Eisenbarth and co-workers[16] have demonstrated that PGA_1 inhibits the cellular incorporation of radioactive precursors into the proteoglycan, protein, RNA and DNA of a well-differentiated rat chondrosarcoma and of a poorly-differentiated murine chondrocarcoma. They suggest a potential therapeutic role for PGA in cases of chondrosarcoma[5,6,16]. The present work shows the cytotoxicity of PGA_1 for non-malignant chondrocytes in organ culture and the inhibition of growth of chick embryonic limb bone rudiments in vitro and, in one sense, supports the proposal that PGA may possess selective effects that act against cancer cell metabolic pathways.

The present observations may also be relevant to the pathogenesis of human connective tissue disease. In rheumatoid arthritis (RA) there is progressive destruction of peripheral articular cartilage[17]. Significant concentrations of prostaglandins (PGE) have been recognised in RA synovial fluid[18] and interpreted as 'reasonable evidence for the postulate that PGE and/or PGA are important contributory factors to the pathogenesis of inflammatory reactions in patients with rheumatic diseases'. It has been shown that the predominant prostaglandin in rheumatoid synovial fluid is PGE_2[19]. In an analogous experiment designed to test the effects of PGE_2 on cartilage (25 µg/ml) PGE_2 was found to have no inhibitory effect on the growth of chick embryonic limb rudiments in vitro, assessed by length and weight measurements, during a 4-day-period. No evidence of PGE_2-induced chondrocyte death was found by light microscopy. Comment on the significance of these observations for the pathogenesis of rheumatoid arthritis must now await experimental data on the effects of prostaglandins on human articular cartilage in organ culture. Consequently, although we report the ability of PGA_1 to cause chick embryonic chondrocyte death and impaired cartilage matrix synthesis, it is not yet known whether damage to, or dissolution of, marginal articular cartilage in rheumatoid arthritis is influenced by the local release of prostaglandins.

16 G. S. Eisenbarth, D. K. Wellman and H. E. Lebovitz, Biochem. biophys. Res. Commun. *60*, 1302 (1974).
17 D. L. Gardner, in: The Pathology of Rheumatoid Arthritis, p. 7. Arnold, London 1972.
18 D. R. Robinson and L. Levine, in: Prostaglandin Synthetase Inhibitors, p. 223. Ed. H. J. Robinson and J. R. Vane. Raven Press, New York 1974.
19 D. R. Robinson and M. B. McGuire, Ann. N.Y. Acad. Sci. *256*, 318 (1975).

A light microscope study of fibre diameter and sarcomere length relationships in rigor skeletal muscles

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Summary. Correlation coefficients between fibre diameter and sarcomere length were significant in relaxed, unfixed muscles in rigor, indicating that fibres with large diameters had short sarcomeres and fibres with small diameters had long sarcomeres. No significant correlations were obtained in muscles from limbs that entered rigor in a folded or stretched position.

Fibre diameter and sarcomere length are important parameters in determining growth potential[1,2] and physiological properties[1] of skeletal muscles. Information is not available on the relationship between the diameter of a fibre and the length of the sarcomeres in individual skeletal muscle fibres in the pre-rigor state. Accurate determination of these dimensional relationships is impossible because of the following properties of pre-rigor muscle: excitability, thaw-rigor changes if the muscle was frozen in the pre-rigor state, and penetration properties of the fixative. Therefore, this study on fibre diameter and sarcomere length was performed on mouse and turkey muscles that had entered rigor mortis. The limbs were in a folded, relaxed or stretched position, thereby providing muscles at varying degrees of contraction and relaxation. Conventional histological methods would involve separate transverse and longitudinal sections in order to obtain fibre diameter and sarcomere length values. Isolation of individual muscle fibres permits the dimensional measurements to be performed on the same fibre. Passively contracted ('kinked') fibres could be observed when the fibres were isolated. Some muscles contain a large number of passively contracted fibres[3-5]. A high proportion of passively contracted fibres would make the fibre diameter measurements meaningless[6]. The proportion of passively contracted fibres in these muscles was small irrespective of the state of relaxation or contraction of the muscles.

