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A collagen and elastic network in the wing of the bat

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INTRODUCTION

The thin and translucent wing membrane of the bat is formed by a double thickness of skin and a central hypodermis in which the large blood vessels, nerves and skeletal muscle fibres are located (Gupta, 1967). The epidermis and dermis of the skin are approximately equal in thickness; the dermis is a single zone that is equivalent to both the reticular and papillary dermis of the skin of other mammals (Gupta, 1967). It is composed predominantly of interwoven collagen bundles and a few elastic fibres, although in some instances elastin may be a component of dermal spicules and cartilaginous plates (Quay, 1970). Elastic fibres, however, are abundant in the hypodermis and are so well organized that they form patterns in the wing that are apparent on gross observation. Allen (1889) recognized bundles of these fibres in an extended, transilluminated bat wing and described them as 'delicate trabeculae'. Systems of elastin fibres are now recognized as important connective tissue superstructures that act in concert with, or in place of, specialized skeletal muscle in the wing membrane (Quay, 1970; Vaughan, 1970*a*).

In recent ultrastructural studies of *Tadarida braziliensis* we discovered a system of fibres that was organized into a net-like scaffolding. The organization and the ultrastructural characteristics of this uniquely regular mesh were studied in untreated, digested and dissected samples of the wing membrane, and from isolated preparations of the component fibre bundles, by light and transmission and scanning electron microscopy. The identification of elastin and collagen in this fibre system was based, first, on the ultrastructural evidence and was determined conclusively by amino acid analysis.

The combination of elastin and collagen in a fibrous net should permit flexibility yet provide tensile strength and limit extensibility of the wing membrane, all properties which are clearly significant for flight. In a broader sense, however, this apparently unique arrangement of fibres should provide a most useful model for inquiry into the mechanical properties of combined elastin and collagen and for study of developmental and physiological aspects of pattern formation, and its control and maintenance.

MATERIALS AND METHODS

Animals

Mexican free-tailed bats, *Tadarida braziliensis* (Family: Molossidae) were caught in the wild and supplied to us by Dr Philip Leitner, St Mary's College, Moraga, California. A colony of these animals is maintained currently under the supervision of Dr Marion Shaw (Department of Physiology and Biophysics and Department of

Table 1. *Amino acid composition of digested and isolated bat wing fibres compared with bovine ligamentum nuchae* and insoluble bovine skin collagen† (residues/1000)*

	Digested bat wing fibres	Isolated bat wing fibres	Ligamentum nuchae elastin	Insoluble skin collagen
Hydroxyproline	12	32	9·6	94
Aspartic acid	6	?	5·4	46
Threonine	15	27	6·3	17
Serine	15	29	5·3	36
Glutamic acid	22	54	15·5	72
Proline	110	100	116	127
Glycine	318	286	334	326
Alanine	230	137	223	112
Valine	77	86	145	23
Methionine	0	2	—	6
1/2 Cystine	0	12	—	—
Isoleucine	30	26	27·6	11
Leucine	52	70	64	25
Tyrosine	28	11	7·5	3
Phenylalanine	4	30	32·4	13
Histidine	29	7	0	5
Hydroxylysine	0	2	—	6
Lysine	3	19	3·5	27
Arginine	15	30	4·3	51

* Francis *et al.* (1973).
† Volpin & Veis (1971).

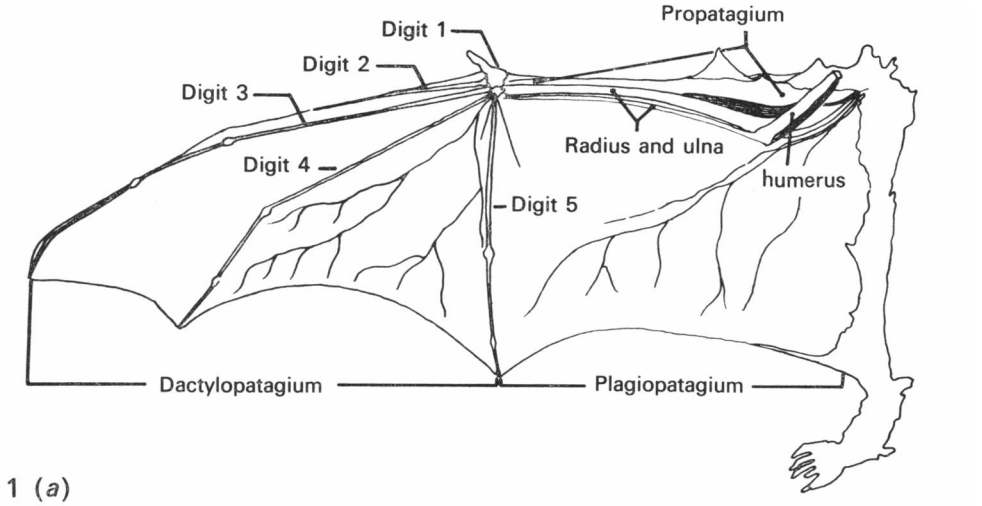
Medicine) who is our collaborator in physiological and morphological studies of microcirculation in the wing membrane.

Preparation of wing specimens

Dissection of the wing and isolation of fibre bundles. To prepare wings for dissection a bat was killed and the wings after removal were extended and pinned to a cork with the ventral surface upward and flooded with cold half-strength Karnovsky (1965) fixative. The tissue in the central plane of the wing was exposed by slitting the skin over the bones of the limb and peeling it away from the surface with a watchmaker's forceps. The dissected, wet specimens were photographed and then one wing was cut into small segments and prepared for scanning electron microscopy (SEM). Individual fibre bundles of the network were teased from the other wing and prepared for transmission electron microscopy (TEM) and amino acid analysis.

Digestion of the wing. The wings were excised, placed between two layers of nylon mesh and framed in a 3×4 lantern slide holder to maintain the flat and extended position throughout the subsequent steps in the digestion procedure. The specimens were treated with 10 % acetic acid for 24 hours, dehydrated through a graded series of alcohols (24 hours) and immersed in methyl salicylate (24 hours). After a second series of dehydrations (24 hours) the wings were submerged in 10 % KOH at 37 °C. In less than 1 hour the majority of the wing was digested and a translucent network of connective tissue fibre bundles was exposed. These wet preparations were photographed, then large segments of the mesh were prepared for SEM, TEM and amino acid analysis.

Control preparations of the wing. Small fragments of untreated bat wings were immersed in cold fixative (Karnovsky, 1965) for several hours, washed in 0·1 M cacodylate buffer and prepared for light microscopy (LM) and TEM.



