Lysozyme is a component of human vascular elastic fibers

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Summary. Lysozyme has been demonstrated in the elastic fibers of normal human arteries and veins by the peroxidase-antiperoxidase technique. Preliminary trypsinization of paraffin sections is necessary to unmask the immunoreactive lysozyme.

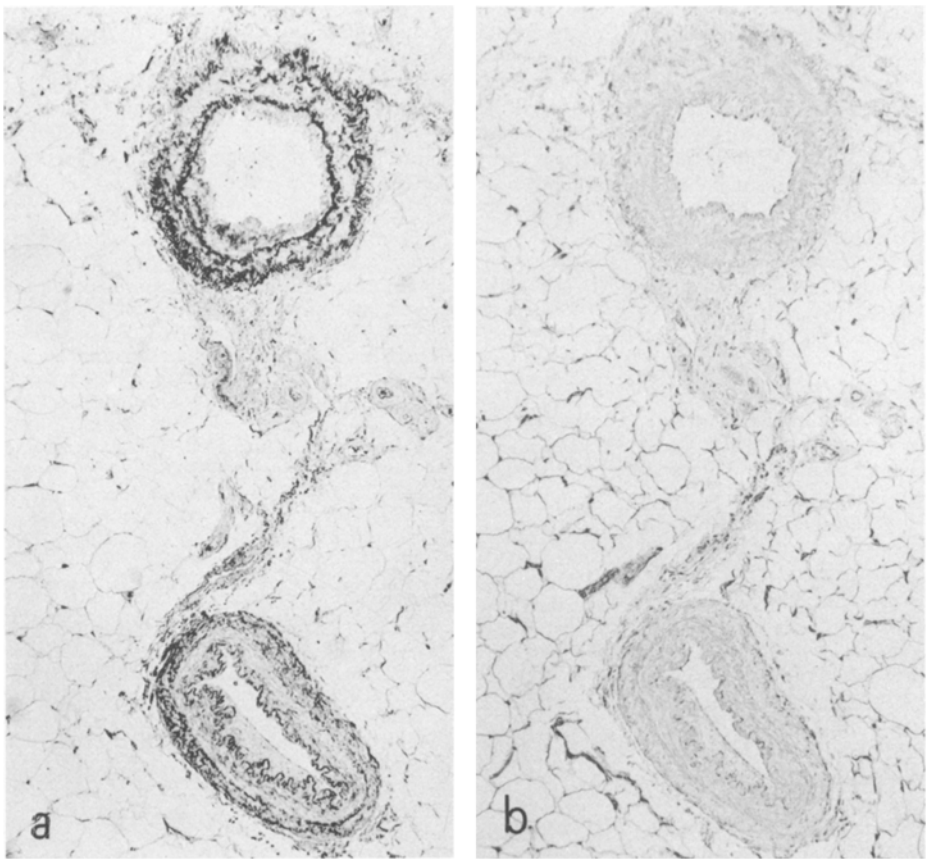
Lysozyme has been detected by immunohistochemical methods in epithelia, their secretions, leucocytes, and in some species, in the connective tissue cartilage[1,2]. Its presence has not hitherto been reported in blood vessel walls. Use of the peroxidase-antiperoxidase (PAP) technique for the immunolocalization of tissue antigens is thought by some[3] to be more sensitive then the sandwich fluorescent antibody technique. PAP methodology certainly has advantages in studying elastic fibers, which exhibit considerable autofluorescence[4] after formalin fixation. The antigen unmasking effect of preliminary trypsinization of tissue sections further enhances the sensitivity of the PAP technique[5].

We wish to report the combined use of trypsinization and PAP methodology for the tissue immunolocalization of lysozyme in normal human blood vessels.

Materials and methods. Routine human tissue biopsies from spleens (3), lymph nodes (6), livers (2), breasts (20), stomachs (3) and skins (5) were fixed in fresh but unbuffered 4% formaldehyde in normal saline for 24–72 h. Thereafter the tissues were dehydrated, cleared in toluene and embedded in paraffin wax (m.p. 56 °C). Endogenous peroxidase activity was blocked by 0.5% H_2O_2 in MeOH and trypsinization (Flow Laboratories) carried out as described previously[6]. Antisera to human lysozyme raised in rabbits (Dako) and goats (Nordic) were applied to the treated 5 µm thick sections for 30 min at a dilution of 1:200 in phosphate buffered saline pH 7.5. After washing in tris-saline, swine anti-rabbit IgG or rabbit anti-goat IgG (Dako) was applied at a dilution of 1:50. After further washing rabbit or goat PAP complex diluted 1:40 was added for 45 min. Staining was achieved with ethylcarbazole and N,N dimethylformamide in acetate buffer pH 5 and H_2O_2[6]. Counterstaining with hematoxylin and mounting in glycerin jelly completed the procedure.

