membrane of the pillar cells were occasionally observed. They were considered to represent communicating junctions[8].

Discussion. Evidence is presented from freeze-fracture replicas that the pillar cells in the respiratory lamellae of the gills of the hagfish are connected by communicating junctions.

The pillar cells share structural and functional characteristics with smooth muscle-like cells (myofibroblasts), such as the renal mesangial cells[9] and the interstitial cells in the interalveolar septa of mammalian lungs[10]. In contrast to the pillar cells these cells are separated from the circulating blood by an original endothelium. However, each of the three cell types is considered to be involved in the regulation of the local blood flow through the corresponding microcirculation at the capillary, i.e. postarteriolar, level, as they are supplied with contractile filaments[10-12]. The three cell types appear also to be responsible for the synthesis and secretion of collagen fibrils and noncollagenous compounds of the extracellular matrix[3,9,10,13-15]. In addition, by freeze-fracture the mesangial cells[16,17] and the interstitial cells in the interalveolar septa of the lungs[18] have been shown to be coupled by communicating junctions. Numerous correlative functional and morphological studies indicate that communicating junctions are the sites where electrotonic and/or metabolic coupling can take place by intramembrane, transcellular, low-resistance pathways[19,20]. The observations reported here suggest a coupling between the pillar cells in the hagfish gills. Thus the communicating junctions between the pillar cells could also allow a synchronized reaction of these cells and thereby facilitate a putative local regulation of the lamellar perfusion as previously discussed for the renal mesangial cells[17] and for the interstitial cells in mammalian lungs[18]. The contraction of the pillar cells is generally considered to be under the control of humoral factors[21], but, although it is disputed[3,21], a direct innervation of the pillar cells has been suggested by Gilloteaux[22] for the secondary lamellae of the gills of the eel.

Moreover, a similar mechanism to that which has been proposed for mammalian lungs[10,23], by which the regional blood flow is regulated by the external oxygen tension, may be operating in the respiratory lamella of fish gills.

Finally, a coordination of the metabolic and secretory activity of the pillar cells, concerning their fibroblast-like functions, could also be achieved by the communicating junctions, as has been shown for various fibroblasts and other secretory cells[20,24].

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2 In this paper the term 'respiratory lamellae' is preferred to 'secondary lamellae' emphasizing the functional similarity of these structures.

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Elastase, collagenase and the radial elastic properties of arteries

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Summary. Studies were performed on 203 pairs of dog carotid arteries subjected to unidirectional radial compression. Treatment with 80 U/ml purified elastase for 90 min decreased radial stress, but treatment with 640 U/ml collagenase for 90 min did not. These data suggest that elastin, but not collagen, contributes to wall resistance to radial compression.

Key words. Elastase; collagenase; radial stress; arterial wall; compression.

Pressurization of arteries causes them to deform in the circumferential, longitudinal and radial directions with little torsion[1]. Deformations in the circumferential and longitudinal directions are tensile, whereas those in the radial direction are compressive. In dog carotid artery, loads in the circumferential direction appear to be borne by both elastin and collagen, while loads in the longitudinal direction appear to be borne almost entirely by elastin[2]. Little is known about the elements that bear compression in the radial direction. Little attention has been given to radial elastic properties because the stress in this direction is only 5–10% of that in the circumferential and longitudinal directions. However, because vascular tissue is essentially incompressible, radial resistance to thinning of the wall can indirectly limit deformations in the remaining directions. The present study was undertaken to assess the effects of enzymatic degradation of elastin and collagen on the uniaxial radial elastic properties of dog carotid artery.

