

L'époxydation sélective I → IV a été réalisée par JÉGER et al.³ avec un excellent rendement (76%) en mélangeant des proportions sensiblement équimoléculaires de manool (I) et d'acide perbenzoïque dans le chloroforme à 0°. Toutefois, la haute dilution (environ $2,5 \cdot 10^{-2} M/l$) du milieu réactionnel utilisé par ces auteurs constituait un inconvénient que nous devons essayer d'écartier. L'étude cinétique de cette époxydation nous a permis de vérifier d'emblée la très grande différence de réactivité des deux doubles liaisons du manool (I) vis-à-vis de l'acide perbenzoïque. En effet, la constante de vitesse de second ordre observée ne change pratiquement pas si l'on effectue la réaction en présence d'un excès de 100% de peracide⁸, c'est-à-dire dans des conditions en principe défavorables pour sa sélectivité. Les travaux de MOUSSERON et LEVALLOIS⁹ ont d'ailleurs déjà montré que l'époxydation d'une double liaison isolée, par exemple d'un groupe isopropylidène, peut être 6 à 7 fois plus rapide que celle d'un groupe allylique $R_1R_2C(OH)CH=CH_2$. A la lumière de ces faits, l'époxydation sélective du manool (I) nous a paru parfaitement réalisable dans un milieu concentré, à condition d'assurer le contrôle thermique complet de la réaction. Ce résultat a été obtenu en introduisant progressivement, en 18 à 20 h, l'acide perbenzoïque (16,6 g ou 121 mM dans 250 ml de chloroforme) dans une solution agitée et refroidie vers +5°/+10° de 29 g (100 mM) de manool (I) dans 400 ml de même solvant. Le traite-

ment habituel permet d'isoler 32,7 g d'époxy-manool (IV) brut, dont 29,4 g distillent sous vide poussé. Ce produit est identique par toutes ses propriétés à l'époxy-manool (IV) préparé selon JÉGER et al.³ dans un milieu 6 à 10 fois plus dilué.

Summary. The semi-industrial production of the odorous oxides IIIab (overall yield 24%) has been achieved by combining the selective epoxidation of manool (I) in a relatively concentrated solution with the ozonolysis of the resulting epoxide (IV).

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Firmenich & Cie., Laboratoires de Recherches, Genève (Suisse), le 23 juillet 1964.

⁸ $k \cdot 10^3 = 8,77$ et $8,59$ en présence, respectivement, de 1 et 2 équivalents d'acide perbenzoïque; valeurs en min^{-1} et mole^{-1} par litre, mesurées en solution chloroformique $0,02 M$ à +2°.

⁹ M. MOUSSERON et C. LEVALLOIS, *Bull. Soc. chim. Fr.* 1960, 788; 1961, 443; 1963, 376; 1964, 297.

¹⁰ Adresse actuelle: M.I.T., Department of Chemistry, Cambridge (Mass. USA).

¹¹ Nous remercions la Direction de la Maison Firmenich & Cie., Genève, ainsi que le Dr. M. STOLL, Directeur scientifique, de l'autorisation de publier ce travail.

Fine Structure of Meiotic Chromosomes of *Gryllus argentinus*

Electron microscope studies on meiotic chromosomes have demonstrated that at the beginning of prophase the chromosomes are composed of three parallelly arranged ribbon-like strands helically twisted along the longitudinal axis of the group (synaptonemal complexes¹; tripartite groups²). The lateral arms of each group is integrated by electron dense microfibrils, whereas the medial component was defined as composed of longitudinal, less dense, smooth filaments². Each lateral ribbon is about 300 Å thick (denser band), and 800 Å wide, the medial one is 250 Å thick. The three elements are separated one from the other by two low-density spaces each measuring about 350 Å wide.

The outer face of each lateral arm is ill-defined as the microfibrils are mingled with the fibrils of the intervening nucleoplasm; the inner face shows a sharper outline. The three elements are equidistant along their length and they were never found fused or coming together. In one case the lateral arms were found diverging one from the other¹. Filamentous continuity between adjacent parts of a lateral arm and the medial component were described in another case² and filaments traversing the intermediate space have been mentioned also^{3,4}. Double-lined structure of the medial component was described in two species (cockroach², coleoptère⁶), and cross striation of the same component was reported for the same coleoptère. Lampbrush loops extending from the lateral arms towards the neighbouring nucleoplasm were reported by NEBEL⁴ et al. The same authors describe in each lateral arm two densities which they think represent sister chromatides⁴.

