X-537A ionophore, with ether or with EDTA. All these agents dissipate the Ca^{2+} gradient across the membrane which loses the capacity to accumulate Ca^{2+} [2–6].

The preincubation of the SR membranes whose ATPase activities are shown in Figure 1 was performed in ionic media (5 m*M* $MgCl_2$, 50 m*M* KCl) which we previously found to preserve optimally the ATPase activity during preincubation at temperatures which in ion free media cause complete denaturation of the enzyme. Figure 2 shows the relative efficiency of Mg^{2+} and K^+ in stabilizing the enzyme activity of SR during preincubation at 45 °C. It is evident from these results that whereas about 5 to 10 m*M* Mg^{2+} already gives maximal stabilization of the enzyme, about 100 m*M* K^+ are necessary for obtaining the same effect. The concentrations for half maximal effect of Mg^{2+} and K^+ were estimated to be 1.0 and 25 m*M*, respectively.

The S-shape curves shown in Figure 2 indicate that the direct plots of ion concentration against ATPase activity are classical saturation curves. The protective effect of these ions is probably due to the binding of Mg^{2+} or K^+ to the membranes which also exhibits saturation[12]. The fact that Mg^{2+} is much more effective than K^+ in protecting SR ATPase from heat inactivation is compatible with the binding results of these cations to the membranes[13], which show that the affinity of the binding sites in the membranes for Mg^{2+} ($pK_M = 4.2$) is much higher that the affinity for K^+ ($pK_M = 1.3$).

The heat activation of the ATPase of SR membranes which occurs between 40 and 45 °C, as well as the effect of divalent cations on the stability of the enzyme, should be taken into consideration when Arrhenius plots of the activity of the enzyme are constructed to obtain information about the various thermodynamic parameters of the enzyme.

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Fibrillation of Tropoelastin Induced by Proteoglycan

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Summary. Electrostatic interaction between tropoelastin, the native precursor of elastin, and proteoglycan results in tropoelastin fibrillation. The finding suggests a possible involvement of proteoglycans in elastogenesis.

It has been shown that simple coacervation of both α-elastin, a degradation product of insoluble elastin, and tropoelastin, the native precursor of elastin, results in elastin fibril formation[2,3]. In a recent work[4], we have described a different mechanism of α-elastin fibrillation, that is ionic interaction with proteoglycan. In the complex coacervate, which is formed as a result of this interaction, fibrillar structures were clearly observable. The present paper gives evidence that this mechanism of fibrillation applies also for the native precursor of elastin, which suggests a possible biological significance of the tropoelastin-proteoglycan interaction.

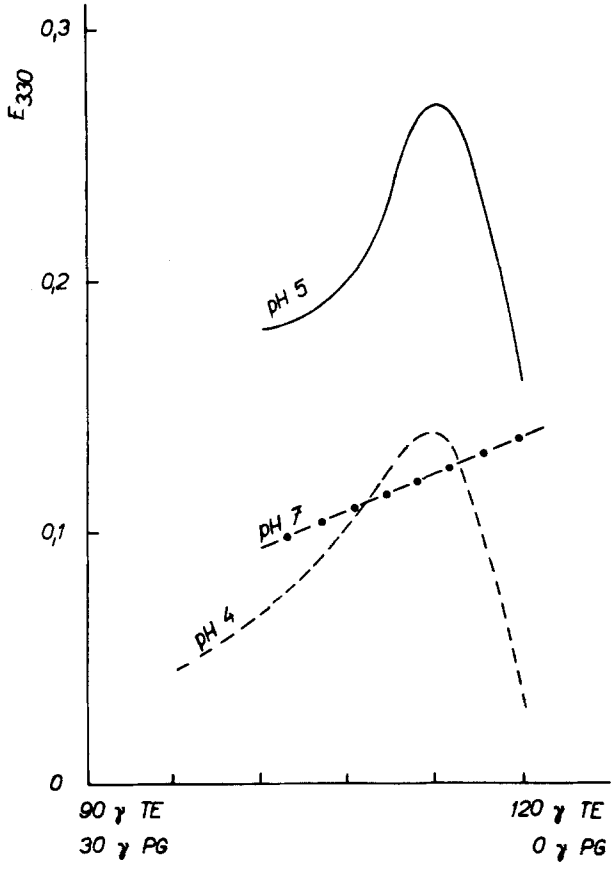

Fig. 1. The plot of optical density at 330 nm vs. tropoelastin: proteoglycan ratio. The amounts given are in μg/ml.