Methods. Adult female mice (30–35 g body weight) were killed by ether anesthesia. Adult male turkeys (7–8 kg body weight) were exsanguinated by cutting the carotid artery. The turkeys were placed in an inverted metal cone to prevent excessive limb and wing movement during exsanguination. Passively shortened and stretched biceps brachii muscles from mice were obtained by pinning one fore-limb inwards maximally, and stretching the other fore-limb outwards maximally. The fully extended limb was pinned at 180° from the trunk. Stretched semitendinosus muscles in the turkey were obtained by securing the limb in a cephalad position. Shortened semitendinosus muscles were obtained by flexing the limbs inwards maximally and securing them in that position. All folding and stretching treatments were performed immediately postmortem. Soleus, sternomastoideus and gastrocnemius muscles in mice, and the pectoralis muscle in turkeys were obtained by placing the animals in a resting position

1 R. I. Close, Physiol. Rev. *52*, 129 (1972).
2 V. R. Young, J. Anim. Sci. *38*, 1054 (1974).
3 C. A. Voyle, J. Food Technol. *4*, 275 (1969).
4 A. C. Hooper and P. V. J. Hegarty, Comp. Biochem. Physiol. *45A*, 411 (1973).
5 C. A. Cardasis and G. W. Cooper, J. exp. Zool. *191*, 333 (1975).
6 M. H. Brooke, in: The Physiology and Biochemistry of Muscle as a Food, vol. 2, p. 131. Ed. E. J. Briskey, R. G. Cassens and B. B. March. The University of Wisconsin Press, Madison 1970.

Table 1. Correlations between fibre diameter and sarcomere length in relaxed mouse and turkey skeletal muscles in rigor mortis

Muscle	Fibre diameter (μm)[a]	Sarcomere length (μm)[b]	Correlation coefficient (r)[b, c]
Mouse			
Soleus	41.2 ± 0.78	2.68 ± 0.03	− 0.33**
Sternomastoideus	58.7 ± 1.03	2.61 ± 0.02	− 0.25*
Gastrocnemius	62.7 ± 0.94	2.15 ± 0.01	− 0.23*
Turkey			
Pectoralis	91.6 ± 2.16	2.23 ± 0.02	− 0.19 (N.S.)

[a] Values represent the mean of 80 fibres ± SEM. [b] Between fibre diameter and sarcomere length. [c] NS = non-significant correlations ($p > 0.05$); * = significant correlations ($p < 0.05$) ($r \geq 0.22$); ** = significant correlations ($p < 0.01$) ($r \geq 0.29$). Number of animals: 4.

immediately postmortem. The mouse and turkey muscles were allowed to go into rigor at 20 °C, which occurred 4 h and 6–8 h postmortem, respectively. These times were determined from postmortem adenosine triphosphate (ATP), pH and extensibility measurements on muscles from mice and turkeys which were killed and treated in a similar manner to those of the present investigation[7].

Unfixed fibres were separated by homogenization[8]. Complete cross-sectional samples were taken from the mid-portion of all muscles so as to avoid the variation in sarcomere length that occurs near the origin and insertion of the muscle[9]. Measurement procedures for fibre diameter and sarcomere length are outlined in detail elsewhere[10]. Briefly, this involved measuring the sarcomere length by counting the number of A-bands along 100 μm of each fibre. Recent unpublished observations in our laboratory indicate that the technique for isolating muscle fibres used in this experiment measures the maximum diameter of each fibre. The statistical analyses were calculated according to procedures and tabular data given by Steel and Torrie[11].

Results. The correlation of fibre diameter with sarcomere length in mouse and turkey muscles that entered rigor in a relaxed position was negative (table 1). This indicates that long sarcomeres are associated with fibres of small diameter and short sarcomeres are associated with fibres of long diameters. The level of significance of this relationship was $p < 0.01$ for soleus and $p < 0.05$ for sternomastoideus and gastrocnemius of the mouse. The pectoralis of the turkey had a correlation that approached significance ($p < 0.07$). Similar analyses are presented in table 2 for muscles which entered rigor with the limbs in a folded or in a stretched position. Folding produced larger fibre diameters and shorter sarcomeres compared to the corresponding muscle in the stretched limb. Sarcomere length was not significantly correlated with the fibre diameter of any of the muscles or treatments in table 2. When the values for the folded and the stretched treatments were pooled for each muscle, the correlation coefficient was significant ($p < 0.01$) for the 3 different muscles examined. This result was expected because presumably this pooling of the data from the muscles of the folded and stretched limbs should logically give a relationship similar to the muscles from limbs in the resting position (table 1).