Histochemical studies were made, first by MOSES⁵, later by NEBEL and COULON⁷ and by COLEMAN and

MOSES⁸. In his latest paper MOSES⁸ concluded that the morphology of the chromosome as a whole, in fixed material, is not dependent on the presence of DNA.

Meiotic chromosomes from araldite embedded testes of *Gryllus argentinus* (fixed in 1% osmium tetroxide dissolved in veronal buffer) were studied after being stained with lead hydroxide (REYNOLDS⁹) or 2% uranyl-acetate in a Siemens Elmiskop I. Electron micrographs were taken at original magnifications of 30,000 to 40,000 and enlarged up to 300,000 ×.

The following terminology is used in this description: frontal views, those in which the three components are seen in the same longitudinal plane; lateral views, those in which only a face of one of the three elements appears in the longitudinal plane; and cross sections, those which show the minimum width of the three components. In this paper frontal views are seen in Figures 1 and 2, a lateral view in Figure 3, an oblique view in Figure 4, and a cross section in Figure 5.

No specific pattern of distribution is noticed in the filaments composing the lateral arms of the groups shown in any of these views. Their medial components show instead

¹ M. J. MOSES, *J. biophys. biochem. Cytol.* 4, 633 (1958).

² J. R. SOTELO and O. TRUJILLO-CENÓZ, *Z. Zellforsch.* 51, 243 (1960).

³ J. G. GALL, *J. biophys. biochem. Cytol.* 10, 163 (1961).

⁴ B. R. NEBEL and E. M. COULON, *Chromosoma* 13, 272 (1962).

⁵ H. A. GUENIN and A. GAUTIER, *Rev. Suisse Zool.* 67, 210 (1960).

⁶ M. J. MOSES, *J. biophys. biochem. Cytol.* 2, No. 4 Suppl., 397 (1956).

⁷ B. R. NEBEL and E. M. COULON, *Chromosoma* 13, 292 (1962).

⁸ J. R. COLEMAN and M. J. MOSES, 3rd Annual Meeting of the American Society for Cell Biology, *J. Cell Biol.* 19, 15A (Abstract) (1963).

⁹ E. S. REYNOLDS, *J. Cell Biol.* 17, 208 (1963).

a complex structural pattern. Three linear densities are depicted in the frontal views. The two lateral ones limit at each side the medial component, the medial one divides the whole group into exactly two halves. As these lines were also seen in cross sections (Figure 5) of the group, it is inferred that they represent three parallel sagittal planes. The individual units composing each plane are of fibrillar appearance and although the majority are aligned in their respective planes, interchange of units between planes is frequently seen. The average thickness of the fibrillar units is about 30 Å, but they present thicker parts (beaded appearance) and regions of different density, the latter condition is probably due to unequal binding of osmium or lead.

In the lateral view of the medial component, a fibrillar array transversal to that seen in the frontal view is seen (Figure 3, A, B). The transversal fibrils (50 Å thick) are regularly spaced along the lateral face (100 Å space) and at the edges of the face they may bend upwards or downwards.

Oblique views (Figure 4) allow one to infer that the transversal fibrils are interconnected with the longitudinal ones. On the other hand, any sectioning angle demonstrates that, from the medial component, filaments traversing the intermediate space come out. These filaments are prolongations of either the longitudinal or the transversal fibrils, but usually the segment traversing the

intermediate space is considerably less dense than their part in the medial component. After traversing the intermediate space they enter the lateral arms where they are lost.