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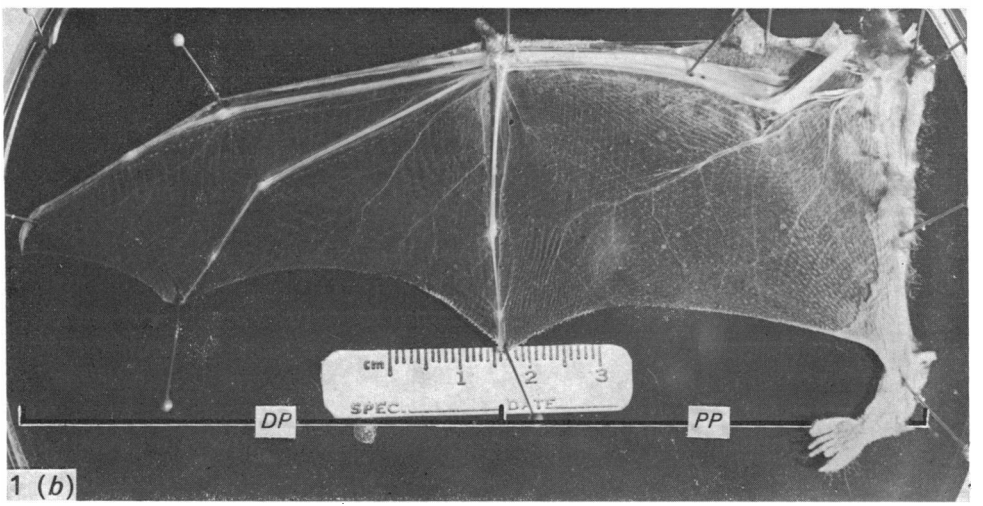


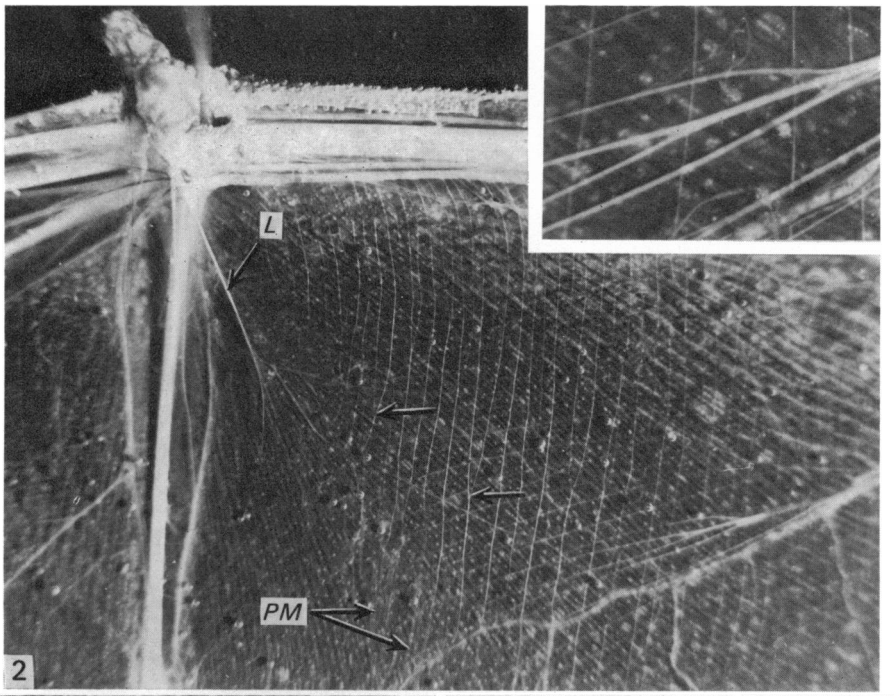
1 (*b*)

Fig. 1(*a*). Anatomical regions of the bat wing membrane. (*b*). Fibre network in a dissected right wing. In the plagiopatagium (*PP*) and dactylopatagium (*DP*) note the network and parallel arrangement of fibres respectively. ×0·93.

Microscopy

Light microscopy and transmission electron microscopy. Samples of untreated dissected and digested wing membranes and isolated fibre bundles that were fixed in Karnovsky (1965) were post-fixed for an additional hour at room temperature in 1 % OsO_4 in distilled water. Tissues and fibre bundles were dehydrated through a graded series of alcohols into propylene oxide and embedded in Epon 812 (Luft, 1961). Thick sections (1 μm) for LM were stained by the method of Richardson, Jarrett & Finke (1960) and with the bichrome stain of Fahrenbach (unpublished). Thin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and viewed in the Philips 201 TEM.

Scanning electron microscopy. The specimens for SEM were treated as for TEM up through the alcohol dehydrations, then were dehydrated further into solutions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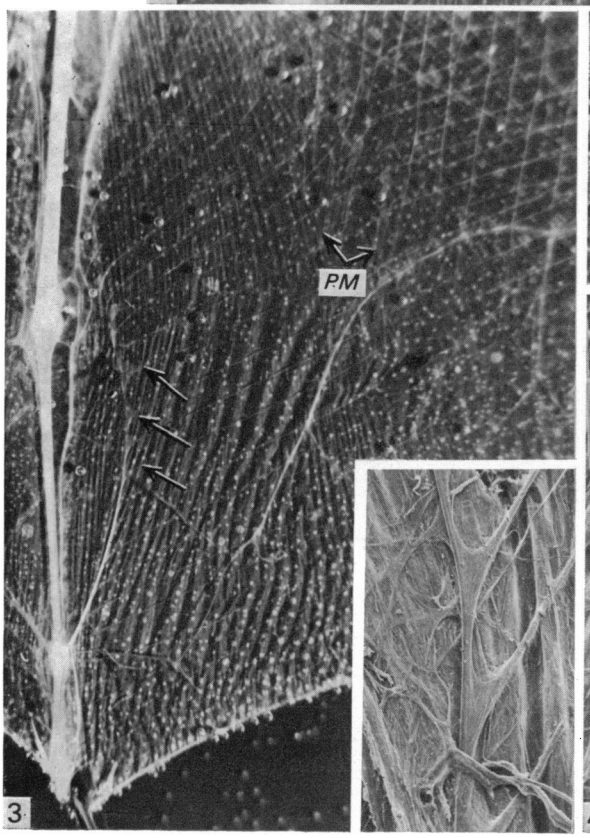
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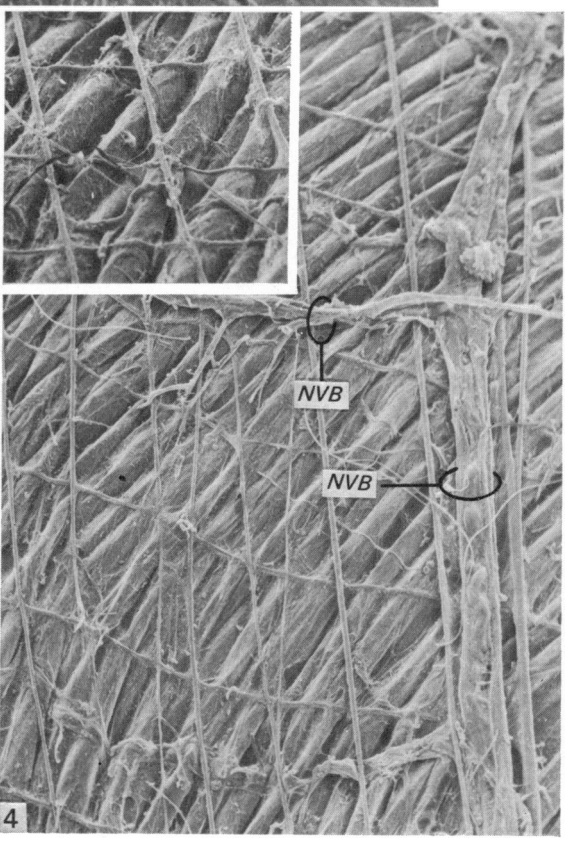
NVB
NVB
4