Discussion. No significant correlation between fibre diameter and sarcomere length in folded or stretched limbs suggests that the constancy of volume of a skeletal muscle during contraction[12] may not always apply to the length and diameter relationships in rigor muscles. Muscle fibre diameter decreases during rigor development due to a movement of fluid into the extracellular space resulting from ATP loss and pH decline[7]. Thus, fibre diameter in muscles in rigor is determined partly by the state of contraction of the muscle and the loss of intracellular fluid, whereas during in vivo contraction the loss of intracellular fluid is not a factor. Furthermore, sarcomere length decreases only in muscles free to shorten during the development of rigor mortis[13]. In a limb extended maximally during the development of rigor the sarcomeres cannot shorten, but the loss of intracellular fluid will occur. Therefore, in studies with muscle in rigor, the transverse dimension is influenced by factors other than limb position, whereas the longitudinal dimension is determined by the position of the limb. The mouse biceps brachii muscle is a good model for such an investigation because the fibres extend from origin to insertion[14]. Since the entire fore-limb of the mouse was securely pinned in a stretched position

7 J. J. A. Heffron and P. V. J. Hegarty, Comp. Biochem. Physiol. *49A*, 43 (1974).
8 P. V. J. Hegarty and R. T. Naudé, Lab. Pract. *19*, L61 (1970).
9 G. Goldspink, J. Cell Sci. *3*, 539 (1968).
10 P. V. J. Hegarty and A. C. Hooper, J. Anat. *110*, 249 (1971).
11 R. G. D. Steel and J. H. Torrie, in: Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York 1960.
12 B. C. Abbott and R. J. Baskin, J. Physiol., Lond. *161*, 379 (1962).
13 D. W. Henderson, D. E. Goll and M. H. Stromer, Am. J. Anat. *128*, 117 (1970).
14 R. W. D. Rowe, Ph. D. Thesis, University of Hull, England.

Table 2. Correlations between fibre diameter and sarcomere length in folded and stretched mouse and turkey skeletal muscles in rigor mortis

Muscle	Pre-rigor treatment	Fibre diameter (μ)[a]	Sarcomere length (μ)[a]	Correlation coefficient (r)[b, c] Separate	Combined[d]
Mouse					
Biceps brachii	Folded	45.1 ± 0.71	2.47 ± 0.03	− 0.13 (NS)	− 0.33**
	Stretched	39.6 ± 0.77	2.80 ± 0.03	− 0.20 (NS)	
Turkey					
Semitendinosus	Folded	78.4 ± 1.67	2.47 ± 0.03	− 0.007 (NS)	− 0.29**
	Stretched	68.5 ± 1.39	3.29 ± 0.03	0.02 (NS)	

[a] Values represent the mean of 80 fibres ± SEM. [b] Between fibre diameter and sarcomere length. [c] NS = non-significant correlations ($p > 0.05$); ** = significant correlations ($p < 0.01$) ($r \geq 0.20$). [d] Correlations derived when folded and stretched fibres ($n = 160$) were statistically analyzed as a combined sample. Number of animals: 4.

immediately postmortem, then all the fibres in the biceps brachii muscle went into rigor mortis in a stretched position. A recent study on only the mouse biceps brachii muscle that entered rigor mortis in a stretched position indicated a significant negative correlation between muscle fibre diameter and the length of the sarcomeres[15]. This observation was not confirmed by the present study on similarly treated skeletal muscles from the mouse and the turkey.

The results of this study demonstrate the importance of limb position in determining the length of sarcomeres and the diameter of fibres in rigor muscles. This is an important consideration in the application of information on the dimensions of muscle fibres to an understanding of the anatomical function of skeletal muscles and in studies on muscle growth and development.

15 A. C. Hooper and J. P. Hanrahan, Life Sci. *16*, 775 (1976).

Ultrastructural and X-ray microprobe comparison of gerbil and human pineal acervuli

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Summary. Only a morphological difference exists between gerbil and the human pineal acervuli. Neither qualitative nor quantitative differences in the chemical composition between both gerbil and human brain sand have been found. The mineral of the gerbil pineal acervuli is, as in the human, also hydroxyapatite.

One of the characteristics of the pineal body of Mongolian gerbils is a constant presence of calcareous deposits (acervuli, corpora arenacea, concretions or brain sand) found in all animals 11 weeks old and more[1,2]. From this point of view, the pineal body of the gerbil is very similar to that of the adult human. The organic matrix of the gerbil acervuli is composed of a carbohydrate, probably an acid mucopolysaccharide, complexed to a protein[1]. The periphery of some calcifications shows an acid phosphatase or an esterase activity[1]. Stained with alizarin red, the gerbil concretions give a positive reaction indicating the presence of calcium salts[2]. This light microscopic finding is in good agreement with the observations of Palladini et al.[3] concerning the human pineal acervuli.

