stalks to exceed 40% and was therefore unable to confirm this finding. The contracted spasmoneme displays the mechanical properties of a swollen rubber[2], therefore its volume may vary with stretching, increasing roughly as the square root of the extension ratio[15]. By restraining the contracted spasmoneme at the relaxed stalk length, it is held at an extension ratio close to three, and therefore the decrease in spasmonemal volume may be even greater during isotonic contraction.

Threshold divalent cation concentrations for glycerolated *Carchesium* spasmonemal contraction are listed in the Table. The threshold calcium ion concentration is similar to that reported for *Vorticella*[5] (included in the Table for comparison). Threshold is independent of both 10^{-2} g ion/l magnesium ion and 2×10^{-3} *M* ATP, and is only slightly sensitive to pH between 6.0 and 7.5. Within a mature colony, no difference in threshold was ever observed between the spasmonemes of older and of younger stalks, nor was any difference in threshold revealed between young and mature colonies. In mature colonies, the fragments of spasmoneme in the main stalk contracted at the same threshold as for the intact spasmoneme. The same was found in young colonies in which the spasmoneme had been disrupted by prolonged incubation in 10^{-6} g ion/l calcium ion. The spasmoneme was less sensitive to strontium and barium ions than to calcium, and much higher free ion concentrations were required to induce contraction. In other respects, stalk coiling and isometric spasmonemal contraction induced by strontium or barium ions was indistinguishable from those induced by calcium. Contraction was not induced by magnesium ion buffers with a free ion concentration of 10^{-2} g ion/l, even in the presence of ATP. Spasmoneme re-extension followed promptly on cation chelation by EGTA. In all cases, contraction re-extension cycles could be repeated many times.

The effectiveness relative to calcium of alkali earth metals in inducing contraction is

$$Ca(1.0) > Sr(0.006) > Ba(0.003) > Mg(0).$$

Similar sequences are typical of the equilibrium binding constants to acidic sites on proteins, for example the skeletal muscle troponin C calcium binding site[16], and of binding to organic polyacids such as α poly-L-glutamic acid[17]. It has been suggested that spasmonemal extension results from repulsion between fixed negative charges, contraction following charge neutralization through calcium binding[2,4]. It is interesting that large dimensional changes in crosslinked polyacid fibres may result from a simple change in the degree of fibre ionization. The high relative sensitivity to calcium, together with the pH and magnesium insensitivity, indicate some ion-binding specificity. It may be that spasmonemal mechanochemistry resembles those polyacid fibre contractions that accompany ion exchange between sodium and calcium, where shortening occurs abruptly at a critical monovalent/divalent cation ratio[18].

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Dense Particles in Subcutaneous Collagen Fibrils

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Summary. Electron microscopy of unstained, fresh air-dried spreads of the subcutaneous connective tissue disclosed dense particles (6–20 nm in diameter) at the dark bands of collagen fibrils with 67 nm periodicity. The particles appear to consist of chlorides from the X-ray microanalysis of collagen fibres.

Electron microscopy of fresh frozen dried ultrathin sections and fresh air-dried tissue spreads has disclosed, without any treatment (i.e. fixation, embedding or staining), various fine structures[2–4]. Escape from any contact with liquid throughout the specimen preparation is essential to study the distribution of diffusible substances in tissues at the electron microscopic level. The specimens thus prepared are applicable to electron probe X-ray microanalysis and dry mount autoradiography.

Energy dispersive X-ray microanalysis affords a good qualitative survey of elements, though not quantitative, in small areas of the electron microscopic level. It gives information on the distribution of mineral elements, with an atomic number of more than 11 (Na), which is not available with the microincineration procedure. In this technique, it is very difficult to overcome the loss and dislocation of the elements during the specimen preparation, and to identify the elements of the ash after the incineration[5,6].

Dense structures in fresh air-dried tissue spreads, which were revealed by a conventional electron microscope, were observed first with a scanning transmission attachment and then analyzed for their elemental content with an energy dispersive X-ray microanalyzer. The platelet dense bodies[7], zymogen granules of the pancreatic acinar cell[8], neurosecretory granules of the mouse pituitary[9], the nucleus of a subcutaneous connective tissue cell[10], and mitochondrial granules[10] were examined

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and have already been reported. In this paper, a study of dense particles of subcutaneous collagen fibrils will be presented.

