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 component[10].

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.

J. R. SOTELO and R. WETTSTEIN

Departamento de Ultraestructura Celular, Instituto de Investigación de Ciencias Biologicas, Montevideo (Uruguay), May 25, 1964.

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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 investigations[1–3], following the earlier findings of LA CASSAGNE[4]. 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 fuchsin[7]. 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 standard[8]. Protease activity was assayed using 1% casein as substrate and measuring the optical density of the supernatant after TCA precipitation, at 280 mμ[9]. Amylase activity was measured by the method of BERNFELD[10]. The NGF activity was tested in tissue culture as previously described[11]. 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 experiment, 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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already described elsewhere[6]. The tubules are enlarged and filled with secretory granules; the excretory ducts are also enlarged and rich in granules. No significant changes are detectable in the acinar portion of the gland. In the actinomycin-treated animals, some signs of cellular damage were visible both in the acinar and in the tubular portions; the cells appeared pale, with some vacuolation in the cytoplasm, in addition to a loss of secretory granules within the tubules. A similar picture was observed in the glands of animals treated with testosterone + actinomycin-D. In agreement with previous observations, testosterone injections into female mice produced a sharp increase of the protease activity and of the NGF concentration in the submaxillary gland (Table II). No significant changes though were observed in the amylase activity, which remained at normal levels after 5 days of treatment. In the presence of actinomycin-D, the testosterone effect on protease and on NGF activity was completely inhibited. Actinomycin alone causes only a moderate fall of activity of both the protease and α-amylase; the NGF concentration appeared reduced to about 50% of the control female glands.

Table II. Effect of testosterone and of actinomycin-D on the specific activities of protease, amylase and NGF in the female submaxillary glands (5 days of treatment). Each value represents the average from 5 separate determinations ± SD

	Protease U/mg	α-Amylase U/mg	NGF[a] μg/ml
Control	6.06 ± 0.32	5.2 ± 0.7	15–30
Testosterone	10.5 ± 0.85	5.4 ± 1.1	1.5– 3
Actinomycin-D	5.7 ± 0.45	5.05 ± 0.62	30–60
Actinomycin-D + testosterone	6.7 ± 0.71	5.8 ± 0.45	15–30

[a] The NGF activity is expressed as the minimum protein concentration required to give a 3+ response in tissue culture (14).

From the results of our experiments, it appears that the action of the male hormone is well localized in only one component of the gland and seems to be exerted on the synthesis or turnover of certain cell constituents. The relative increase of the soluble proteins in the testosterone-treated glands shows that net protein synthesis has taken place under the hormonal stimulation. So far as is known, the complete inhibition of the testosterone effect on the submaxillary gland by actinomycin can be accounted for to a blockage at the level of *m*-RNA synthesis[12]. More definite evidence, however, should come from *in vitro* experiments on cell-free preparations. They should also provide an answer as to whether the NGF protein is actually produced in the salivary gland rather than stored there and simply activated by the testosterone treatment. Experiments along this line are now in progress[13].

Riassunto. La somministrazione di testosterone in topi femmina produce notevoli modificazioni della ghiandola sottomascellare, che acquista i caratteri di tipo maschile. Actinomicina-D, somministrata contemporaneamente all'ormone maschile, inibisce tale effetto sulla ghiandola sottomascellare. I risultati suggeriscono che l'azione del testosterone si svolga al livello della sintesi di RNA nucleare.

P. U. Angeletti, M. L. Salvi, and G. Tacchini

Laboratori di Chimica biologica, Istituto Superiore di Sanità and Istituto di Chimica, Università Cattolica del Sacro Cuore, Roma (Italy), June 1, 1964.

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Electrical Properties and Glucose Transfer in the Goldfish Intestine

Transmural potential differences (PD) have now been recorded from many *in vitro* intestinal preparations[1–4]. In all cases the serosa shows a positive potential with respect to the mucosa and the magnitude of the current needed to short circuit the PD correlates approximately with the net transfer of sodium from mucosa to serosa. The intestine of the marine teleost *Cottus scorpius* is unusual in that it transports sodium from the mucosa to serosa in the absence of a recordable PD[5]. The following work was undertaken to test whether this property was common to the fresh-water teleost, *Carassius auratus*, whose salt requirements are very different from those of its marine counterpart.

The preparation used was a 1.5–3 cm sac of everted goldfish intestine consisting of the intestinal bulb and part of the anterior intestine. The sac was suspended in 60 ml of Krebs-Henseleit[6] medium containing 27.7 m*M* glucose and gassed with 95% O_2 + 5% CO_2. 0.1 ml of the same medium was placed within the sac at the start of the experiment and the transmural PD then recorded over a 60 min period with a Vibron electrometer using calomel electrodes and agar bridges made with 0.9% w/v NaCl. The PD was corrected for the junction potential. At the end of incubation the medium within the sac was weighed and the amount of glucose present determined by the method of Hansen[7]. In every case a definite

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