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.

[11] S. FITTON JACKSON, Proc. R. Soc. B *146*, 270 (1957).
[12] M. U. NYLEN, D. B. SCOTT and V. M. MOSLEY, in *Calcification in Biological Systems* (Ed. R. F. SOGNNAES, Am. Ass. adv. Sci., Washington D. C. 1960), p. 129.
[13] M. J. GLIMCHER, Revue mod. Phys. *31*, 359 (1959).
[14] R. A. LUBEN, J. K. SHERMAN and C. L. WADKINS, Calc. Tiss. Res. *11*, 39 (1973).
[15] M. J. GLIMCHER and S. M. KRANE, in *Treaties on Collagen* (Ed. B. S. GOULD; Academic Press, London and New York 1968), vol. 2, part B, p. 67.
[16] A. J. HODGE and J. A. PETRUSKA, in *Aspects of Protein Structure* (Ed. C. N. RAMANCHANDRAN, Academic Press, New York 1963), p. 289.
[17] J. F. MANERY, in *Mineral Metabolism* (Eds. C. L. COMAR and F. BRONNER; Academic Press, New York and London 1961), vol. 1, part B, p. 551.
[18] J. F. MANERY. Physiol. Rev. *34*, 334 (1954).

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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[2] J. S. ALEXANDROWICZ, Q. Jl. microsc. Sci. *92*, 163 (1951).
[3] C. A. TERZUOLO and Y. WASHIZU, J. Neurophysiol. *25*, 56 (1962).
[4] M. C. BROWN and R. B. STEIN, Kybernetik *3*, 175 (1966).
[5] H. L. FIELDS and D. KENNEDY, Nature, Lond. *206*, 1235 (1965).

On examination of this postulate, it would seem that the most potentially rewarding parameter to measure in the receptors and muscles, besides the frequency, is the strain $\left(\frac{\Delta l}{l}\right)$ where l is the length. Diffraction is a reliable and convenient way of monitoring strain; measurements can be made simultaneously from several different points of the preparation and one can work on the closed system, since the receptors and the extensors need not be dissected. Control theory techniques can then be used to analyze the system and correlate strains and impulse frequencies. Although diffraction has been used to study striated muscle[6,7], we believe that it is the first time that diffraction from mechanoreceptors has been utilized.

Methods. In this part of the program the mechanoreceptors were dissected from the 2nd and 3rd abdominal segments of the crayfish, *Jasus verreauxi*, by first cutting a rectangular segment from the shell using a dentist's drill, removing some tissue and, in this case, removing the receptors. The receptors were mounted horizontally in a glass bath containing COLE's solution[8] (NaCl, 455 mM; KCl, 15 mM; $CaCl_2$, 25 mM; $MgSO_4$, 10 mM; buffered with 17.57 ml of 0.5 M H_3PO_4 and 0.956 ml of 0.5 M NaOH per l of solution to give a pH of 7.4 at 21 °C). The sensory nerves SN_1 and SN_2 were placed on a platinum electrode and bathed in paraffin oil, the action potentials being detected by a unity voltage gain amplifier (input impedance 10^{11} Ω) and recorded using a storage oscilloscope. Equal stretches were applied simultaneously from both ends of the receptor using 2 micromanipulators, so as not to disturb the nerve on the platinum wire. A 7 mW HeNe laser beam (wavelength λ = 632.8 nm) was passed through the receptor muscles RM_1 and RM_2 near the dendritic region, and a translucent screen was used to display the diffraction. This enabled us to photograph the diffraction as well as to measure the separation of the fringes using vernier calipers. If the separation on the screen of the zero and first order fringes is x and D is the perpendicular distance between the preparation and the screen, then the sarcomere length S is given by $S = \lambda[(D/x)^2 + 1]^{1/2}$ (Eq. 1).

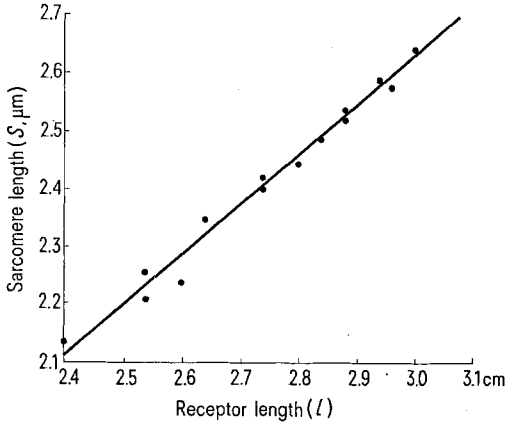

Fig. 1. Correlation between the sarcomere length S, monitored by means of diffraction, and the receptor length l.