Absorption of the antisera with neat and diluted human tears, saliva, purified human lysozyme (ex Dr E. F. Ossermann, New York), hen's egg lysozyme and *a*-elastin peptides[7,8] was performed by incubation with an aliquot for 16 h at 4 °C. Corresponding paraffin sections from the same tissue biopsy blocks were stained by H & E, orcein, Weigert's elastic, and Congo blue stain for elastic fibers[9]. Snap-frozen unfixed breast tissue was examined by indirect fluorescence and the above PAP method. For indirect fluorescence the rabbit anti-human lysozyme was applied at a dilution of 1:10 for 30 min, and after washing, a sheep anti-rabbit IgG 1:10 for 30 min.

Results. Both rabbit and goat antisera to human lysozyme yielded marked immunostaining of elastic fibers in the media and adventitia of normal arteries and veins (fig. a) in paraffin sections of the formalin-fixed organ biopsies. All 3 elastic stains corresponded precisely with the fibers



Vein (top) and artery (lower) in breast adipose tissue. *a* Lysozyme immunostaining of elastic fibers in the media and adventitia of both vessels. *b* Total abolition of lysozyme staining after absorption of primary antiserum by human lysozyme. Paraffin sections, PAP preparations with hematoxylin counterstaining. Both × 70.

stained in the lysozyme PAP preparations. Omission of the primary antisera, substitution of rabbit antisera to unrelated human antigens (α-lactalbumin, casein), or of inappropriate PAP reagents abolished the immunostaining.

Absorption of the antisera to human lysozyme with neat and 1:10 dilutions of human tears, saliva and 1 mg human lysozyme in 1 ml saline completely abolished immunostaining (fig. b). Hen's egg lysozyme and α-elastin peptides were without effect.

Trypsin digestion was a necessary preliminary step; no lysozyme immunostaining of elastic fibers in the paraffin sections was found with either antiserum in its absence. Only relatively weak elastic-fiber immunoreactivity was found with the indirect fluorescence and PAP methods on normal vessels in snap-frozen sections.

Discussion. Use of the techniques described here have confirmed the immunolocalization of lysozyme in the other tissue components described previously[1,2]. It seems likely that the unmasking of tissue antigens by preliminary trypsinization[5], and the sensitivity of the PAP method[3] are responsible for the demonstration of lysozyme in vascular elastic fibers of formalin-fixed, paraffin-embedded tissue.

Lysozyme is a cationic protein. Charge attraction appears to be responsible for its interaction with mucin[10] and cartilage proteoglycan[11]. Only limited anionic groups are found in elastin[12]. They may contribute to the affinity of lysozyme for elastic fibers, but the presence of adjacent glycoprotein microfibrils with a greater proportion of diacidic aminoacids[13] and anionic glycosides seem more likely to account for the selective localization of lysozyme in elastic fibers.

The biological role of lysozyme in elastic fibers is currently completely unknown. In the absence of an appropriate glycosidic substrate it is tempting to speculate the lysozyme may assume a nonenzymatic function[14]. In high concentration lysozyme inhibits the activity of collagenase[15], and has been shown to inhibit the proteolysis of proteoglycans by elastase[16]. Possibly, lysozyme may thus protect elastic-fiber components from in-vivo proteolysis.

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On the distribution of plasma L-asparaginase[1,2]

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Summary. The guinea-pig, *Cavia porcellus,* is unusual in possessing plasma L-asparaginase, an enzyme with anti-tumor activity. 21 additional species have been examined as to the presence of this enzyme: the results confirm and extend its remarkably limited species distribution.

Recently we drew attention to the fact that there are several significant biochemical differences between the guinea-pig and a number of other mammals[4]. Two of the most striking of these are the presence of plasma L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) in the guinea-pig, and an insulin amino acid sequence that is strikingly different from that of porcine and bovine insulin. These biochemical peculiarities are shared by the guinea-pig (and a few other members of the same superfamily, i.e., Cavoidea) and New World monkeys, species which otherwise have little in common except that their evolutionary ancestors arrived in South America during the late Eocene or early Oligocene[5-10].

The appearance of similar traits in unrelated species sharing the same biospace might logically be considered as convergent evolution. One can speculate, for example, that New World parasites might have existed which lacked the capacity to synthesize asparagine, and that guinea-pigs and New World monkeys independently developed resistance to these parasites by evolving a serum asparaginase, although there is no evidence to support this suggestion.

No obvious biochemical purpose is met by plasma L-asparaginase but the enzyme is of interest, aside from questions about its limited distribution, because of its anti-tumor activity (see Wriston, Jr et al.[11] for review). In fact, it was the anti-tumor activity of guinea-pig serum against certain transplanted mouse tumors that led to the discovery and isolation of L-asparaginase from guinea-pig serum[12-15] and indirectly to the discovery of an *E. coli* asparaginase with anti-tumor activity[16].

22 species were examined for plasma asparaginase in connection with the early work on the guinea-pig serum enzyme. We recently became interested in screening additional species because of the possibility that the results might shed light on the still unsettled question as to whether the ancestors of present day South American rodents and primates came from North America or Africa. These results, together with those of the earlier workers, are summarized in the table.

Methods. Asparaginase activity was determined by direct nesslerization as previously described[17]. Samples were obtained from the frozen sera collections of the National Zoological Park/Smithsonian Institution, Washington, D.C., and the Philadelphia Zoological Garden, Philadelphia, PA.

Results. Because most of the recent assays were done on a single serum sample which had often been stored frozen for