Materials and methods. Tropoelastin was a generous gift from Dr. L. B. Sandberg (University of Utah, Salt Lake City, USA). It was prepared from Cu-deficient pig aorta and its amino acid composition was in agreement with the criteria of purity. Proteoglycan prepared from bovine nasal cartilage by the dissociative method[5] was the same preparation as in the previous report[4]. It did not contain any contaminating substances.

Optical density measurement at 330 or 440 nm was used for the detection of the interaction between the tropoelastin and proteoglycan. The measurement was carried out at various pH values and at varying weight ratios of both components while the total concentration remained constant and was 120 μg/1 ml.

The interaction products (complex coacervates) for electron microscopic observation were prepared either by mixing the compounds in solution directly at pH 4.0 and 20 °C (see the results) or by dialysis at 20 °C of mixed solutions prepared at pH 7.0–8.0 (no interaction occurs at these pH values) against several changes of water ad-

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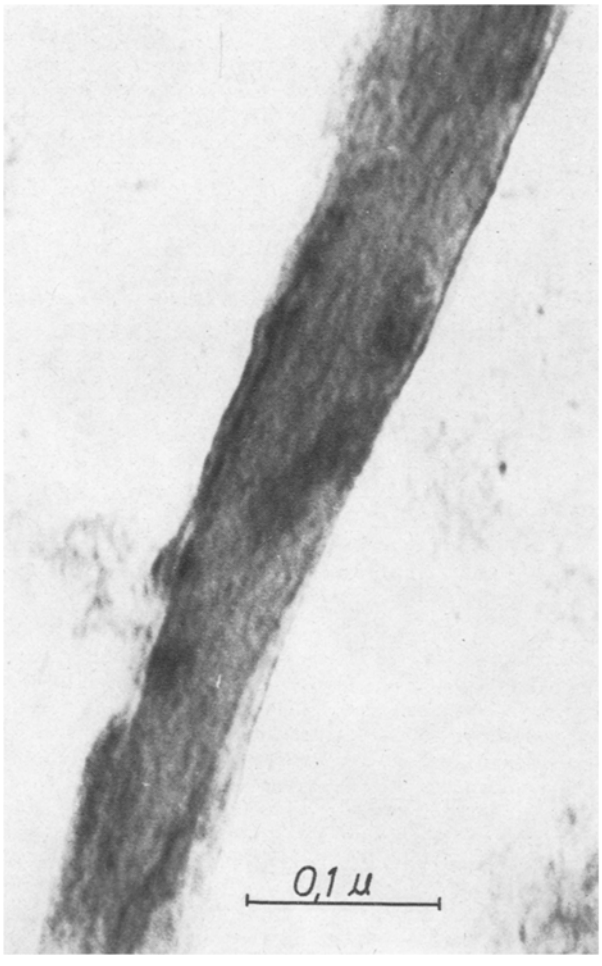

Fig. 2. Electron micrograph of the tropoelastin – proteoglycan interaction product formed at pH 4.0 (dialysis procedure). Negative staining with 12 m*M* uranyl acetate, 19.5 m*M* oxalic acid solution, pH 3.0. × 40,000. The bar represents 0.1 μm.

