probably depends on higher enzyme level. As far as we know, no earlier investigations are available on the stimulatory effect of thyroid hormones on this nuclear protein kinase activity.

Although the physiological roles of the nuclear protein kinases are yet to be clearly understood, it is tempting to speculate that the stimulated protein kinase activity, induced by T_3 administration, may result in an increase in non-histone protein phosphorylation, which could lead in turn to enhanced template activity of chromatin and hence to the well-known increase in liver RNA biosynthesis. The possibility of an enhanced template activity of rat liver chromatin following thyroid hormone administration warrants further investigation.

Riassunto. Si è dimostrato che la somministrazione di 3, 5, 3′-Triiodo-L-Tironina esalta nettamente l'attività protein cinasica associata alle proteine non istoniche della cromatina epatica del ratto tiroidectomizzato. Tale cinasi, che fosforila le proteine non istoniche della cromatina, la caseine e la fosvitina, non agisce sugli istoni o sulla protamina e non viene stimolata dal 3′, 5′-AMP ciclico. Si prospetta l'ipotesi che nel fegato del ratto trattato con l'ormone tiroideo la maggiore attività della cinasi associata alla cromatina possa aumentare il contenuto in fosfato delle proteine non istoniche ed esaltare il processo della trascrizione.

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Fibrillation of α-Elastin Induced by Proteoglycan

α-Elastin, a high-molecular weight polypeptide derived from insoluble elastin[1], undergoes reversible coacervation at high temperatures. On prolonged coacervation, an insoluble product is formed which shows elasticity typical for native elastin[2]. It has been shwon recently that coacervation of α-elastin results in fibril formation[3].

Formation of an insoluble elastic product from α-elastin has been observed to take place also due to interaction of α-elastin with a number of sulphated polysaccharides[4], some of which are contained in connective tissue in the form of proteoglycans. It has been well established that these proteoglycans interact with collagen[5,6] and are able to influence the course of its fibrillation[7].

The present paper reports on fibrillation of α-elastin as a result of interaction of α-elastin with connective tissue proteoglycan. This observation suggests a possible involvement of proteoglycans in elastogenesis.

Materials and methods. Insoluble elastin was prepared from bovine ligamentum nuchae by hot alkali treatment[8]. The insoluble elastin was hydrolyzed with oxalic acid[1] and α-elastin was isolated from the hydrolysate and purified by repeated coacervation at 60–80 °C and adsorption of coacervated α-elastin on Super Hyphlo Cell. No contaminating material was detected in the purified product.

Proteoglycan was prepared from bovine nasal cartilage by the dissociative procedure[9]. Isoelectric focusing revealed typical proteoglycan bands with isoelectric points of 3.6–3.9. No contaminating material was present.

The occurrence of interaction between α-elastin and proteoglycan was established by optical density measurements at 440 nm of α-elastin – proteoglycan mixtures in water solutions at varying ratios of both components and constant total concentration.

Electron microscopic observation was carried out on a Tesla-BS 613 electron microscope.

α-Elastin – proteoglycan interaction product (complex coacervate) was prepared from 108 μg of α-elastin plus 24 μg of proteoglycan per 1 ml of water (maximum interaction) at pH 3.0. The samples were negatively stained with a 12 m*M* uranyl acetate – 19.5 m*M* oxalic acid solution[3], pH 3.0.

Results. The plot of optical density at 440 nm vs. α-elastin/proteoglycan ratio at 20 °C demonstrated the occurrence of interaction at pH values 2.5 (the lowest pH followed) to 4.0–4.5. As an example, the dependence is shown of the complex coacervate formation on α-elastin/proteoglycan ratio at pH 3.0 (Figure 1).

Electron microscopic observation revealed in the complex coacervate the presence of a certain amount of amorphous material together with a significant proportion of typical fibrillar structures (Figure 2). In the fibrils, fine filaments were clearly discernable. The maximum observed width of fibre was 5000 Å.

Discussion. Interaction between α-elastin and proteoglycan under suitable ionic conditions results in the formation of complex coacervate. The first stage of the complex coacervate formation is the electrostatic interaction between oppositely charged macroions through which

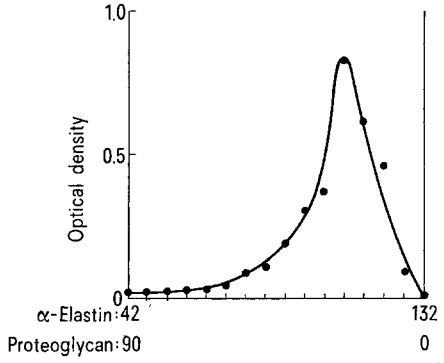

Fig. 1. Optical density at 440 nm vs. α-elastin/proteoglycan ratio. The amounts given are in μg/ml.

