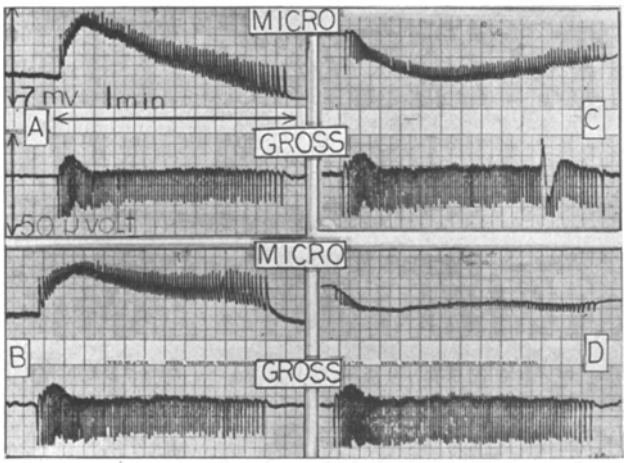

Fig. 3. Reversal of polarity of slow signals from microelectrode tip touching explant in 4 successive positions 100–200 μ apart in a straight line. The 1st, 3rd, 5th and 7th traces are DC records from microelectrodes at these positions and for each there is a simultaneous AC record from gross electrodes (2nd, 4th, 6th and 8th traces). The distortion at the end of record C is an artefact. The similarity between all gross records shows no change in the basic activity of the focus while a change in polarity of some of the signals in each sequence was recorded by the microelectrode.

contact with microelectrode in sufficient numbers is the glial cell. The slow signals have a definite resemblance to those produced by glial cells in response to associated neuronal action[4,5].

Resumén. La exploración con microelectrodos, de explantes telencefálicos de embrión de pollo, registra espontáneamente señales de dos tipos: lentas y rápidas, confirmando hallazgos similares previamente reportados con macroelectrodos. Observandose un patrón en la distribución de las señales lentas, las cuales son originadas por la misma fuente en cada explante pudiendo ser ésta, las células de glía o el producto de potensiales dendríticos.

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Fluctuations in Sarcomere Length in the Chick Anterior and Posterior Latissimus dorsi Muscles During Isometric Contraction

Recently, LARSON et al.[1] reported the detection and measurement of sarcomere fluctuation during isometric contraction of the frog sartorius using the optical diffraction patterns obtained by transmitting a laser beam through the muscle. In order to ascertain whether sarcomere fluctuation is a property peculiar to the frog sartorius it was decided that the laser beam diffraction method should be applied to some other muscles. In practice very few muscles are suitable for this kind of study as it is necessary to use a muscle that is very thin and which possesses sarcomeres of more or less uniform length. After a number of muscles had been tried it was found that the chick posterior latissimus dorsi muscle gave a good workable diffraction pattern. This muscle is a fast or phasic muscle whereas the adjacent anterior latissimus dorsi muscle is a slow or tonic muscle. It was possible to obtain a reasonable diffraction pattern from the anterior latissimus dorsi and, therefore, the extent of sarcomere fluctuation in the phasic muscle could be compared with that in the tonic muscle.

Materials and method. The posterior latissimus dorsi and anterior latissimus dorsi were dissected out from 20-day-old White Mountain Cross chicks. They were suspended in the muscle chamber as shown in Figure 1. The muscle chamber was filled with Krebs bicarbonate ringer solution at 38 °C, equilibrated with a 95% O_2/5%CO_2 mixture. The optical arrangement for recording sarcomere fluctuations during contraction was as follows: The laser beam (Optics Technology, Inc., Model 170, Helium-Neon gas Laser = 6328 Å, power 0.3 mw) was transmitted through the muscle in the muscle chamber and the diffracted beam was then focused into the lens of a 16 mm movie camera (Bolex 16 mm H reflex). Also the screen of an oscilloscope showing the stimulation pulses was reflected and focused into the top left-hand corner of the camera lens using several mirrors and lens. The length of the muscle was adjusted to its maximum resting length by lowering the bottom hook using the rack and pinion. It was then gently flattened between the glass plate and the muscle chamber wall using the other rack and pinion which moved the muscle chamber backwards and forwards in the horizontal plane. The laser beam was then switched on and moved up and down the muscle until a region was found which gave a good diffraction pattern. Care was taken not to expose any region of the muscle to the beam for more than a few seconds. The muscle chamber was then drained and the camera set in motion (60 frames per sec) and after a second or so the muscle was stimulated via the hooks from which the muscle was suspended, with a burst of 30 volt DC square wave pulses of 10 msec duration delivered at a frequency of 50 pulses per sec. The tension developed during the contraction was recorded using the myograph (Grass straingauge) with the output connected to a pen recorder (Dynograph Offner Type RS). After processing, the film was analyzed frame by frame on an isodensitometer (Joyce and Loebl Ltd., England) and the distance between the diffraction lines was measured and plotted for each individual frame of the film.