of Etoh-Freon TF (2:1, 1:1 and 1:2) for 20 minutes each and in 100% Freon TF for 30 minutes. The network of the digested specimens was oriented on Freon-wetted filter paper. All samples were transferred into the pressure chamber of a Bomar SPC-50 critical point bomb, flushed with Freon 13 and taken through the critical point for drying (Cohen, Marlow & Garner, 1968). Dried network specimens were lifted off the paper and, like the tissue samples, were affixed to aluminium stubs with silver conducting paint and coated with a thin layer of gold–palladium alloy in a Technics Hummer II sputter coating instrument. Specimens were viewed in an Etec Autoscan SEM operated at 20 kV in the secondary electron mode.

Amino analysis

Samples of the digested bat wing network and of isolated fibre bundles were analysed for amino acid (aa) composition. Each preparation was dried and then hydrolysed for 24 hours at 110 °C in constantly boiling HCl under nitrogen. The hydrolysate was dried by rotary evaporation, the sample applied to a Durram 500 amino acid analyser with an automatic integrator, and the amino acids quantitated. No corrections were made for hydrolytic losses. The percentages of elastin, collagen and 'other protein' in the untreated, isolated fibre bundles were calculated from the data (Table 1) by assuming that, typically, elastin contains 12 hydroxyproline/1000 aa, collagen has 100 hydroxyproline/1000 aa, collagen and elastin have 340 glycine/1000 aa and 'other protein' consists of 100 glycine/1000 aa (Francis, Rhys & Thomas, 1973; Volpin & Veis, 1971).

RESULTS

Regions of the wing membrane

Portions of the wing membrane are named according to their relationship with the body wall and the bones of the appendages (Fig. 1*a*). Together, the plagiopatagium (PLP) and the propatagium (PP) form a nearly rectangular segment of the membrane that is bounded medially by the bones of the hind limb and the lateral body wall, anteriorly by the leading edge of the wing, laterally by the bones of the first and fifth digits and posteriorly by the trailing edge. Included within the boundaries of the PP and PLP are the bones of the upper limb. Portions of the wing membrane between the digits comprise the dactylopatagium (DP).

Organization of the fibre patterns: light microscope and scanning electron microscope observations of dissected specimens

In the living bat, a network-like scaffolding was observed in the PP and PLP portions of the semi-transparent wing membranes that were extended over a light

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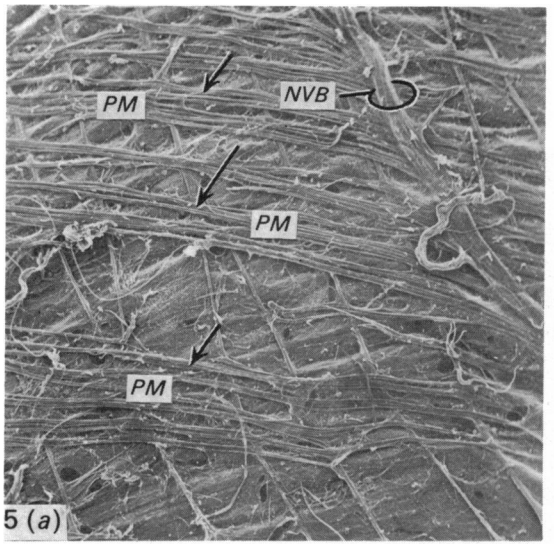
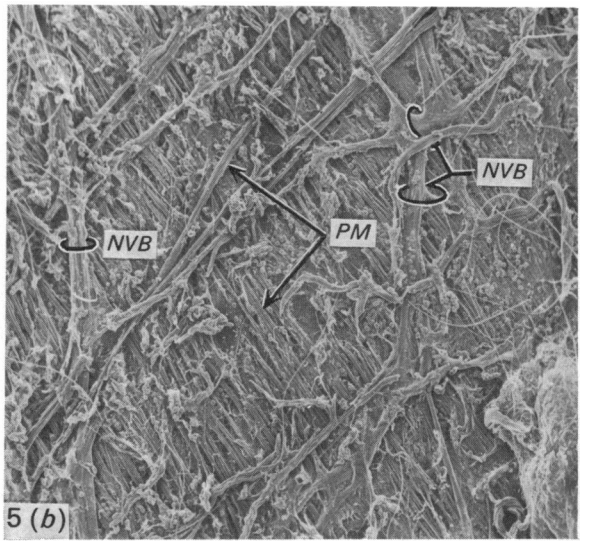
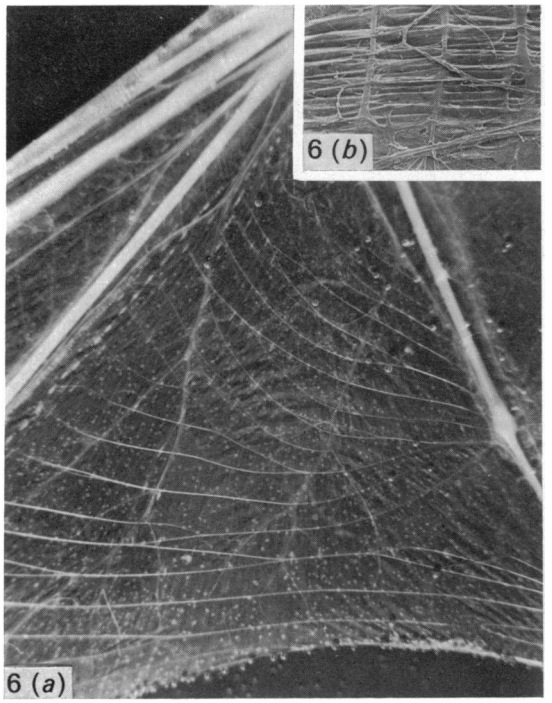
Fig. 2. Fibre network in the plagiopatagium. Arrows indicate vertical and horizontal fibre bundles that branch, coalesce and change direction. A ligament (*L*) from the radio-ulnar–carpal joint and a branched tendon (inset) integrate into the network. Plagiopatagiales muscles (*PM*) are visible as parallel, vertical lines. ×3·3. Inset. ×10.