Three adult mice (male and female) weighing about 25 g were decapitated and pieces of subcutaneous connective tissues were removed from the dorsal skin. Pieces of the tissue were spread with a pair of needles over copper grids (400 mesh) which were covered in advance with a thin collodion (1.5% isoamyl acetate) and evaporated with a carbon coating on the slide glass. The spreads were immediately dried in air at room temperature. They were cut at the edge of the grids and removed. The grids with the spread were then observed under an electron microscope (Hitachi HS-7) at an acceleration voltage of 50 kV. Electron probe X-ray microanalysis was performed with an energy dispersive spectrometer (Nuclear Diodes, EDAX) with a scanning transmission apparatus (JEM-ASID) fitted on an electron microscope (JEM-100 C). The acceleration voltage was 60 kV. The subcutaneous connective tissue spread for the electron probe X-ray microanalysis was prepared by spreading small pieces of the tissue with a pair of needles over a clean plastic plate. Copper grids which were washed in acetone were inserted between the plastic plate and the tissue spread. The specimens were first examined with the ASID and the fine structure was observed on the screen. The electron beam was then directed on the spots where the microanalysis was to be performed. The size of the beam spot was about 10 nm in diameter. The X-ray counting time was 300 sec. Spots on collagen fibres were analyzed. Spots in a relatively lucent area between fibres, as well as nuclei of connective tissue cells, were used as controls, the results of which have already been reported[10]. The spots on the spreads of blood plasma[7] and pancreatic acinar cells[8] were also used as controls.

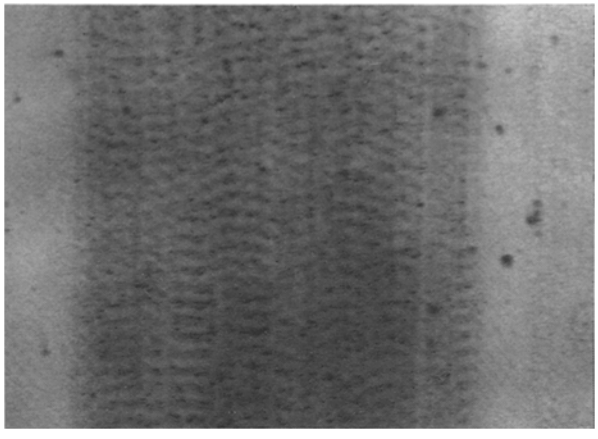

Fig. 1. Collagen fibrils in the fresh air-dried spread of the subcutaneous connective tissue of a mouse dorsal skin, showing about 67 nm periodicity. The specimen was observed without fixation or staining under the electron microscope. Particles of various density, ranging from 6 to 20 nm in diameter, were confined to the dark bands, which were about 32 nm in width. The bands probably corresponded to 'holes' of the collagen fibrils. Note the periodicity of light and dark bands. × 32,000.

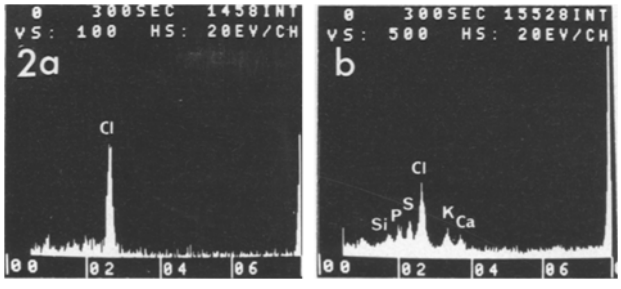

Fig. 2a) Spectrogram of a spot on a collagen fibril in the fresh air-dried spread of the subcutaneous connective tissue, which was spread on the copper grid without any supporting film and examined by an energy dispersive X-ray microanalyzer. The acceleration voltage, 60 kV, and the integrated pulse counting was made for 300 sec. Note only a peak for chlorine. b) Spectrogram of a spot on one of the nuclei in the same spread as in a), shown as a control. Lines characteristic of silicon, phosphorus, sulfur, chlorine, potassium and calcium are revealed.

The conventional electron microscopy of the fresh air-dried spread of the subcutaneous connective tissue demonstrated various substructures, as was the case with spreads of other tissues[7–10] and with fresh frozen dried ultrathin sections[4]. Dense fibrous structures of various thicknesses were spread over the relatively lucent area. They showed various sizes, interlacing over the spread. Broad and thin fibres as well as very fine fibrils were found. The broad fibres were from 1.5 to 2 μm wide and showed a tortuous course. The thin fibres were about 200 nm wide and had a straight course. The very fine fibrils were also found down to about 60 nm wide. The fine fibrils were frequently connected with the above two types of fibres. The broad fibres corresponded to collagen fibres and the thin ones to elastic fibres. A higher magnification of the broad fibres and the fine fibrils disclosed distinct dark and light bands characteristic of collagen fibrils with the periodicity of about 67 nm (Figure 1). Dark bands ranged from 25 to 38 nm in width; the mean being about 32 nm. Light bands ranged from 16 to 48 nm in width; the mean being about 35 nm. The broad fibres were found to be an assemblage of fine fibrils which were about 60 nm across. No periodicity could be found in straight elastic fibres. Dense particles in collagen fibres were conspicuous, appearing globular in shape of various sizes ranging from 6 to 20 nm in diameter. They filled the dark bands and each of them exhibited quite different densities. Denser particles were most likely to be found along the edge of the dark bands. The dense particles were clearly seen on the relatively thick collagen fibrils but few could be found on the very fine fibrils (less than 200 nm in width). Nuclei of connective tissue cells were seen as large dense oval structures, adhering to dense fibres or dispersed in relatively lucent areas. The type of cells was not identified in this study. Within the cytoplasm showing an unclear boundary, there were round, oval or elongated bodies with homogeneous-looking, medium-dense materials containing abundant round dense granules of 20–100 nm in diameter. They seemed to correspond to mitochondria. When the spreads were observed with the ASID, almost the same ultrastructure, as was seen with a conventional electron microscope, was recognized. However, neither the dense particles nor the periodicity of collagen fibrils were discernible.