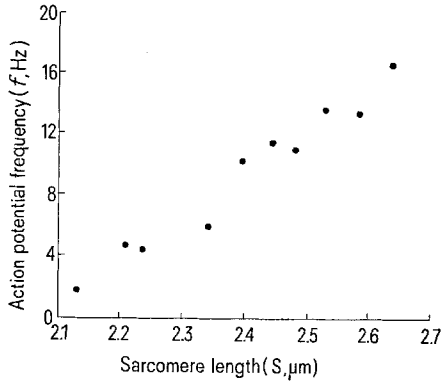

Fig. 2. Action potential frequency f from the sensory nerve SN_1 plotted against the receptor length l.

Results. The zero, first and second order fringes were easily distinguished in the laser speckle background, but care must be exercized in determining which of the diffraction fringes belong to RM_1 and which to RM_2. As the receptors were stretched the separation of the 2 first order fringes was observed to decrease. The sarcomere length as calculated using Eq. 1 was found to be proportional to the length of the receptor as shown in Figure 1.

Usually we have been able to measure the separation of the fringes on the screen more accurately for RM_2 than for RM_1, because those from RM_1 are not as well defined, which is consistent with ALEXANDROWICZ's[2] statement that the striations in RM_2 are more ordered than those in RM_1. We have found that the sarcomere lengths for RM_1 and RM_2 are both governed by equations of the form $S = ml + c$(Eq. 2), where m and c are constants and l is the length of the receptor, and therefore either could be used to monitor strain. By considering Figures 1 and 2 we can see that action potential frequency is a smooth function of length or sarcomere length which illustrates that diffraction can be used to monitor strains.

Because there is some controversy, which we have not solved, as to whether action potential frequency is linearly related to length or not, we have refrained from drawing a straight line through the points of Figure 2. We merely want to indicate in this paper that sarcomere length can be related to action potential frequency.

Discussion. The main purpose of this paper is to report the advantages of using laser diffraction to study mechanoreceptors and the related feedback systems. The first essential is to prove that strain can be monitored by using diffraction, that is to show that sarcomere length S is a function of length of the receptor l. As can be seen in Figure 1, we have found S to be linearly related to l as described in Eq. 2. We have also found that S is related to the action potential frequency, f, as shown in Figure 2. In order to ensure the most meaningful correlation between S and f, we have obtained diffraction from a region in the muscles very close to the area of sensory nervous response, that is near the dendritic area of the sensory nerves.

Accuracy of results is governed by the clarity of the fringes which depend upon the power of the laser used (which we find must be greater than 2 mW), the condition of the preparation, and the care exercized in dissection. However, diffraction enables strain to be monitored without removing the receptors from the abdominal shells so that the receptors, nerves and muscles are retained as a closed system, thus allowing study of postulates such as that of FIELDS and KENNEDY[5]. In these circumstances, measurement of length by any other means would be

[6] J. BOREJDO, P. MASON and J. UNSWORTH, Experientia *30*, 373 (1974).

[7] J. A. BARDEN and J. UNSWORTH, Physiol. Chem. Phys. *7*, 31 (1975).

[8] W. H. COLE, J. gen. Physiol. *25*, 1 (1941).

difficult because the points of insertion of the receptors onto the shell are inaccessible.

Diffraction also has the advantage that strains in RM_1, RM_2 and, for that matter, in nearby extensor muscles can be monitored simultaneously, provided one has a sufficient number of laser beams. It can also be pointed out that unlike mechanical means, diffraction pattern can follow the structural changes in the fibres with negligible timelag and hysteresis, the only limitations thus being imposed by the system used to pick it up.

