). The nuclei were then collected on parlodium (1.5%) covered grids, stained in ethanolic uranyl acetate (1:3 aqueous uranyl acetate plus 95% ethanol, 1 min), washed in 95% ethanol and air-dried. Chromatin fibres with nucleosomes of 300 Å were prepared through a modification of the method of Miller and Beatty[11]. Oocytes were isolated in 0.1 M KCl, then transferred to 5:1 of 0.1 M KCl + 10^{-3} M $Ca(NO_3)_2$ for ~ 1 h. Each nucleus was put on a drop of bidistilled water (pH 8.7, adjusted with sodium borate buffer) and allowed to swell for 15 min. Then, the material collected on parlodium (1.5%) covered grids was fixed with 10% formalin in 0.1 M sucrose for 10 min. The grids, washed in water, were stained in ethanolic uranyl acetate, as described above. Spermatocyte nuclei from pieces of seminiferous tubules

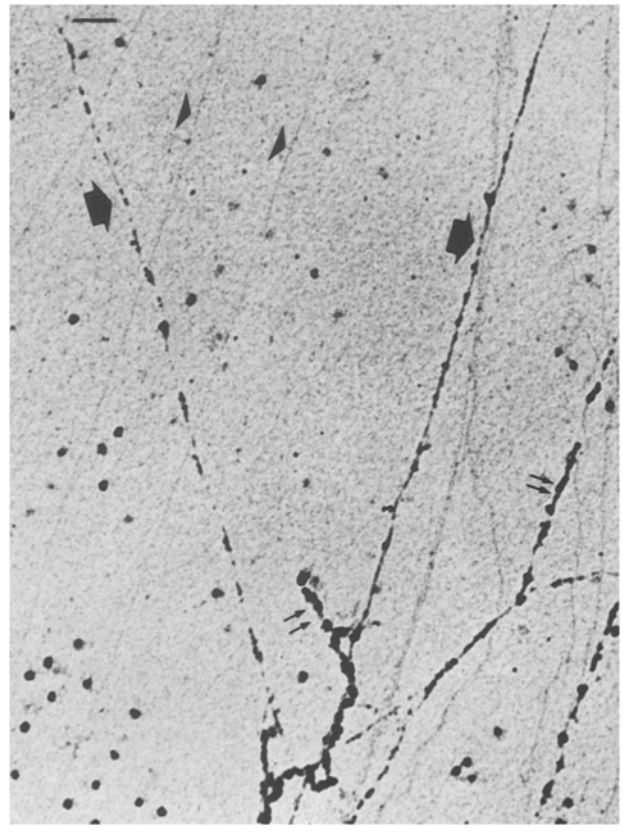

Fig. 1. Spermatocyte nucleus obtained by the NaCl procedure. Fibres with nucleosomes of 80 Å (arrowheads), of 270 Å (double arrows), and smooth threads (triangles). Alcoholic uranyl acetate. Bar = 1000 Å.

shortly left in 0.1 M KCl were transferred to 0.1 M KCl + 10^{-3} $Ca(NO_3)_2$ and left for 1 min. After squashing, the mixture was spread in water (pH 9.0), collected on grids and stained in ethanolic uranyl acetate, as described. The electron micrographs were obtained in a Siemens Elmiskop I, 60 kV.

Results and discussion. Chromatin fibres organized in beads of 80 Å and 270–300 Å were observed in the same nucleus, this variation in diameter appearing in preparations submitted to NaCl treatment. Some fibres did not contain globules but were smooth, fine threads 30–50 Å in width (figure 1). Thicker fibres were obtained in preparations