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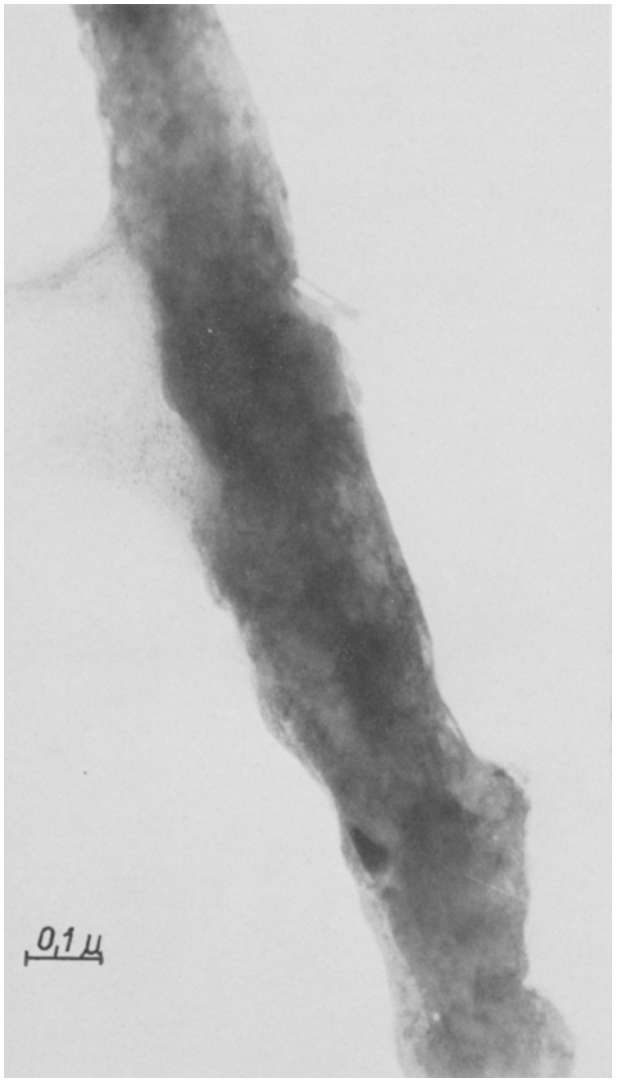

Fig. 2. Electron micrograph of α-elastin – proteoglycan interaction product (complex coacervate) formed at pH 3.0. Negative staining with 12 m*M* uranyl acetate – 19.5 m*M* oxalic acid solutions, pH 3.0. ×40,000. The bar represents 0.1 μm.

electroneutral complex arises[10]. In the case of α-elastin and proteoglycan, the ionic interaction may be assumed to take place through basic groups on α-elastin and ester sulphate groups on proteoglycan, similarly as in the collagen – proteoglycan interaction[11].

At high temperatures, α-elastin undergoes simple coacervation as a result of the association between hydrophobic side chains. This simple coacervation results in fibril formation[3]. It has been suggested[3] that the increase in order of α-elastin is offset by a decrease in order of the solvent.

In the present work, fibrillation of α-elastin has been ascertained in the presence of proteoglycan under conditions which do not give rise to coacervation of α-elastin alone. In this case, the increase in order of α-elastin will be compensated for mainly by a decrease in free electrostatic energy of the system which is the driving force for complex coacervation[12]. Due to the presence of proteoglycan in ground substance in which elastin (and collagen) fibres are deposited, we presume that the kind of interaction described above is implicated, at least partly, in the formation of fibrils from native soluble elastin in connective tissue.

Further experiments comprising tropoelastin, the native precursor of elastin, are now underway.

Zusammenfassung. Die Wechselwirkung zwischen α-Elastin und Proteoglykan in mässig saurem pH-Bereich hat die Fibrillation von α-Elastin zur Folge.

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Cyclomorphosis and Amphigony in *Brachionus calyciflorus* Pallas (Rotatoria)

It has been shown by selection experiments that the offspring of a single amictic female of heterogonic rotifers is not genetically homogeneous. For this reason genetic recombination in parthenogenetic lines has been postulated[1].

The hypothesis that the morphologic variability, which is found in the offspring of a parthenogenetic female, is correlated with the appearance of males and with the consequent amphigonic processes has therefore been subjected to experimental test.

The experiment was initiated with one amictic female of *Brachionus calyciflorus* Pallas, which was collected from a small pond near Turin. From this female, bearing postero-lateral spines of medium length (S/B = 14, see below), a line was cultured in an inorganic medium (No. 10 of CHU)[2] which was also used for rearing green unicellular algae (*Oöcystis* sp.) employed as food. High temperature (26 °C), crowding (> 4 individuals/ml) and excess of food insured an environmental pressure which determined the appearance of mictic females[3]. Every 3rd h the animals have been counted and typed as mictic or amictic, according to the method proposed by GILBERT[3].

Amictic and mictic females were fixed and stored for further morphological analysis, and a constant population density of 5 individuals/ml was maintained throughout the experiment, which was carried on over a month (about 22 generations). Resting eggs were stored for further hatching. The percentage of mictic females increased gradually during the experiment, until it

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