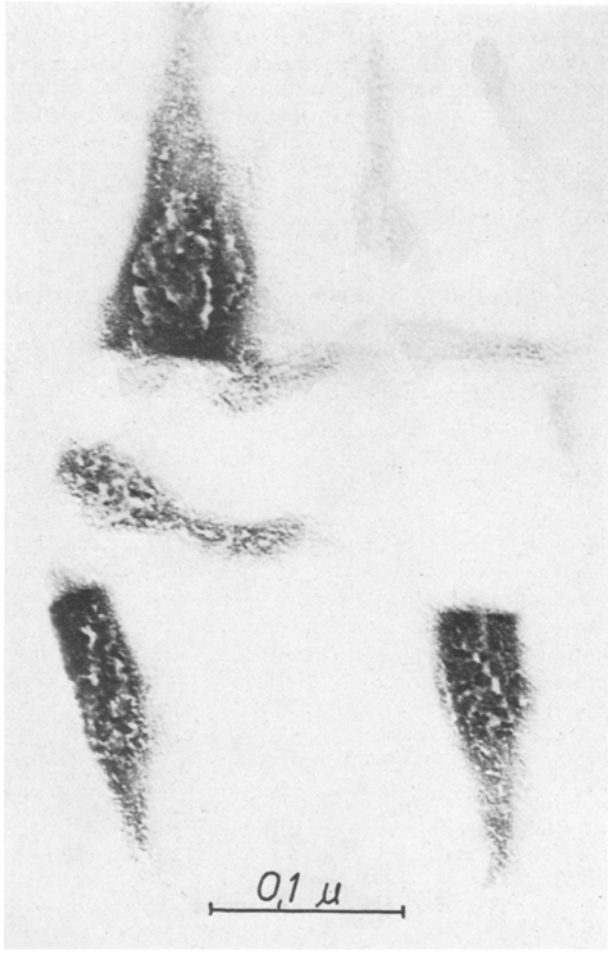

Fig. 3. Electron micrograph of the tropoelastin-proteoglycan interaction product formed at pH 4.0. Staining and magnification the same as in Figure 2.

justed with acetic or hydrochloric acids to gradually decreasing pH values until pH 4.0 was reached.

Electron microscopic observation was carried out on a Tesla-BS 613 electron microscope. The samples were negatively stained with 15 m*M* uranyl acetate, 19.5 m*M* oxalic acid solution[2], pH 3.0.

Results. The plot of optical density vs. tropoelastin: proteoglycan ratio at pH 4.0, 5.0 and 7.0 is shown in Figure 1.

A distinct peak in the optical density vs. tropoelastin: proteoglycan plot at pH 4.0 and 5.0 proves the occurrence of interaction (complex coacervate formation) at these pH values with the optimum of tropoelastin:proteoglycan ratio approximately 19:1. No interaction took place at pH 7.0. In view of these results, pH 4.0 was chosen for the preparation of the interaction products as stated in the forgoing. The weight ratio tropoelastin:proteoglycan was 19:1.

Electron microscopic observation revealed the presence in the complex coacervate either of typical fibrillar structures (Figure 2) or in some cases of characteristic segments (Figure 3) in the dependence on the conditions of the complex coacervate preparation.

Discussion. It is generally accepted that insoluble elastin fibres are formed as a result of cross-linking of the soluble precursor, tropoelastin. It can be assumed that, before cross-linking, the individual tropoelastin molecules have to be arranged in such way that the correct apposition is achieved of the lysine side chains which are responsible for the cross-links formation[6,7].

Hydrophobic associations, which take place at elevated temperatures, have been suggested as the ordering force for such alignment of tropoelastin molecules prior to cross-linking[3]. The increase in order of tropoelastin connected with the alignment results in the filament formation[3] which takes place in the absence of any other high molecular weight component.

In this report, another mechanism is described for tropoelastin fibrillation, namely electrostatic interaction with a connective tissue proteoglycan. Similarly as in the fibrillation of α-elastin reported by us earlier[4], the in-

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crease in order of tropoelastin can be expected to be compensated for by a decrease in free electrostatic energy of the system. The biological significance of the present observation rests in that proteoglycans are present in the ground substance in which elastin fibres are deposited. This suggests that the type of interaction described above can take place also during elastogenesis in vivo.