A further advantage of using diffraction to monitor strain is that it can easily be converted into electrical signals which can be automatically and rapidly measured. For instance, by passing the 2 first order fringes through a mechanical chopper and then on to two photodiodes, the time interval T between the resulting pulses measures the sarcomere length. We have set up such a system, in which the time interval T is transmitted via interfacing to a control computer, Hewlett-Packard 2100S, which can also be used to apply stretches, monitor action potentials and record force. Since a resolution time of the order of 1 msec is easily achieved, not only can slow postural adjustments be monitored but also fast flexions of the crayfish tail can be followed.

The Stimulus Transmitting Apparatus in the Trichobothria of the Bugs *Pyrrhocoris apterus* L. and *Dysdercus intermedius* (Dist.) and its Influence on the Dynamic of Excitation in these Sensilla

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Summary. Correlations exist between ultrastructural characteristics in the stimulus-transmitting apparatus of the trichobothria on the 4th abdominal segment of the bug *Pyrrhocoris apterus* (L.) and the functional characteristics of these sensilla.

In several investigations of sensory organs – work done on the crayfish receptor organ[2] and on the Pacinian corpuscle[3,4] for example – it could be proved that the properties of the structures which provide the coupling between the stimulus and the receptor cells can cause a decline of the stimulus effect with time, or can filter away certain components of the stimulus, even prior to transmission to the excitable structures.

On the ventral side of the 4th abdominal segment of the bugs *Pyrrhocoris apterus* (L.) and *Dysdercus intermedius* (Dist.), groups of 3 trichobothria are situated laterally, one at each side. In *Pyrrhocoris* these trichobothria differ in their functional properties[5]. Apart from differences in their size and the length of their hair shafts, all trichobothria display the same external construction (Figure 1).

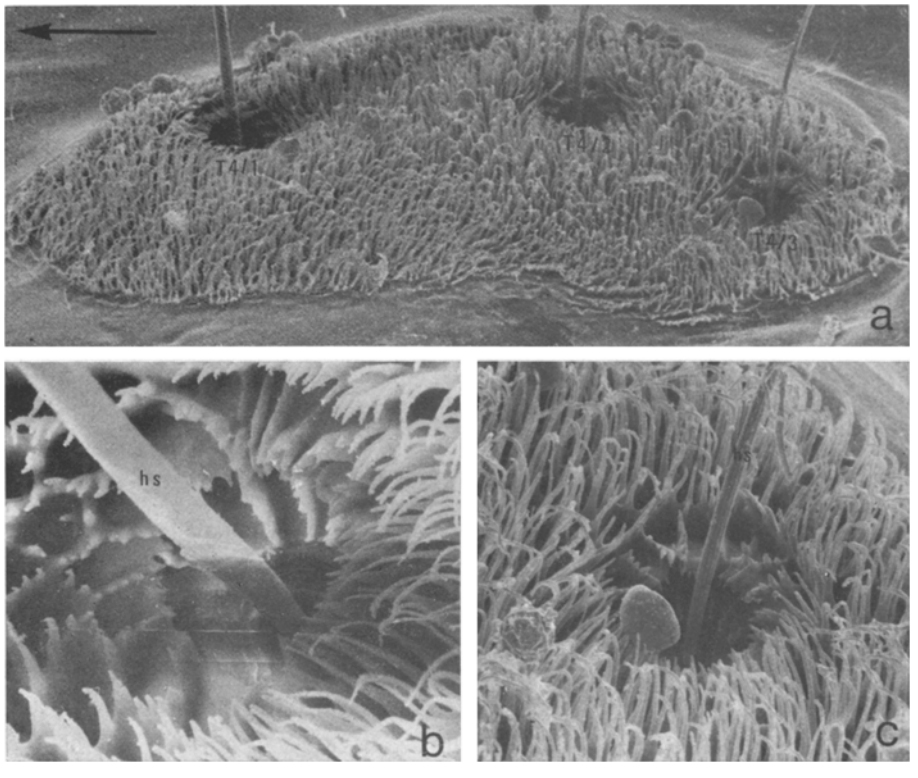

Fig. 1. a) Group of three trichobothria (T1–3/4) on the 4th abdominal segment of *Pyrrhocoris apterus*. Caudal direction is indicated by arrow. (Courtesy of B. BEY and G. THIES, Regensburg.) × 750. b) T1/4 trichobothrium. × 2900. c) T3/4 trichobothrium. × 2000.