[1] R. E. LARSON, M. J. KUSHMERICH, D. H. HAYNES and R. E. Davies, Biophys. J. Soc. Abstr. 12th Ann. Mtg. 8, February 19–21 (1968), p. A8.

Results. The measurements of sarcomere length fluctuation before and during contraction of the chick posterior latissimus dorsi are shown in Figure 2. From this figure it will be seen that the fluctuation in the distance between the diffraction lines was very small whilst the muscle was at rest. When stimulation commenced the distance between the diffraction lines increased indicating that the mean sarcomere length in the area of the laser beam decreased slightly even though there was no appreciable change in the overall length of the muscle. During the development and maintenance of isometric tension the fluctuations increased. For instance, the mean fluctuation between frames 40 and 75 was 2.22 mm per frame whereas the mean fluctuation in the resting muscle, 20 to 0 frames, was 0.58 mm per frame. In other words, the fluctuation had increased in magnitude by approximately 4 times. The maximum amplitude of the fluctuation was about 6 mm which in terms of actual movement at the level of the individual sarcomere approximates to 900 Å (calculated using the Bragg equation). Thus in the chick posterior latissimus dorsi whilst the muscle is maintaining isometric tension there is a considerable amount of internal work taking place in the individual sarcomeres even though there is no external work being performed.

The slow chick muscle, namely the anterior lattissimus dorsi, was less suitable for this type of investigation as the diffraction lines were less distinct and the 'background noise' of the measurements whilst the muscle was resting was greater. However, if the mean resting fluctuation is subtracted from the mean fluctuation during contraction, then values for the fast and slow muscles may be compared. For the fast muscle (posterior latissimus dorsi) the value was 1.7 mm and for the slow muscle (anterior latissimus dorsi) the value was 0.7 mm per frame. The extent of the sarcomere dither in the fast muscle was, therefore, about 2.5 times greater than in the slow muscle. The fluctuations in the slow muscle were in fact due mainly to periodic dither of about the same size of those in the posterior latissimus dorsi but they occurred at about $^1/_3$ of the frequency.

Discussion. The fact that the slow muscle exhibited far less sarcomere fluctuation is of considerable interest as the tension developed by both the fast and the slow muscles was approximately the same. This means that the slow muscle was performing far less internal work in order to maintain about the same tension. Recent work on hamster muscles[2] has shown that the slow soleus muscle is able to maintain tension at a much lower cost, in terms of ATP used than is the fast extensor digitorum longus or the biceps brachii muscle. It seems feasible, therefore, that the

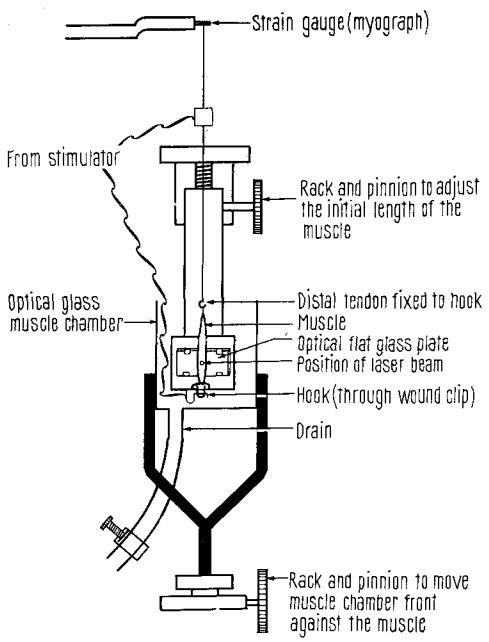

Fig. 1. The apparatus used for suspending and measuring the isometric contraction of the muscle whilst simultaneously recording sarcomere fluctuations.

[2] G. Goldspink, R. E. Larson and R. E. Davies, in preparation for publication.

