refolded to give the original pattern, or whether some other pattern from the original will result. The extent of denaturation can vary from slight structural changes to complete rearrangement of the peptide chains.

If denaturation is due to heat, the protein remains in the zwitterionic state. Hydrogen bonds between peptide chains are cleaved and bonds between hydrophobic groups may disappear. The insolubility of heat-treated proteins is probably caused by the S–S interchange reaction, and the resultant formation of new intermolecular S–S bonds[7].

The present experiment shows that heat has significantly different effects on α-crystallin compared to β- and γ-crystallins. α-Crystallin is not significantly precipitated even at 100 °C, in contrast to β- and γ-crystallins which precipitate increasingly with rise in temperature above 50 °C.

α-Crystallin is, however, distinctly modified by the rise in temperature, since its electrophoretic mobility is altered. The change in molecular conformation which must account for this, does not affect its antigenic sites, since the immunologic specificity of α-crystallin remains identical from 22–100 °C. Further evidence of a change in α-crystallin structure is offered by its more intense color reaction with ponceau xylidine after heating (Figures 1 and 2). This phenomenon has been found for other proteins, and ascribed to the unfolding of the peptide chains. The higher color reactivity shows that some of the reacting groups are buried inside in the native protein or screened off in another manner.

JANSEN et al.[8], studied the thermal coagulation of bovine and human serum albumin and reported that the physical properties depend upon the free sulfhydryl group and if this sulfhydryl group is destroyed by heating or other chemical reagents, clear clots result.

KINOSHITA and MEROLA[9] reported that there is a masking of sulfhydryl groups as the lens matures being strikingly absent in cataractous lenses[10]. This suggested that during cataract formation, sulfhydryl groups are oxidized to disulfide bonds.

β- and γ-crystallins contain free sulfhydryl groups while they are absent in α-crystallin as revealed by a negative nitroprusside test[11]. It may be concluded that during heating the free sulfhydryl groups of β- and γ-crystallins are converted into disulfide linkages with the formation of an insoluble precipitate, while the solubility of α-crystallin is unaffected by heat because it lacks free sulfhydryl groups[12].

Résumé. La chaleur est capable de précipiter les protéines du cristallin bovin β et γ, alors que l'alpha cristallin n'est pas précipité à des températures allant jusqu'à 100 °C. Cette constatation semble être en relation avec la formation de ponts disulfures moléculaires.

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[13] Post-Doctoral Fellow of the National Council to Combat Blindness.
[14] Supported by N.I.H. Grant No. NB 04765.

Chitin in Tunicata

The occurrence of cellulose as so-called tunicin in the mantle of tunicates is a well-established fact. Obviously this sort of cellulose is the same as that occurring in plants[1,2]. The analogy concerns not only the chemistry but also the structure, as the texture of the microfibrils exactly resembles the arrangement of fibrils found in the cell walls of certain plants[3,4]. The tunicates are the only group of animals known so far in which cellulose occurs; chitin, which is closely related to cellulose, has never been found in tunicates[5,6] or in other chordates[6]. Thus it was quite surprising when, in connection with investigations on the distribution of peritrophic membranes in animals not belonging to the Arthropoda, it was possible to find in the intestine of several ascidians membranes containing considerable amounts of chitin. Until now only some of the larger ascidians have been examined: *Phallusia mammillata, Ciona intestinalis, Halocynthia papillosa, Clavelina lepadiformis* and *Corella* sp. *Phallusia* and *Ciona* from the Mediterranean (Banyuls and Naples) were fixed in formalin; *Clavelina* from Heligoland was fixed in bouin; *Halocynthia* was the only living material, coming from the Adriatic; *Corella* was fixed in formalin and supplied by the Carolina Biological Supply Comp., Burlington N.C., USA.

All these species filter plankton in their branchial sacs, wrap it in mucus and transport it by means of their cilia into the intestine. Here the band of mucus is loosely wrapped in additional membranes, being secreted by the cells of the intestine. The secretion of these peritrophic membranes may easily be seen in cross sections of the intestine. Histochemical reactions, such as staining with alcian blue, toluidine blue, bromophenol blue, and the PAS reaction, indicate that these peritrophic membranes are built up of, or at least contain, glycoproteids. The band of mucus with plankton coming in from the branchial sac, is sometimes transported through the intestine as a rather straight band, and is sometimes coiled up between membranes which are wrapped loosely around these remarkably flat coils. It may be that it depends on the