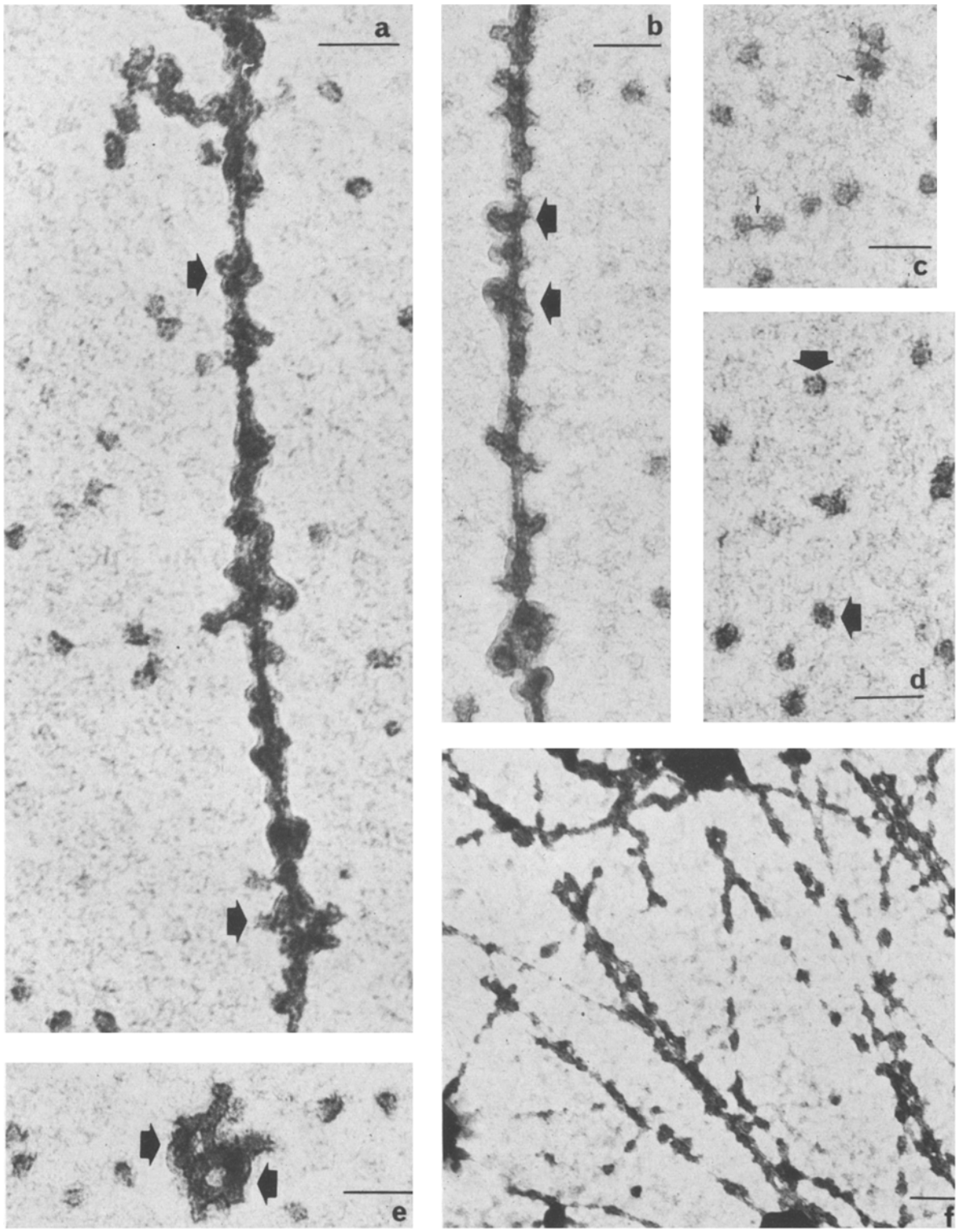

Fig. 2. Condensed chromatin fibres obtained by the Ca^{++} treatment of oocytes[a-e] and spermatocytes (f). *a* and *b* Aspect of the fibre with globules of 300 Å sometimes disposed as large coils (arrows); *c* the interglobule filament (arrows) of a disrupted fibril; *d* loosed globules, 2 of them showing ~ 8 nucleosomes (arrows). Bar = 1000 Å.

using Ca^{++} from both female (figure 2, a-e) and male (figure 2, f). These fibres form globules of 300 Å (figure 2, a and b) which are interconnected by thin (~ 30 Å) filaments (figure 2, c). Each globule consists of about 8–10 nucleosomes (80 Å) disposed in a circular way at the globule periphery (figure 2, d). We also observed that these globules are arranged in larger coils along the fibres (figure 2, a, b and e).

The findings in this and also in our previous work[9] indicate that the condensed chromatin fibre is organized in globules composed by the assembly of nucleosomes. We think that, although these globules might correspond to the knobs or supernucleosomes reported by Hozier et al.[10], the arrangement of the nucleosomes in the globules suggests that each globule could be an uncoiled turn of the superhelix described by Finch and Klug[8]. The agglomerates of globules observed suggest also that the supercoils form major coils along the fibre.