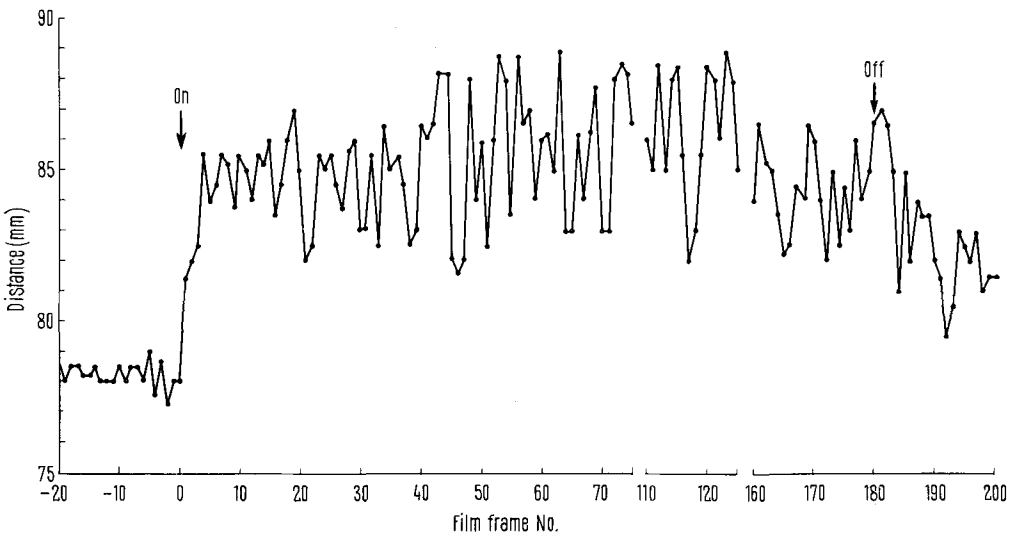

Fig. 2. Sarcomere fluctuations before and during isometric contraction of the chick posterior latissimus dorsi muscle. These fluctuations are seen as a result of plotting the zero and 1st order peak distance measured from the isodensitometer traces of each individual frame. The film speed was 60 frames per sec and the combined magnification of the optical system and isodensitometer was approximately × 65.

extent of sarcomere fluctuation may be one of the main factors which determines the energy requirements of the muscle for maintaining isometric tension and also possibly the rate at which the muscle fatigues. Presumably sarcomere fluctuation is related to the length of time that each cross link on the myosin filament is engaged with the actin filament. The fluctuations probably only occur with the random release or engagement of many cross links. In the fast muscle there is presumably a greater probability of sarcomere movement as the length of time that each cross link is engaged is probably far less than in the slow muscle[3].

Zusammenfassung. Es wird gezeigt, dass die Schwankung der Sarkomerenlänge bei Benützung der «laser beam» – Methode während der isometrischen Kontraktion der M. latissimi dorsi beim Hühnchen verschieden war. Der Schwankungsumfang war in den vorderen und hinteren Muskeln ungefähr gleich (900 Å), hingegen war die Frequenz in den hinteren phasischen Muskeln dreimal höher als in den vorderen tonischen Muskeln.

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Effect of Light and pH on Diazotized Bilirubin

While studying the enzyme, bilirubin glucuronyl transferase, variable results had been obtained on the determination of diazotized non-conjugated bilirubin. Previous workers have shown that undiazotized bilirubin is very sensitive to light and pH[1–10] and that extinction and absorption maximum values of azo dyes depend on such factors as pH, alcohol, and albumin[11]. It was therefore desirable to note whether light and pH had significant effects on the spectrophotometric assay of diazotized non-conjugated bilirubin under the conditions of the aforementioned experiments. As noted below, normal room light did not significantly affect the spectrophotometric determination of diazotized bilirubin whereas pH had a marked influence on the optical density readings. These observations will undoubtedly be of interest to other laboratories doing bilirubin determinations.

Effect of pH. To study the effect of pH on spectrophotometric properties of bilirubin, a bilirubin solution was made as follows: 4.5 ml ethanol, 95%; 0.6 ml of 0.25*M Tris* buffer, pH 8.0 at 25 °C; 0.5 ml bilirubin solution (19 mg bilirubin (Sigma) rapidly dissolved in 10 ml of 0.2*N* NaOH and 28.5 ml distilled water); and 1.95 ml of distilled water. 3 ml of concentrated diazo reagent[12] was then added. After shaking for 30 min, the solution was diluted to 100 ml with methanol. Series of samples were set up, using NaOH and HCl solutions, to give various hydrogen-ion concentrations. Equal volumes of diazotized bilirubin solution were then added. Optical density was read at 535 nm (Spectronic 20) and the pH was determined (Beckman Expanded Scale Model 76). Water was used in each series as a control replacing the acid or base. Conditions were reversed by adding HCl solution to the more alkaline samples (compared to the control at pH 2.3). Each sample was brought to the same volume so that the concentration of bilirubin was the same in each cuvette. Optical density and pH were determined again.

The effects of acid and base on diazotized bilirubin colorimetry are shown in the Figure. Upon addition of NaOH, optical density readings decreased as the pH was increased from the control sample pH value. Acid lowered the pH but did not produce changes in the optical density readings compared to the control. To determine whether the diazotized bilirubin was acting as an indicator[13], HCl was added to the samples whose pH values had been increased. It was presumed that changing the pH back to the control level might give optical density readings similar to that of the control samples. This was not the case. The addition of HCl resulted in changing the pH to a value around 2 but produced only a minimal increase in the optical density readings.

Diazotized bilirubin was therefore markedly affected by the hydrogen-ion concentration of the solution. Different optical density readings were obtained with the same concentration of bilirubin just by changing the pH through the addition of NaOH. It is therefore important to keep a pH of 2 or less throughout the procedure after the addition of concentrated diazo reagent to obtain consistant results.

Effect of light. For the study on light, diazotized conjugated and non-conjugated bilirubin samples were obtained by the extraction and chromatographic tech-

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