A surprisingly great morphological and histochemical resemblance between human and gerbil pineal calcification has encouraged Japha et al.[1] to suggest that the latter should be used as an excellent model to explore the phenomenon of pineal calcification. In order to give more accuracy to this unexpected opportunity, it is necessary to compare the fine morphology and the mineralogical composition of the human brain sand with the gerbil pineal acervuli.

Material and methods. The pineal bodies of 5 male Mongolian gerbils (Merio unguiculatus) weighing 80 g were fixed by intracardial perfusion with 2% glutaraldehyde and 1% formol in 0.1 mol cacodylate buffer (720 mosmol; pH 7.2). After a brief rinsing in the same buffer, the pineal bodies were postfixed in 1% OsO_4 and embedded in Durcupan. Ultrathin sections were cut with a diamond knife, contrasted with uranyl acetate and lead citrate and observed in Zeiss EM 9A electron microscope. For the microprobe analysis, some pineal bodies were dehydrated and embedded without OsO_4 treatment in Durcupan. The 1000 Å thick sections of these blocks were mounted on nylon grids and analysed for 600 sec at 25 kV in a Hitachi HU-12 transmission electron microscope equipped with a Princetone Gamma Tech X-ray energy dispersive device.

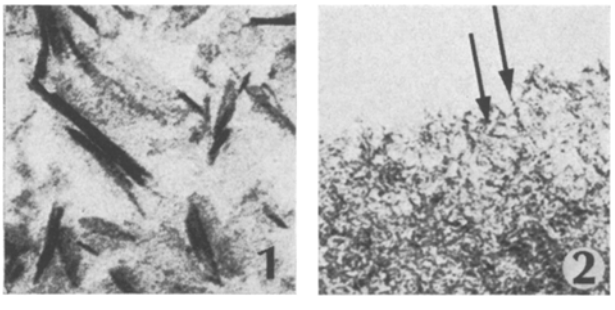

Fig. 1. Gerbil pineal calcification. A widely interspaced needle-like crystals can be seen. × 140,000.

Fig. 2. Human pineal calcification. This material is composed of smaller and considerably more condensed crystals (arrows). × 140,000.

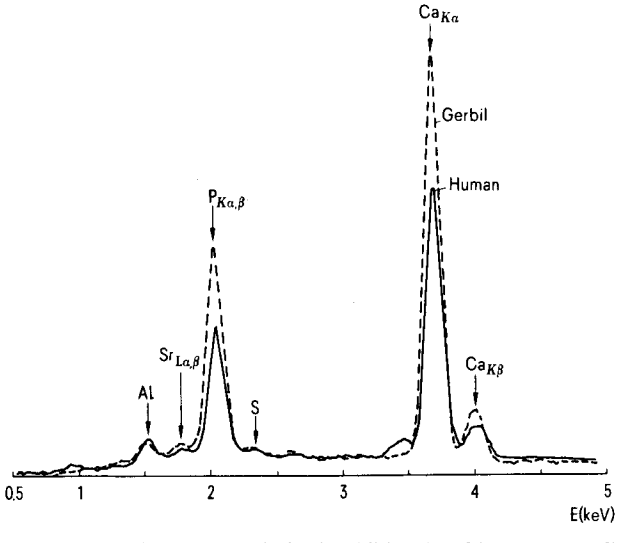

Fig. 3. X-ray microprobe analysis of gerbil (---) and human acervuli (——). Note an identically qualitative and quantitative composition. Aluminium peak corresponds to the specimen holder.

1 J. L. Japha, T. J. Eder and E. D. Goldsmith, Anat. Rec. *178*, 381 (1974).
2 R. J. Reiter, M. G. Welsh and M. K. Vaughan, Am. J. Anat. *146*, 427 (1976).
3 G. Palladini, L. Alfei and L. Appicciutoli, Archo ital. Anat. Embriol. *70*, 253 (1965).
4 S. M. McGee-Russel, J. Histochem. Cytochem. *6*, 22 (1958).
5 J. C. Russ, in: Microprobe Analysis as Applied to Cells and Tissues, p. 269. Ed. T. Hall, P. Echlin and R. Kaufmann. Academic Press, London, New York 1974.
6 G. Boivin, Arch. Anat. micr. *64*, 183 (1975).
7 L. Angervall, S. Berger and H. Rockert, Acta path. microbiol. scand. *44*, 113 (1958).
8 K. M. Earle, J. Neuropath. exp. Neurol. *24*, 108 (1965).
9 R. Krstić, Cell Tiss. Res., *174*, 129 (1976).