Methods. Segments of relaxed dog carotid artery 4–5 cm in length were opened longitudinally with a sharp scalpel. These tissues then were divided into a pair of vessel segments and were

placed in a 10-ml tissue bath with a smooth floor. Alternate pairs of preparations were placed intima-side up, and intima-side down, and the bath was filled with physiological salt solution (PSS) composed of 120.0 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.7 H_2O and 15 mM phosphate buffer consisting of 10 mM $Na_2HPO_4 \cdot H_2O$ and 2 mM HCl adjusted to pH 7.4. The temperature of the fluid in the bath was maintained at 37 ± 0.5 °C by a thermostatically-controlled water jacket. Each vessel was placed in the bath for 90 min and was subjected to graded compression with a Grass force transducer suspended from a depth gauge micrometer with a nonrotating blade. Vessel contact was made with a polished stainless steel disc, 0.56 cm in diameter, mounted on the sensing arm of the force transducer. This method of compression is similar to that employed by Patel et al.[3]. The micrometer was advanced in 0.01-cm steps to compress the tissue while force was recorded. Radial compressive stress was computed as the force recorded by the force gauge divided by surface area of the contact disc. The thickness of the undeformed vessel sheet was determined at the start of each experiment by subtracting the micrometer reading obtained with the disc resting on the vessel from that obtained with the disc resting on the floor of the tissue bath. All data were corrected for the compliance of the force gauge, 0.00007 cm/g, over the range of forces recorded. One segment of each pair of vessels was studied after 90 min in the bath with no treatment (control), and the second segment was studied after 90 min treatment with either 80 U/ml purified elastase (Worthington ESFF) or 640 U/ml purified collagenase (Worthington CLSPA). These doses were used to degrade, but not destroy the wall, as other studies revealed that higher doses caused destruction of the wall[4]. Several of the arterial specimens were examined histologically after treatment and mechanical study using formaldehyde fixation, paraffin-embedding and staining with either Verhoff's elastica stain or Masson's trichrome stain for collagen. Histologic examinations were performed solely to disclose whether the enzymes had detectable effects. No quantitative histologic measurements were made, nor were any mechanical changes inferred from these observations, as loss of staining would not necessarily be correlated with loss of load-bearing in any given direction.

An additional group of arteries was studied with the tissue extended biaxially before compression. These vessel segments were clamped along their edges, extended 50% biaxially to narrow the tissue, and pinned securely in the bath before radial loads were applied.

Results and discussion. Elastase experiments. The wall thickness of 97 arterial segments under control conditions was 0.113 ± 0.001 cm (mean and SE), whereas the wall thickness after treatment with elastase was increased to 0.116 ± 0.001 cm. This slight increase in thickness (2.6%) was statistically significant ($p < 0.05$) as determined by a paired t-test, and was thought to result from the uptake of water by the wall following enzymatic degradation by the enzyme; in fact the wall assumed a 'fluffy' appearance following treatment with the enzyme. Figure 1 presents radial compressive stress data (mean and SE) for the 97 pairs of arterial segments. Data are plotted as a function of wall compression, where contact between the disc and the wall (zero change in thickness) was defined as the position at which the force gauge first recorded a nominal 1.0 g force (0.098×10^4 N/m^2). This represented less than 3% of the maximum force recorded in this study. Stress and tissue deformation are plotted downward and to the left because they both are compressive. Treatment with elastase caused a decrease in radial compressive stress (vertical shift upward) with significantly different values ($p < 0.05$) observed at all but the lowest levels of compression. The arrow indicates the wall compression at which, and greater, differences were statistically significant. These data suggest that