The information provided by cross sections and by frontal or lateral views of longitudinal sections allows one to infer the following: (1) the medial component is integrated by three longitudinal planes of filaments; (2) each

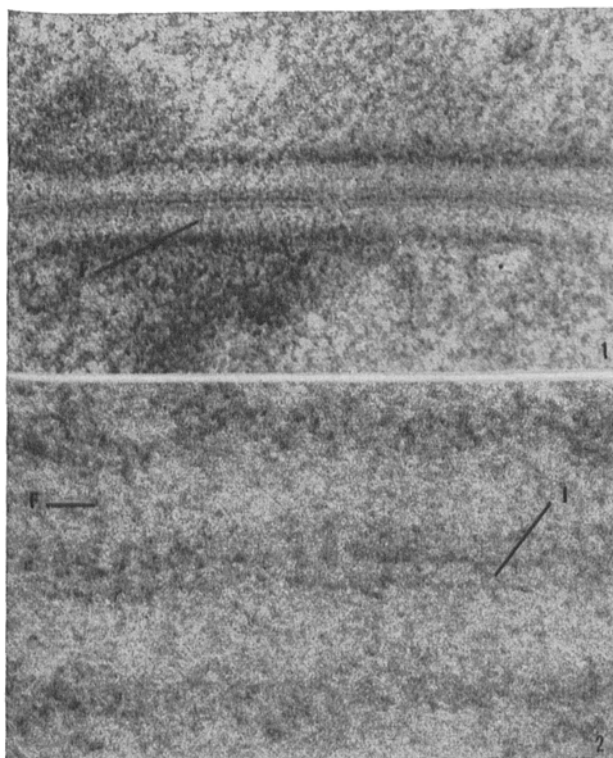


Fig. 1. At relatively low magnification (75,000 ×) the three planes of filaments integrating the medial component of tripartite group are seen as three linear densities. The filaments connecting the medial component to the lateral arms are also seen (F).

Fig. 2. This high magnification picture (300,000 ×) shows: (1) interconnection between longitudinal fibrils of two neighbouring planes (I), and (2) the filaments traversing the intermediate space (F).

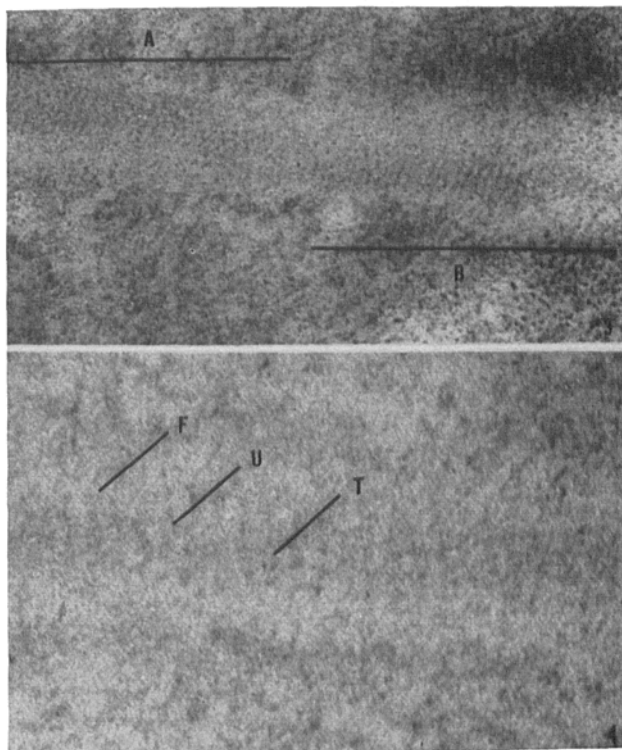


Fig. 3. Lateral view of the medial component (60,000 ×). The segment marked A probably represents one of the planes of the medial component, whereas the segment marked B may be another one. The transversal striation is clearly seen in both of them.

Fig. 4. This oblique section (300,000 ×) shows: (1) the transversal fibrillar array (T); (2) the uniting tracts between longitudinal and transverse fibrils (U); (3) the elements interconnecting medial component and lateral arms (F).

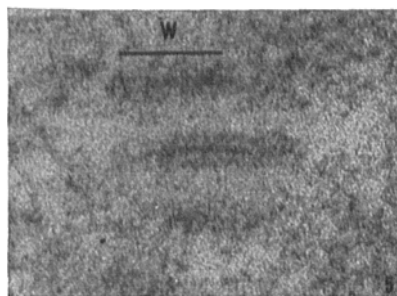


Fig. 5. The width (W) of the tripartite group is indicated by the line in this cross-section. The medial component shows distinctly the three linear densities shown in Figure 1 (160,000 ×).

plane is integrated by longitudinal and transversal units; (3) adjacent planes are united by short fibrillar bridges; (4) filamentous bridges exist at places, between the material of the lateral strands and the three-dimensional fabric of the medial component¹⁰.