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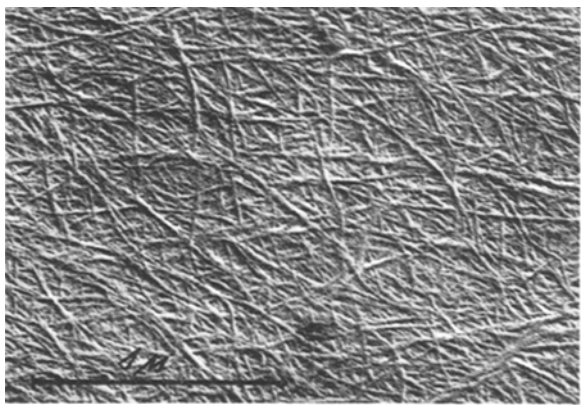
amount of plankton being filtered as to how the band is transported through the intestine. Perhaps coils are formed, when the secretion of peritrophic membranes cannot follow the rapid intake of food.

The peritrophic membranes did not dissolve even after prolonged treatment with 40% potassium hydroxide at 80 °C. As they did not stain with chlorinezinciodine and as they did not dissolve in copper oxide-ammonia (Schweizer's reagent), it seems unlikely that they contain cellulose. But they gave a very distinct chitosan reaction, which shows that chitin is an essential element in the construction of these peritrophic membranes.

An investigation of their fine structure confirmed this view. There are microfibrils in these membranes, showing a texture that resembles the texture of microfibrils found in plants[3,4], insects[7,8], and other animals[9]. It is an irregular, felt-like texture. Frequently the microfibrils are not interwoven in one level, but pass through several levels (see also [7]). Sometimes a number of microfibrils are united to form coarser fibres, which are strewn irregularly over the surface of the ground texture or form a flat band. The single microfibrils show notches, suggesting that these chitin-containing microfibrils of tunicates as well as the cellulose-containing microfibrils of plants[10-12] (and tunicates[1,2]) consist of smaller units[13].



Phallusia mammillata: Irregular network of microfibrils containing chitin. Shadowed with platinum. × 33,600.

Zusammenfassung. Peritrophische Membranen kommen nicht nur bei Arthropoden vor, sondern sind im Tierreich weit verbreitet. Chitin enthaltende peritrophische Membranen werden auch von den bisher daraufhin untersuchten Ascidien gebildet. Die frühere Auffassung, nach der Chitin bei Deuterostomiern nicht auftritt, muss daher endgültig aufgegeben werden.

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Inhibition of Secretion and Secretory Potentials in the Submandibular Gland of the Cat by Acetazolamide

Lundberg[1-4] recorded transmembrane potentials in the acinar cells of the submandibular and sublingual glands of the cat. He showed that stimulation of the secretory nerve increased the intracellular negativity, and he was able to demonstrate that secretory potentials were due to an active inward transport of anions, largely chloride, through the outer acinar cell membrane.

It is well known that the potent carbonic anhydrase inhibitor acetazolamide (diamox) is able to inhibit a number of secretory processes[5]. Among others Maren and Robinson[6] suggested that the diamox-inhibition of cerebrospinal fluid formation is due to an inhibition of a chloride pump in the chorioid plexus.

Carbonic anhydrase has been found in salivary glands of many species, and recently Morris and Swayne[7] showed that most of this carbonic anhydrase is located at the acini.

In the present work it was shown that diamox is able to inhibit salivary flow rate as well as secretory potentials.

Methods. Young cats (2–3 kg) anaesthetized with chloralose (70–90 mg/kg i.p.) were used. Salivary flow rate was measured by collecting the saliva obtained from the cannulated submandibular duct in a tuberculin syringe. Secretory rate was measured in 10 successive periods, of which the first 2 lasted $^1/_2$ min each, the rest having a duration of 1 min each. The salivary flow rate decreased during the first periods but remained relatively constant in the last 4 periods. The flow rate measured in the last 1 min period was taken as a 'steady state'. Salivary secretion was elicited by electrical stimulation of the chorda tympani (10 c/sec, 10 V).

Transmembrane potentials were measured by the technique of Lundberg[1] with the only difference that the potentials were recorded from the gland in situ. Therefore, only a small part of the gland, needed for the micropuncture, was exposed. The potentials were recorded with a Mingograf writer. Secretory potentials were obtained after stimulation of the chorda tympani (15 c/sec, 10 V).

Diamox was administered intravenously according to Birnbaum and Hollander[8] in some experiments, in

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