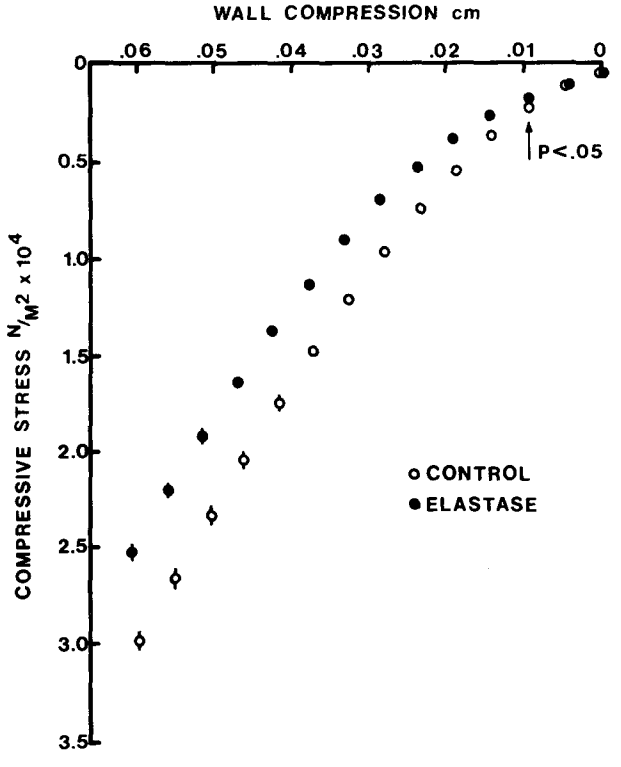

Figure 1. Tissue compression-radial stress curves for 97 pairs of arterial segments. Data show means and SE. SE are absent where they were smaller than the symbols used to designate means. Treatment with elastase decreased stress (shift upward) at all points to left of the arrow ($p < 0.05$).

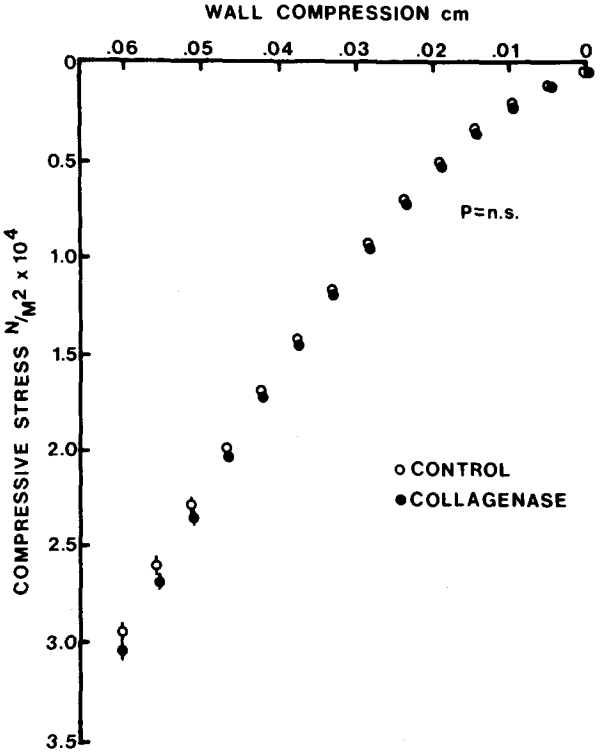

Figure 2. Tissue compression-radial stress curves for 106 pairs of arterial segments. Means and SE are shown. Treatment with collagenase did not significantly alter radial stress.

elastin bears compressive loads in the radial direction, i.e., that elastin contributes to the resistance of the wall to thinning. Histologic examination of several vessels after treatment with elastase disclosed decreased uptake of Verhoff's elastica stain and occasional broken elastic lamellae.

Collagenase experiments. The wall thickness of 106 arterial segments under control conditions was 0.110 ± 0.001 cm, whereas the wall thickness after treatment with collagenase was 0.104 ± 0.001 cm. This 5.5% reduction was statistically significant ($p < 0.5$). Figure 2 presents radial compressive stresses for the 106 pairs of arterial segments plotted as a function of wall compression. These data show that degradation of wall collagen did not significantly alter wall compressive stress (p = NS), in spite of the reduction in wall thickness. These data suggest, but do not prove, that collagen does not bear a significant portion of radial compressive loads. Histologic examination of several vessels after treatment with collagenase disclosed decreased uptake of Masson's trichrome stain.