Zusammenfassung. Die Chromosomen weisen in der Meiose elektronenmikroskopisch einen aus dreigeteilten Gruppen bestehenden Bau auf. Der Mittelteil einer solchen Gruppe wurde bei 300 000facher Vergrößerung untersucht. Dieser Mittelteil besteht aus einem dreidimensionalen Filamentgewebe. Die Filamente sind in jeder Hauptebene vorwiegend in zwei Richtungen orientiert:

longitudinal in der Frontalansicht und transversal in der Lateralansicht.

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Inhibition of the Testosterone Effect on the Submaxillary Gland by Actinomycin-D

The influence of male hormones on the submaxillary glands has been established by several investigations¹⁻³, following the earlier findings of LA CASSAGNE⁴. In previous work we have also reported on the sexual dimorphism of the mouse submaxillary gland in relation to a specific nerve-growth-factor (NGF), which is present in high concentration in the protein extract of such glands^{5,6}. It was then demonstrated that injections of testosterone into female mice call forth a marked increase of the NGF content of the submaxillary glands and, conversely, castration of adult male mice results in a sharp decrease of the NGF. Analogous changes in the protease content of the submaxillary gland were observed upon similar hormonal manipulations. The present study is concerned with the effect of actinomycin-D, a powerful inhibitor of RNA-synthesis, on the testosterone-effect in the mouse submaxillary gland.

Adult female mice (4 months old) of Swiss strain were used in all the experiments. The animals were divided into 4 groups of 10 each; one group (control) received no injections; one group received daily injections of testosterone propionate (1 mg/animal); one group, actinomycin-D alone (5 µg/animal); one group was injected with testosterone (1 mg/animal) and actinomycin-D (5 µg/animal). The animals of each group were sacrificed after 5 days of treatment. The whole lobes of the submaxillary glands were dissected out, freed of connective and adipose tissue, and promptly weighed on a torsion balance. Pieces of the glands were fixed and stained with Alcian blue and basic fuchsin⁷. In each group the glands were homogenized in 5 volumes of cold saline solution. The homogenates were centrifuged at 10,000 g in a refrigerated centrifuge and the clear supernatant was removed and used for the various assays. Proteins were measured by the LOWRY method, using bovine albumin as a standard⁸. Protease activity was assayed using 1% casein as substrate and measuring the optical density of the supernatant after TCA precipitation, at 280 mµ⁹. Amylase activity was measured by the method of BERNFELD¹⁰. The NGF activity was tested in tissue culture as previously described¹¹. Table I shows the effect of testosterone and of actinomycin-D, alone or in combination, on the total body weight, on submaxillary gland weight and on the total soluble proteins from the same glands. It appears that, within the 5 days of the experi-

ment, testosterone induces a moderate increase of the total body weight and actinomycin alone produces a slight loss in body weight. Noticable changes are quite evident in the submaxillary gland weight. Testosterone caused a 13% increase of the wet-weight and a 25% increase of the soluble protein content. Actinomycin-D alone causes a 14% loss in the wet-weight and a similar decrease in the soluble protein content. The combined treatment of testosterone + actinomycin-D results in only a slight decrease of the weight of the glands. The histological aspect of the submaxillary gland after testosterone treatment appears characteristic, as we have

Table I. Effect of testosterone (1 mg daily), actinomycin-D (5 µg daily), and testosterone (1 mg) + actinomycin-D (5 µg) on body weight and submaxillary gland weight (5 days of treatment). Average values from 5 animals in each group

Treatment	No.	Body weight g	Submaxillary glands wet-weight mg	Soluble proteins mg
Control	5	25	164	12.5
Testosterone (1 mg daily)	5	27	186	15.2
Actinomycin-D (5 µg daily)	5	24	140	10.1
Actinomycin-D + testosterone	5	26	152	11.2

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⁴ A. LA CASSAGNE, C. R. Soc. Biol. 134, 223 (1940).

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⁹ M. KUNITZ, in *Methods in Enzymology* (1955), vol. 2, 33.

¹⁰ P. BERNFELD, in *Methods in Enzymology* (1955), vol. 1, 149.

¹¹ R. LEVI-MONTALCINI, R. H. MEYER, and V. HAMBURGER, Cancer Res. 14, 49 (1954).