Fig. 3. Fibre network and ligamentous arcades in the plagiopatagium. Note the origin of horizontal–diagonal fibre bundles (arrows) from the ligamentous arcades. Plagiopatagiales (*PM*) muscles are evident. ×3·3. Inset. ×23.

Fig. 4. SEM of the fibre network and neurovascular bundles (*NVB*) in the plagiopatagium. ×20. Inset. Wrinkling of the skin indicates the direction of tension on the skin by the fibres. ×35.







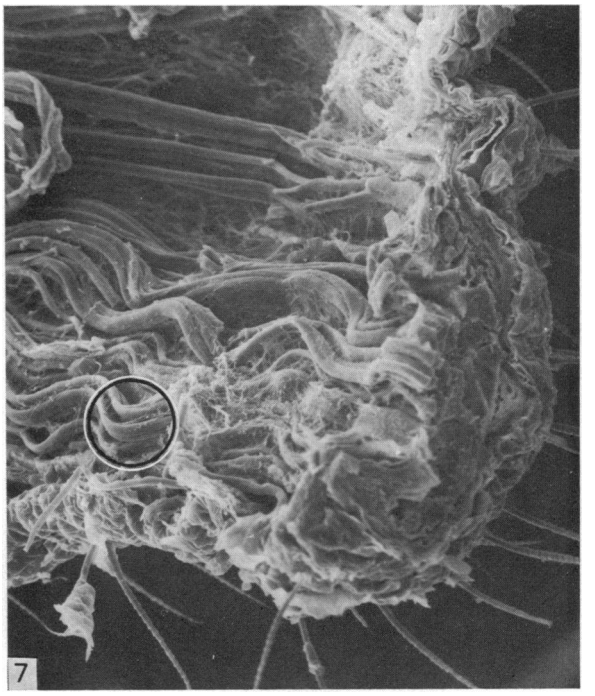


Fig. 5. SEM of plagiopatagiales muscles (*PM*). (*a*) Horizontal muscle fibres are associated with the fibrous network (arrows) and neurovascular bundles (*NVB*) in a region distal to the body. (*b*) More proximally, horizontal and vertical muscle fibres and NVB obscure or replace the fibrous network. (*a*) ×20. (*b*) ×18.

Fig. 6. Parallel fibre bundles in the dactylopatagium. (*a*) Branched fibrous bundles occur between digits 4 and 5. ×3·3. (*b*) SEM of the single direction fibres. Note the direction of wrinkling of the underlying skin. ×10.

Fig. 7. SEM of skeletal muscle bundles that reinforce the trailing edge of the wing. Note the banding (circled) of the individual fibres. ×140.

box. In the DP parallel 'braces' were observed *in lieu* of a network. Details of both patterns were seen more clearly by LM and SEM in dissected specimens that had had the skin removed from one surface (Fig. 1*b*). In general, the network in the PLP consisted of parallel, equally spaced, vertical fibre bundles that extended from the humerus, radius and ulna to the trailing edge of the wing membrane (Fig. 2). The vertical fibre bundles were crossed by a system of also parallel and equally spaced horizontal–diagonal fibre bundles which branched from ligamentous arcades between the joint capsules of the metacarpal and phalanges of the fifth digit (Fig. 3). The skin beneath the fibre network was corrugated in a direction which suggested that equal tension in the net-like region was exerted on the wing membrane by both the vertical and horizontal–diagonal fibre bundles (Figs. 2, 4).

Complication of this basic system was imposed by (1) branching of fibre bundles of both directions, (2) interjection of fibre bundles from tendons of appendicular muscles, (3) intercalation of radiating ligaments from the radio-ulnar–carpal joint (Fig. 2), (4) intrinsic skeletal muscle of the wing, and (5) the pattern of the neuro-vascular elements (Fig. 4). Several of the vertical fibre bundles gave off a horizontal branch (or changed direction without branching) in the mid-portion of the wing which could be traced to the ligamentous arcade (Fig. 2). Other fibre bundles originated independently, then coalesced (Fig. 2). In all instances, branched and confluent fibre bundles were integrated into the pattern of the network. Similarly, fibre bundles from other sources were inserted into the network without loss of ordered structure; the tendon of the humeropatagialis muscle subdivided and contributed 8–10 horizontal branches to the mid-part of the wing, then inserted into the ligamentous arcades (Fig. 2, inset). Finally, it was observed that intrinsic plagiopatagiales muscles were oriented in parallel with the vertical fibre bundles (Figs. 2, 3) and, to a lesser degree, other muscles proximal to the body wall were in parallel with the horizontal–diagonal fibre bundles (Fig. 5*a*). The abundance of the latter muscle partially obscured the fibre network and, in some areas, may have replaced it (Fig. 5*b*).

Branched fibre bundles in the DP (Figs. 5, 6) spanned the wing membrane between the digits in a single direction only. This was apparent from the direction of wrinkling of the skin relative to the fibre bundles (Fig. 6, inset). Between digits 4 and 5, fibre bundles were oriented in a horizontal–diagonal direction, but assumed a nearly vertical–diagonal course between the third and fourth digits. The area of the wing membrane between digits 2 and 3 was too limited to make observation of fibre bundles feasible (Fig. 6).

The trailing edge of the wing membrane was reinforced along its length by a large bundle of skeletal muscle fibres (Fig. 7).

Organization of the fibrous network: scanning electron microscopy of digested specimens

The fibre network, suspended only by its bony and connective tissue supporting structures, was isolated from the rest of the wing by digestion. In wet preparations the fibre bundles appeared as well defined, refractile tendon-like bands that lay in two (and in some areas in three) separate planes (Fig. 8*a*). When the same specimen was prepared for SEM, further detail of the network was apparent (Fig. 8*b*). Each fibre bundle in the network was ensheathed by a finely filamentous reticulum (Fig. 8*c*) and was assembled from smooth surfaced, 1 μm fibres that branched (Fig. 8*d*).