Pre-extended arterial sheets. The above studies were carried out on arterial segments that were not stretched in the circumferential or longitudinal directions to avoid multidirectional interactions. However, studying unstretched vessels permitted the connective tissues to retract, possible reorienting them into the radial direction. Elastic lamellae may be observed to retract and reorient in this fashion in vessels that are fixed while undistended[5]. In order to avoid such retraction, 21 additional pairs of vessel segments were pre-stretched simultaneously 50% in both the circumferential and longitudinal directions, and then subjected to stepwise compression. These expriments showed that treatment with elastase (N = 8) significantly decreased radial compressive stress ($p < 0.05$), but that treatment with collagenase (N = 13) did not do so (p = NS). Therefore, it was concluded that in both unstretched and prestretched vessels, treatment with elastase, but not collagenase, decreased radial stress elicited in response to wall compression.

Specificity of enzymes. Use of enzymatic agents raises questions of specificity. Ideally one would employ mammalian granulocyte elastase and mammalian collagenase, but unfortunately neither of these enzymes are commercially available. Therefore, the present study used Worthington ESFF elastase derived from porcine pancreas, and Worthington CLSPA collagenase derived from *Clostridia histolyticum*. Both of these agents are among the most purified and most specific commercially available (Worthington Biochemical Manual, Freehold, New Jersey, USA), but both may act on a variety of substrates besides pure elastin and pure undenatured collagen[6,7]. Moreover, there are some amino acid sequences that are common to both elastin and collagen[8] raising the possibility that some degradation of both connective tissues may have occurred with either enzyme. However, in the present expriments it was found that treatment with the commercially available enzymes produced 1) different histological effects, 2) opposite changes in wall thickness, and 3) different mechanical responses. These observations suggest that, for the most part, mutually exclusive responses were obtained.

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Effect of coenzyme-A, NAD, alpha lipoic-acid and cocarboxylase on survival of rats with galactosamine-induced severe hepatitis

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Summary. Galactosamine, a selective hepatotoxin, produces in rats histologic alterations, which show the characteristics of severe human viral hepatitis. In the present study the efficacy of two different cofactor regimens (coenzyme A, NAD, alpha lipoic-acid, cocarboxylase) in rats with fulminant galactosamine hepatitis were tested. The results showed an improvement of the short-term survival with a short-term treatment and definitely better survival with a long-term regimen with cofactors.
Key words. Liver failure; hepatitis; galactosamine; coenzyme A; NAD.

Introduction. Fulminant liver failure induces important disturbances of liver function including decreased synthesis of essential substances, reduced detoxification of metabolites and alteration of the intermediary metabolism. We previously reported alterations in the decarboxylation of pyruvate with increased blood levels of pyruvate, lactate, acetoin and 2,3-butylene glycol in patients with fulminant hepatitis[1,2]. Pyruvate has to be decarboxylated and oxidized in order to enter the tricarboxylic acid cycle[3]. These key reactions require coenzyme A, NAD, alphalipoic acid and cocarboxylase as cofactors[4]. The lack of one or several of these cofactors might be responsible for the metabolic disorders in patients with acute liver disease. On the basis of this hypothesis, we conducted an uncontrolled clinical study with cofactor treatment[5,6]. The study was performed prior to the general availability of intensive care unit facilities, mannitol treatment and hemoperfusions in the treatment of fulminant hepatitis. Ten out of 26 (38.5%) comatose patients – 20 in stage IV and 6 in stage III – survived[6]. In a later cooperative international study without cofactor treatment only 10/100 (10%) patients with liver coma survived[7].

Since the uncontrolled study indicated a benefit of the cofactor treatment, we were encouraged to check this effect in an animal model and chose the recently described galactosamine (gal-N) hepatitis rat model for this purpose[8–11]. The ideal animal model for cofactor treatment in acute liver failure has to show the histologic characteristics of human disease, namely a combination of necrotic, damaged and intact hepatocytes; the alterations in the intermediary metabolism mentioned above are induced by damaged and not by necrotic liver cells. Gal-N, a selective hepatotoxin, fulfills this requirement by imitating the histologic alter-