Energy dispersive X-ray microanalysis of spots on several collagen fibres disclosed a high peak for chlorine (Figure 2a). The microanalysis of a spot in a dense region of a nucleus in the same spread, as in the above, detected lines characteristic of silicon, phosphorus, sulfur, chlorine, potassium and calcium, (Figure 2b) which was previously reported[10]. This also indicates that the spectrometer is useful to detect these elements. Analysis of a spot in the lucent area between fibres as a control showed a distinct but very low peak for chlorine. The control spots in the

previous reports[7,8] gave no peaks for specific elements. It may be concluded that there is a high amount of chlorine in collagen fibres and chlorides appear concentrated in the dense particles of the collagen fibrils.

Round dense particles associated with collagen fibrils have been reported at the early stages of calcification in the periosteal bone and bone cartilage of the chick embryo by Fitton-Jackson[11]; in mineralized turkey tendons[12]; in the reconstituted collagen fibrils in vitro[13]; and recently in the calcifying tendon matrix[14]. GLIMCHER and KRANE[15] have explained that dense particles are deposited within 'holes' in the collagen fibrils which are inherent in the model of collagen fibrils[16]. They ascribed the particles to the amorphous phase of calcium phosphate precipitation although the exact formula of the compound is unknown[15].

Only a high peak for chlorine could be detected in the collagen fibres in the fresh air-dried spread of the subcutaneous connective tissue by the present electron probe X-ray microanalysis, in spite of the expectation of a high amount of calcium and phosphorus. It has been considered that the chloride ion is mostly extracellular and only a small proportion of cells, such as those of the mesenchymal origin, were known to contain large amounts of chloride[16]. However, as MANERY[17] described, the extracellular space of the connective tissue contains abundant chloride. Comparing the sodium space with the chloride space, he concluded that chloride exists as 'dry' form in the connective tissue especially in tendons and subcutaneous tissues[18]. He and his associates also suggested that the chloride ion is in collagen fibrils[17].

Dense particles at the dark bands of collagen fibrils in the subcutaneous connective tissue in the fresh air-dried spread appears to contain a high amount of chloride, to which the density of the particles is probably due. Their exact, biological significance is still to be determined. Chlorides may act as inhibiting the depositing of phosphates or calcium at precipitation sites in the collagen fibrils of the subcutaneous connective tissue. A further study is needed to know the exact location of the dense particles, and their chemical composition. Moreover, a study is needed concerning the factors which differentiate the collagen fibrils of the bone and the cartilage which calcify, from those of other connective tissues which do not calcify.

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Laser Diffraction Used to Monitor Strain in Mechanoreceptors of *Jasus verreauxi*

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Summary. Laser diffraction patterns from crayfish abdominal mechanoreceptors have been observed and the corresponding sarcomere lengths calculated and then correlated with sensory nerve discharge frequencies.

Mechanoreceptors were first reported by ALEXANDROWICZ[2] in the lobsters *Homarus vulgaris* and *Palinurus vulgaris*. These organs are present in other crustacea such as the marine crayfish, *Jasus verreauxi*, which we use in our experiments. Every abdominal segment of a crayfish has 2 such organs, situated with lateral symmetry, each consisting of 2 muscles of unequal size, which have associated sensory and motor nerves. The muscular parts of the organs were named RM_1 and RM_2, RM_1 responding to slow stretches with sustained discharges that last for hours, and RM_2 responding to only rapid stretches, the response lasting for about 30 sec. This suggests that RM_1 may be concerned with slow postural changes and RM_2 with phasic changes. In RM_1 length has been found to be related to the frequency of firing of the associated nerve SN_1[3,4].

While it is believed that these receptors serve an overall function similar to that of muscle spindles in the mammalian system, a number of alternatives have been suggested as to the role played by the mechanoreceptors and to the exact nature of the feedback system. One of these alternatives is the postulate of FIELDS and KENNEDY[5] which is 'that the slow stretch receptor functions as part of a negative feedback servo system ... Tail position (output) represents the balance between the activity of slow extensor motoneurones, which tend to shorten the segment, and the opposing forces of gravity, flexor tone and contraction of extensors in adjacent segments which tend to lengthen the segment. The stretch receptor detects differences between the set point (at which its discharge frequency is zero) and the actual tail position. This difference may be thought of as the error; its magnitude is coded by the frequency of impulses in the stretch receptor neurone'.

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