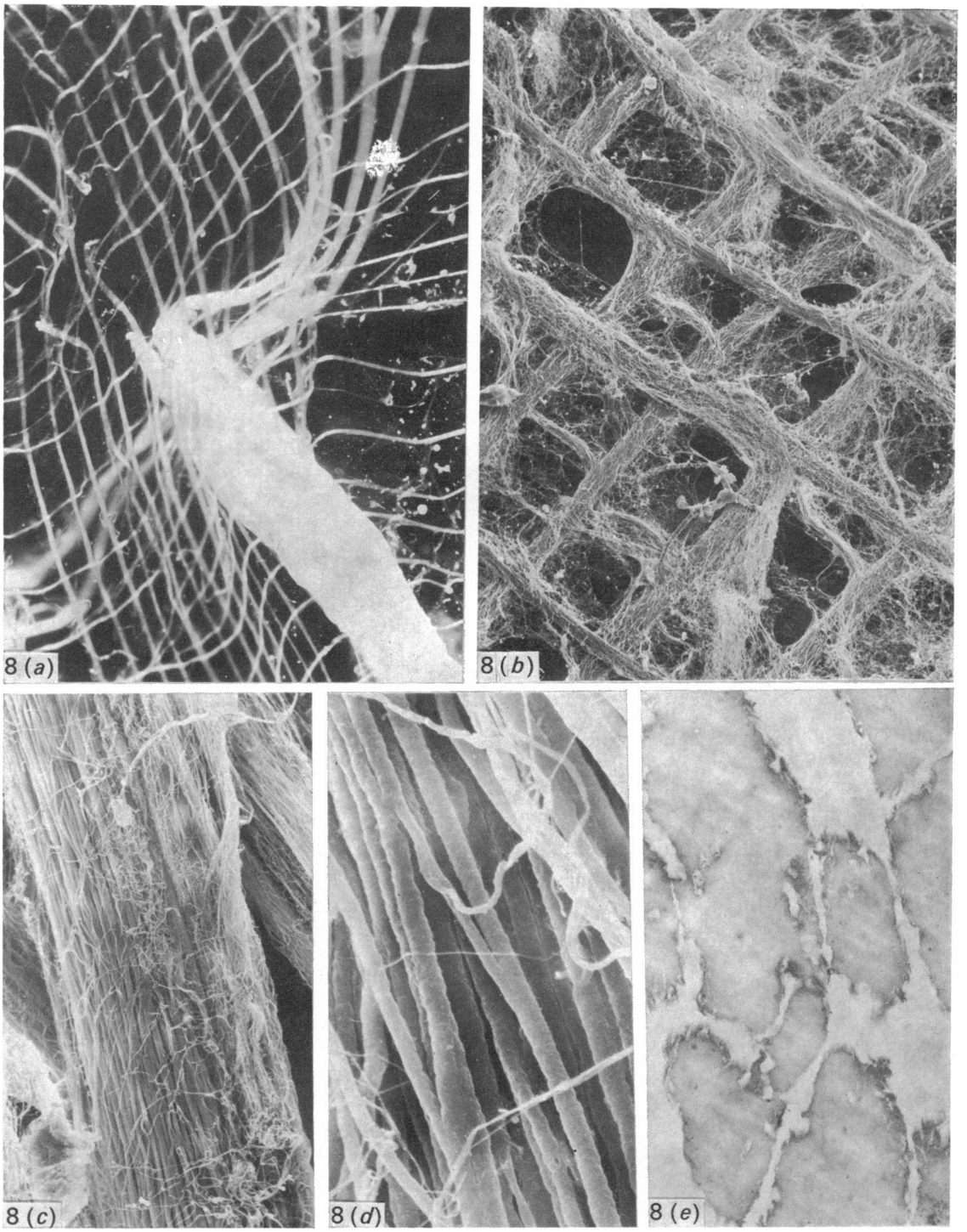
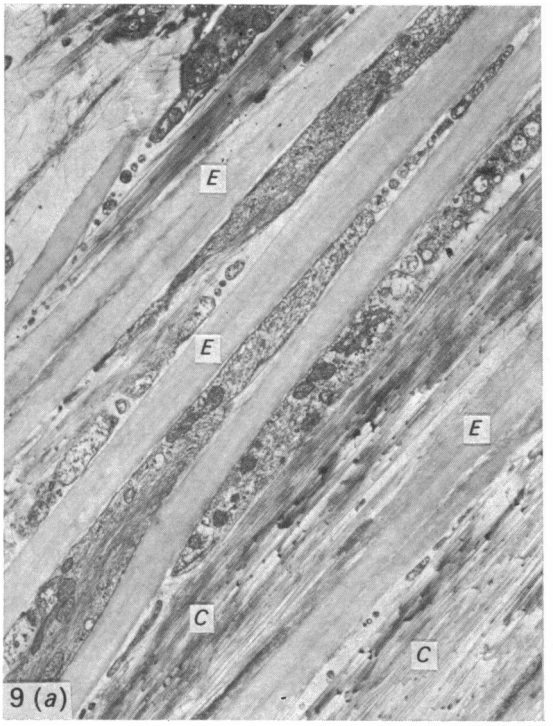
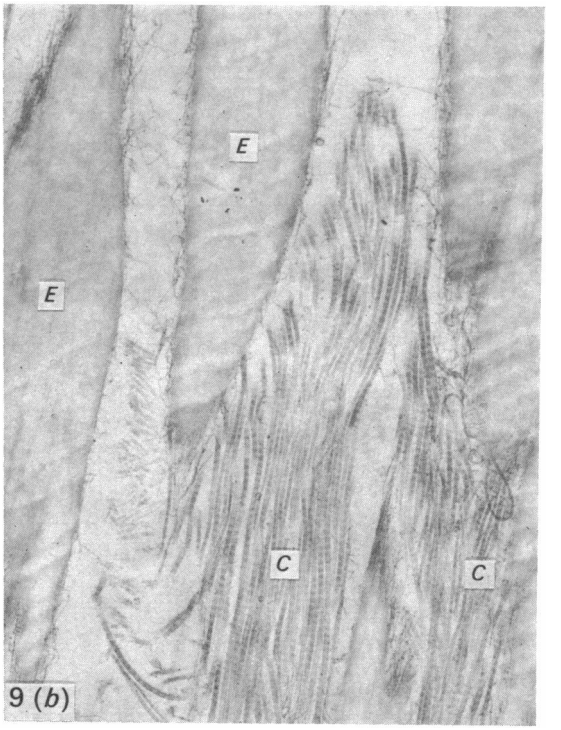
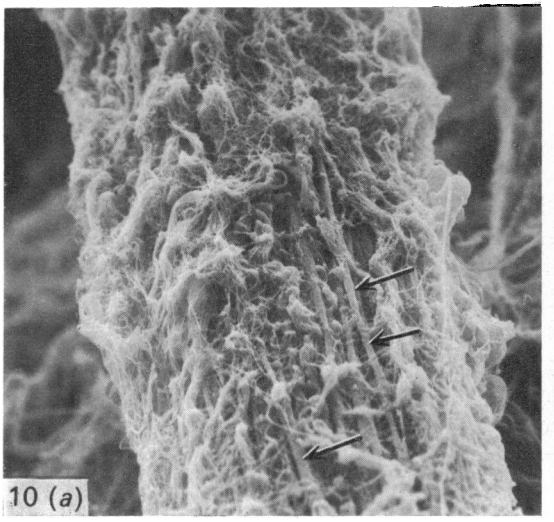


Fig. 8. Digested bat wing preparations. (*a*) Fibrous network of a wet preparation. ×16. (*b*) SEM of the fibrous network. ×60. (*c*) SEM of a fibre bundle of the network. Note the large smooth-surfaced fibres and the finely filamentous sheath. ×590. (*d*) SEM of smooth-surfaced fibres of a bundle. Note branching. ×1800. (*e*) TEM of fibres in a fibre bundle. Note the absence of internal structure. ×10 000.