The presence of smooth and fine threads on the NaCl preparations suggests they could be products of changes in the protein-DNA complex[12]. Alternatively, they could belong to the category of free DNA as found in 10% of the genome[13]. As to the 30 Å interglobule fibrils, these may correspond to the internucleosomal filaments observed by others[6]. They presumably contain DNA in its B-structure configuration of 3.4 Å/base pair. We do not know whether such connecting fibril is a structural component or is an artefact resulting from stresses during the preparation.

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A duplication involving the esterase 6 locus in a wild population of *Drosophila melanogaster*

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Summary. A possible duplication of the esterase 6 locus in *D. melanogaster* is reported. Recombination between the duplicated loci is about 30%.

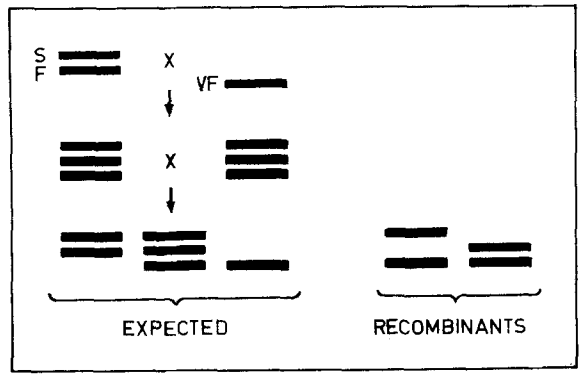
In *Drosophila melanogaster,* the esterase 6 locus *(Est-6)* maps on the 3rd chromosome at position 36.8; 6 different alleles have been detected so far by acrylamide gel electrophoresis[1]. Isoelectrophoretic alleles with reduced enzymic activity or with thermal lability have also been described[2,3]. The electrophoretic variants show codominance, without hybrid bands in the heterozygotes[4].

In the course of an extensive study on the dynamics of the *Est-6* polymorphism in wild populations[5], in a sample of 1327 males collected near Vicenza (Italy) 9 individuals with an unusual 3-band phenotype were observed[6].

From stocks of the original population reared in the laboratory, males were singly crossed with virgin females homozygous for the allele $Est\text{-}6^{VF}$. This allele is quite rare in the wild and shows the greatest electrophoretic mobility (1.21). By scoring the F_1, in the progeny of some crosses, individuals with a 3-band phenotype were observed. These invariably showed a pattern corresponding to the simultaneous presence of the allelic variants S, F, and VF with mobilities 1.0, 1.07, and 1.21, respectively. Since the allelic variant VF was contributed by the parental female, homozygous for $Est\text{-}6^{VF}$, it was clear that the S and F variants were transmitted together by the male parent. By inbreeding the F_1, it was possible to obtain an homozygous stock for the 2-banded paternal pattern. A detailed analysis, performed with the use of different substrates revealed that the 2 bands show differential affinity: in a mixture of α- and β-naphthyl acetate, one of the 2 bands (mobility 1.07) shows α-specificity, while the slow band (mobility 1.00) shows the usual β-specificity peculiar for the *Est-6* variants. The α-specific band is generally more heavily stained in females than in males.

The analysis of the F_2 progeny showed the presence of some recombinants between the α- and the β-specific esterase loci (figure). A further experiment was set up in order to estimate the frequency of recombination between these loci. The results are reported in the table. The recombination frequency calculated according to Fisher's formula resulted in about 28.7%. On the basis of these results, the hypothesis of the existence of 2 different cistrons appears unquestionable. However, the transposition of a large chromosome segment appears unlikely, since in salivary gland chromosomes preparations no evident morphological changes were noted.

The location of the presumed duplication was attempted by crossing individuals homozygotes for the duplication, with those of the marker stock 'sepia'. Unfortunately, in most of the F_2 individuals the α-specific band was extremely faint, so that a nonambiguous classification of the electrophoretic



Parental, F_1 and F_2 esterase phenotypes observed in a cross S, F × VF with the presumed parental genotypes $Est\text{-}6^{S}$, $Est\text{-}6^{F}/Est\text{-}6^{S}$, $Est\text{-}6^{F} \times Est\text{-}6^{VF}/Est\text{-}6^{VF}$.