Depending on reaction conditions, 2 different types of structure have been observed: a) fibrils, b) segments. In this a certain analogy can be seen to the other connective tissue protein, collagen, which can be prepared either in fibrillar form or in the form of segments. The detailed investigation of the respective reaction conditions is now under way.

Some Metabolic Disorders Affecting the Carotenoid-Linked Haemolymph Proteins in *Rhynchosciara americana* (Diptera, Sciaridae)

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Summary. Disorders in the carotenoid metabolism are proposed to explain the absence of the yellow and violet or only the violet carotenoid-linked proteins from the haemolymph of some *R. americana* larvae. The lack of only the yellow chromoprotein is considered to be due to a failure in the biosynthesis of its apoprotein.

The study of metabolic disorders affecting carotenoid-linked proteins can provide useful data on the metabolism of carotenoids by the animal under study, as well as on the assembly of these proteins. On the other hand, following the physiological and/or morphological changes associated with those metabolic disorders, one may have an insight into the function of the carotenoid-linked proteins. Findings in this field are desirable, since the only firmly established function of those proteins in the animal is to provide protection against photodynamic action, although they certainly have other functions[3].

Rhynchosciara americana have 3 pigments in the haemolymph[4] from which one has an unknown nature and the two other are carotenoid-linked proteins[5]. BASILE et al.[4] found in *R. angelae* (*R. americana*)[6] larvae with only one pigment in the haemolymph, and they were able to show that it was a consequence of a sex-linked mutation. In this paper we describe some other metabolic errors affecting the haemolymph pigments of *R. americana*. Although the animals did not survive for a genetic analysis be accomplished, sufficient data were collected for the establishment of a tentative explanatory model of the metabolic disorders from a biochemical point of view.

Figure 1 shows electrophoretograms of the pigments found in normal larvae and in larvae showing metabolic disorders which we will call 'mutants'. LI-mutant corresponds to that one previously described[4] and has only the lemon-coloured pigment which has an unknown nature[5]. This mutant, therefore, does not have any protein-bound carotenoid but, except for its color, it is similar to the wildtype. The LII-mutant does not have the violet chromoprotein in the haemolymph. No changes

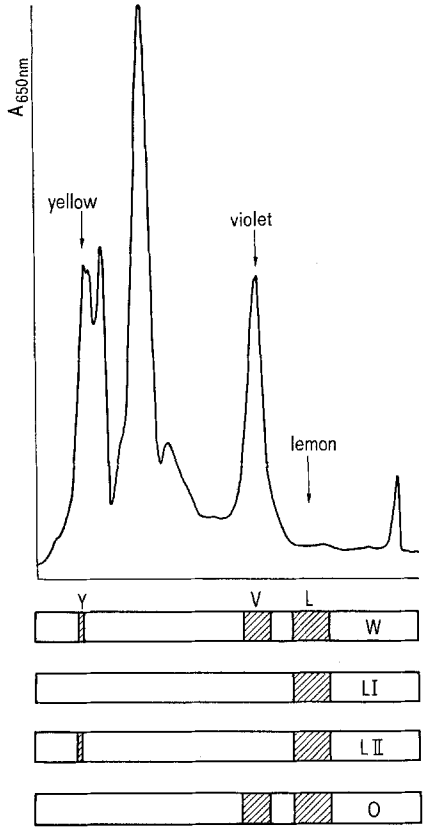

Fig. 1. Densitometric scan of a 7% acrylamide gel electrophoretogram of protein[7] from the heamolymph of wild-type larvae. The diagram below the densitogram shows the migration of the lemon (L), violet (V) and yellow (Y) pigments displayed by the haemolymph of the wild-type larvae (w) and of the mutants lemon I (LI), lemon II (LII) and orange (O).

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