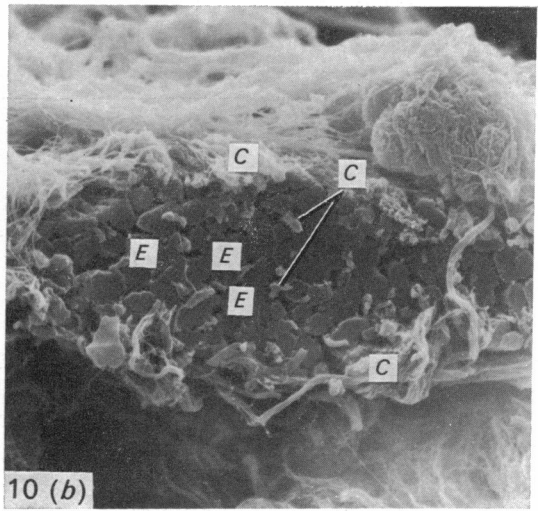


Fig. 9. TEMs of fibre bundles from control specimens. (*a*) Collagen fibrils (*C*), elastic fibres (*E*) and fibroblasts are organized in parallel. ×4700. (*b*) Collagen fibrils and the microfibrillar portion of the elastic fibres associate in close contiguity. ×15000.

Fig. 10. SEMs of fibre bundles from dissected wings. (*a*) Bundles of collagen fibrils ensheath fibres of the bundle. Note the smooth-surfaced elastic fibres internally (arrows). ×1570. (*b*) Fibre bundle sectioned transversely; note the superficial collagen and the small collagen bundles (*C*) interspersed with the large diameter elastic fibres (*E*). ×2270.

The structure of these component fibres was characteristic of elastic connective tissue, although the fibre diameter was about 5 times less than that reported from SEM studies of ligamentum nuchae (Gotte, Mammi & Pezzin, 1972).

Composition of the fibre bundles

Digested specimens. Identification of the fibres as elastic fibres was confirmed from TEM studies of embedded, digested preparations and by amino acid analysis. They were comparable morphologically with the elastin component (the central amorphous portion of the fibre) but were devoid of the fibrotubular elastic microfibrils that are characteristic of chemically unaltered elastic connective tissue (Ross & Bornstein, 1969) (Fig. 8*e*). The amino acid analysis of the fibre network (Table 1) showed a high content of glycine and alanine, a small amount of hydroxyproline, no hydroxylysine and almost no polar amino acids. Such a composition is characteristic of elastin, as can be seen by comparison with the alkali-resistant elastin from the bovine ligamentum nuchae (Francis *et al.* 1973), and differs from collagen in its lack of hydroxylysine, relatively low hydroxyproline and lack of polar amino acids (Volpin & Veis, 1971). The differences between bat wing elastin and elastin from bovine ligamentum nuchae probably reflect species differences as well as the presence in the bat wing preparation of a small amount of non-elastin protein left as a result of incomplete digestion.

Untreated control specimens. To determine whether the fibrous systems were composed exclusively of elastin, sections of untreated wings were studied by LM and TEM. In thick sections a second type of fibre was identified on the basis of the staining reactions. This fibre was dark blue, did not branch, and its staining was equivalent in colour and intensity with that of the fibrous connective tissue of the dermis; other pink fibres showed frequent sites of branching. The nature of this heterogeneous composition of the fibre bundles was resolved by TEM; elastic fibres, bundles of collagen fibrils, and fibroblasts, were organized compactly and in parallel (Fig. 9*a*). Spaces between the fibrillar components were seen only at the interface between cells and fibres, and could be accounted for most probably by the shrinkage that is associated with processing of tissue through organic solvents. Again, elastic fibres were identified by their characteristic branching, predominantly amorphous internal structure and, in these preparations, by the surface-related microfibrillar component. Collagen fibrils were non-branching and were diagnosed by their typical pattern of banding. The two types of fibres occurred in a close anatomical relationship (Fig. 9*b*). The microfibrillar portion of the elastic fibres and the collagen fibrils appeared to be contiguous. A large number of fibroblasts was integrated into these bundles and formed a sheath-like layer about the surface.

Dissected specimens. By SEM it was seen that coarse accumulations and finely interwoven fibrils of collagen were oriented randomly about the surface of the fibrous bundles (Fig. 10*a*). The relative contribution of these fibrils was appreciated by comparing the fibrous bundles of dissected specimens with those of the digested wings (Fig. 8*c*). From SEM observations of transversely sectioned fibre bundles (Fig. 10*b*) it seemed that the oriented *internal* bundles of collagen fibrils accounted for only 5–10 % of the fibres.

Isolated fibres. The contribution of collagen to the fibre bundles was determined with greater accuracy by amino acid analysis of horizontal and vertical fibre bundles which had been stripped from the PLP and DP of dissected wings. At the same time, a portion of these fibre bundles was embedded and studied by TEM for morph-

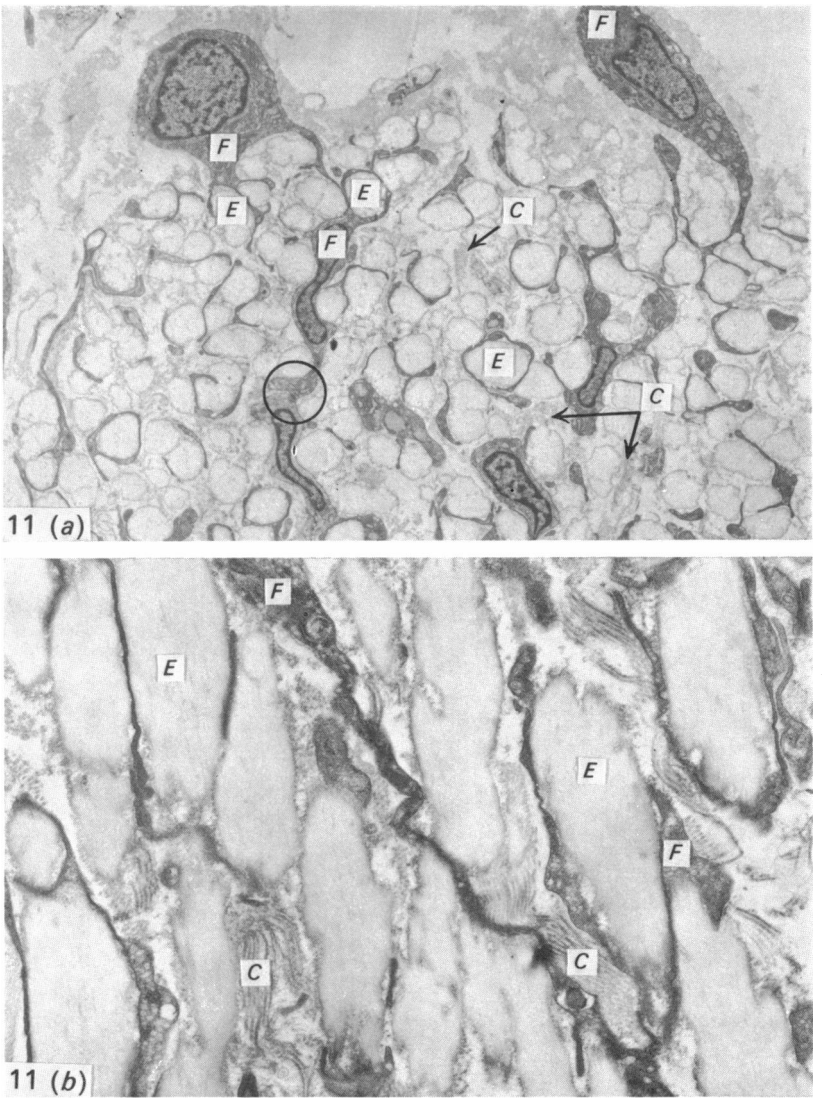


Fig. 11. TEMs of isolated fibre bundles. (*a*) Fibroblasts (*F*) join with one another by focal junctions (circled) and extend slender processes that ensheath individual elastic fibres (*E*). Small bundles of collagen fibrils (*C*) are also apparent. × 3660. (*b*) Elastic fibres (*E*) and collagen fibrils (*C*) and fibroblast processes (*F*) are associated closely in the fibre bundles. × 10500.

ological verification of the material analysed. The results of the amino acid analysis indicated that approximately 50 % of the total protein in the bundles was elastin, 25 % was collagen (internal and surface associated) and another 25 % represented 'other protein' (Table 1). The last protein fraction may be accounted for by the unsuspected isolation of skeletal muscle fibres, and by the large numbers of cells that were part of the bundles. The TEM studies of isolated fibres revealed occasional skeletal muscle fibres in the preparations and showed extensive involvement of fibroblasts with individual elastic fibres. These cells appeared to form a reticulum within the fibre bundle; they joined with one another by focal junctions and, in any one plane of section, nearly all elastic fibres appeared to be enveloped individually

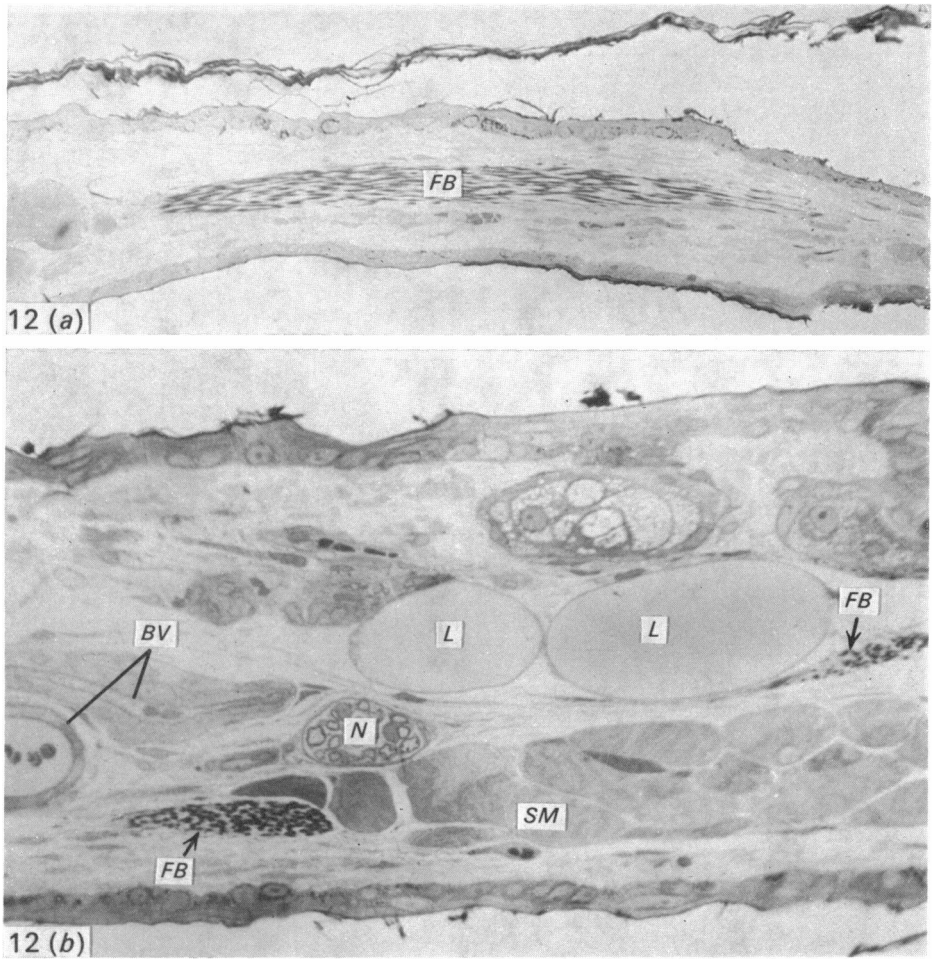


Fig. 12. Fibre systems in relation to other tissues in two wing regions. (*a*) The fibre bundle (*FB*) occupies a major portion of full thickness wing in the dactylopatagium. Collagen fibrils are stained lightly and elastic fibres are dark. ×100. (*b*) Fibre bundles (*FB*) in the plagiopatagium are seen in relation to skeletal muscle (*SM*), nerves (*N*), blood vessels (*BV*) and lipid droplets (*L*). ×120.

by a slender process of the cytoplasm (Fig. 11*a*, *b*). Collagen fibrils also were in close contact with the fibroblasts, but were not enclosed by processes in the same manner. From the nature of the cytoplasm, in particular the dilated rough endoplasmic reticulum, and the intimate association of cells with both elastin and collagen, it appeared that the fibroblasts were metabolically active.

Relationships of the fibre systems with other tissues of the wing

The fibre systems were located in the hypodermis where, in the DP, they filled the central plane and occupied as much as 50 % of the thickness of the entire wing membrane (Fig. 12*a*). In the thicker, proximal portions of the wing (PLP) the fibre system accounted for less than 10 % of the wing thickness and was surrounded both dorsally and ventrally by blood vessels, nerves, skeletal muscle fibres and large lipid droplets (Fig. 12*b*). Muscle fibres were absent from the hypodermis of the DP. The

sparsity of fibrous connective tissue in the hypodermis of all areas permitted all structures, including the underlying (dorsal) skin, to be seen easily in dissected specimens (Figs. 4; 5*a*, *b*; 6*b*).

DISCUSSION

The ubiquity of elastic fibres in the wings of bats has been recognized for nearly a century, but their organization has been described in only a few studies (Vaughan, 1966, 1970*a*, *b*; Iverson, Bhangoo & Hansen, 1974). Usually, the fibres are regarded as being arranged in parallel and branching bands, although Iverson *et al.* (1974) do sketch it as a network. Failure generally to recognize the network is surprising because in published photographs and museum drawings the organization may be clearly seen through the thin wing membrane. Regardless of the overall manner of organization of the fibre systems, however, functional interpretations appear to have been based largely upon the intimate associations between the fibres and the specialized muscles of the wing.

In this context it should be noted that the fibre bundles provide for the insertion of skeletal muscle (Quay, 1970; Vaughan, 1970*b*). When the muscles contract they tense the fibres and so tense the wing membrane. The effect on the wing membrane is seen clearly in the scanning preparations, where the skin underlying the fibres is corrugated (Figs. 4, 6*b*). Tension on the membrane is important in flight when the wing is partly flexed and yet must remain taut, particularly the proximal segments (PLP and PP) which have the major role in providing the lift force during flight (Vaughan, 1966). Tautness of the wing membrane not only maintains the shape of the wing as an efficient air foil, but also limits distortion of this surface from the forces of the airstream (Vaughan, 1966).

Anatomical specializations, such as fibre systems, which reinforce the wing, increase its resistance to puncture (Studier, 1972) and enable it to withstand the negative and positive forces on the superior convex and inferior concave surfaces respectively, would appear to be particularly essential in *Tadarida*, and indeed in Molossid bats in general, for the long, narrow wings of these bats are adapted for rapid and sustained flight (Struhsaker, 1961). The well organized net-like system of fibre bundles in *Tadarida* would seem to be an effective superstructure for providing strong, equally distributed reinforcement of the critical wing segments. The collagenous component of this system, which has not been recognized previously, would contribute tensile strength and limit extensibility. Collagen in other elastic systems, such as elastic ligaments, is believed to limit strain at high levels of stress (Gibson & Kenedi, 1970).

In general, connective tissue structures which are organized from both elastic and collagen tissue usually occur in organs which are prone to mechanical stress and to stretching in two or more directions. Examples of such tissues include ligaments, tendons, periodontium, epineurium, perineurium, fibrous cartilage and the adventitia of blood vessels (Fullmer, 1958; Gawlik, 1965). In each structure it is typical for one fibre type to predominate. The bulk of elastic ligaments (ligamentum nuchae and ligamentum flavum) and elastic laminae is elastic connective tissue, although a small quantity of collagen is present as well. In this respect, the individual fibre bundles of the network in the PLP and the parallel bundles of the DP appear to be comparable to systems of elastic ligaments. The elastic fibres dominate the bundles, but the microfibrillar portion of the elastic fibre appears to be contiguous with collagen fibrils (Figs. 9*b*, 11*b*). Adhesion of these two components in aggregations

of collagen and elastic connective tissue has been reported by others (Karrer, 1960; Schwarz, 1964; Kobayasi, 1968; Albert, 1972).

When compared with other elastin dominated structures, the bat's wing is possibly unique in the marked linearity of its elastic and collagen fibres. In most tissues where the two types of fibres occur together such linearity is observed primarily when the tissue is under tension. To a degree, this may also be true for the bat wing fibre bundles. In specimens where the wing was extended (stretched) for dissection, digestion or microcirculatory experiments and subsequently fixed for electron microscopy, the alignment of both fibre types was perfectly in parallel (Fig. 9*a*). This parallel alignment of collagen and elastin was still apparent, although less perfect, in isolated fibre bundles that were not processed under tension. In these specimens, all elastic fibres were oriented in the direction of the bundles; and although the collagen was aligned similarly, it appeared to assume a slightly sinuous course (Fig. 11*b*).

The cells, too, of the bat fibres have characteristics in common with fibroblasts of elastic ligaments. They project long slender processes that encompass elastic fibres and they connect with one another by specialized focal junctions. Similarly, elastic fibres in culture (Schwarz, 1964) and in the fetal calf ligamentum nuchae form in 'deep embayments' of the cell (Fahrenbach, Sandberg & Cleary, 1966). This relationship during elastogenesis has been interpreted as an indication of control over structural organization of the fibre (Greenlee & Ross, 1967). In the bat, we have now demonstrated a continuing relationship between cell processes and much more *mature* elastin fibres which has not been described in other elastic structures (Fig. 11*a*).

The specialized junctions seen among bat fibre bundle fibroblasts (Fig. 11*a*) have been reported also in the ligamentum nuchae of the fetal and newborn calf (Ross & Greenlee, 1966; Fahrenbach *et al.* 1966), fetal rat tendon, osteoblasts and odontoblasts (Ross & Greenlee, 1966). The observation that these contacts formed early in development and were maintained even after the accumulation of considerable numbers of fibres suggested that the cells performed a structural role in specialized connective tissue assemblies (Ross & Greenlee, 1966). Junctions of this nature are not found in connective tissue where cells are motile (e.g., in wound repair) (Ross & Greenlee, 1966).

For many reasons, the fibre system in the bat wing should prove a useful model for studies of mechanics, development, physiology and ageing of combined elastic and collagen connective tissue systems. First, individual fibre bundles in both the PLP and DP can be isolated with relative ease and with the advantage that mechanical properties of collagen and elastin in concert can be tested in a discrete morphological entity where both fibre types are aligned in parallel. Secondly, the network-like organization of the fibre bundles in the PLP would appear to be an intriguing system for inquiry into development, control and maintenance of a complex connective tissue system. It would be of particular interest to explore what, if any, role external forces play in the formation of this complex pattern. The influence of mechanical forces in moulding the arrangement and dimensions of elastic connective tissue during elastogenesis has been reported or discussed by others (Gawlik, 1965; Greenlee & Ross, 1967). In the bat system, the question of activity versus structure under varied conditions could be tested easily. Finally, one could explore whether the proportions of the collagen and elastin fibres, and their pattern of organization in the wing, might be related to age.

SUMMARY

Bundles of collagen fibrils, elastic fibres and fibroblasts are organized into a network that lies in the plane of a large portion of the bat wing. By ultrastructural (TEM and SEM) and biochemical analyses it was found that individual bundles of the net are similar to elastic ligaments. Although elastic fibres predominate, they are integrated and aligned in parallel with small bundles of collagen. A reticulum of fibroblasts, joined by focal junctions, forms a cellular framework throughout each bundle. Because of the unique features of the fibre bundles of the bat's wing, in particular their accessibility, and the parallel alignment of the collagen fibrils and elastic fibres in each easily isolatable fibre bundle, they should prove a most valuable model for connective tissue studies, particularly for the study of collagen–elastin interactions.

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