Measurements of unfixed mammalian muscle fibres from *pre-* and *post-rigor* muscles of animals at different stages of development

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INTRODUCTION

It is difficult to accurately measure the size of muscle fibres from farm animals because of limitations in the sampling, preparation and measurement of muscle samples. Goldspink (1962 a, b) found that the fibres in the mouse *biceps brachii* extent from origin to insertion. Therefore, the problems of fibre measurement caused by the interdigitation of fibres in the muscles of farm animals is eliminated. Furthermore, the mouse *biceps brachii* is small, sampling errors may be reduced because transverse sections of the whole muscle can be obtained. The observations made by Goldspink (1962 a) on this mouse muscle have been referred to by many workers reviewing the results of fibre size studies on muscles from farm animals (Boccard, 1966; Lawrie, 1966; Livingston, Blair and English, 1966; and others).

The muscle fibre measurements of Goldspink (1962 a, b) were made on fixed and embedded muscles. Shrinkage and distortion of fibres is caused by fixatives (Goldspink. 1961) and by the embedding medium (Schilling, 1966; Drury and Wallington, 1967). In this paper results are presented of fibre size measurements on unifixed and unembedded *pre-* and *post-rigor* *biceps brachii* from mice at various stages of development.

MATERIALS AND METHODS

Male mice from Falconer's Q strain were fed a stock diet *ad lib.* throughout the experiment. The animals were killed by ether anaesthesia, and the *biceps brachii* from the right side was immediately dissected out. The muscle is 'pear shaped', and Goldspink (1962 a) found that the fibres exhibit their maximum cross-sectional area in the 'belly' of the muscle. In the present experiment a complete transverse portion approximately 0.5 mm thick was cut from the *biceps brachii* from one side, and placed in a flatbottomed tube

which contained 5 mM ethyleneglycol *bis* (β-amino-ethylether)-N, N^1-tetra-acetic acid (EGTA) in physiological saline. The EGTA prevented the severe contractions, and therefore distortions, caused by the homogenisation of *pre-rigor* muscle. This technique is rapid, measurements on the unfixed fibres can be commenced within 5—10 minutes *post-mortem*. Details of the procedure will be presented elsewhere (Hegarty and Naude, in preparation). A few drops of the suspension of separated fibres were placed on a hanging drop slide. The image of the fibres was projected using a projection microscope (x 1,050). The diameter of 100 fibres were measured with a micrometer calipers. The *biceps brachii* from the other side was allowed to go into *rigor mortis in situ* with the limb in the resting position. The skin was removed after 24 hours *post-mortem*. The muscle was then dissected and processed in a similar manner to the *pre-rigor* muscle except that EGTA was omitted from the physiological saline. There was no difference in mean fibre diameter between the left and right *biceps brachii* from the same animal when compared in the *pre-rigor* condition. There was also no difference in *post-rigor* muscles similarly compared. EGTA had no effect on fibre diameter; no difference was found in mean fibre diameter between *post-rigor* muscles from one side of animals which were separated in physiological saline plus EGTA and the corresponding muscles from the other side of the animals which were separated in physiological saline only.

RESULTS AND DISCUSSION

The effect of body weight on mean fibre diameter of *pre-* and *post-rigor biceps brachii* is shown in Table 1 and Fig. 1 (A). The mean fibre diameter of both *pre-* and *post-rigor* muscles increased linearly with an increase in body weight over the range 6.8—35.3 grams. *Rigor mortis* caused a significant decrease in mean fibre diameter. The percentage decrease was not influenced

Table 1. *Pre-* and *port-rigor* mean fibre diameter of *biceps brachii* from mice of different body weights.

Body weight (grams)	*Mean fibre diameter (μ)*			*Body weight (grams)*	*Mean fibre diameter (μ)*		
	Pre-rigor	*Post-Rigor*	*% difference*		*Pre-rigor*	*Post-rigor*	*% difference*
6.8	26.83	22.25	17	21.8	38.97	32.85	16
10.0	33.96	29.08	14	25.5	44.58	33.65	25
10.5	32.72	25.81	21	27.7	44.76	38.33	14
14.8	37.17	29.70	20	30.1	49.04	41.11	16
16.0	36.51	30.93	15	32.6	48.47	42.01	13
16.9	35.90	31.75	12	35.3	52.21	42.14	19
20.4	39.39	29.85	24				

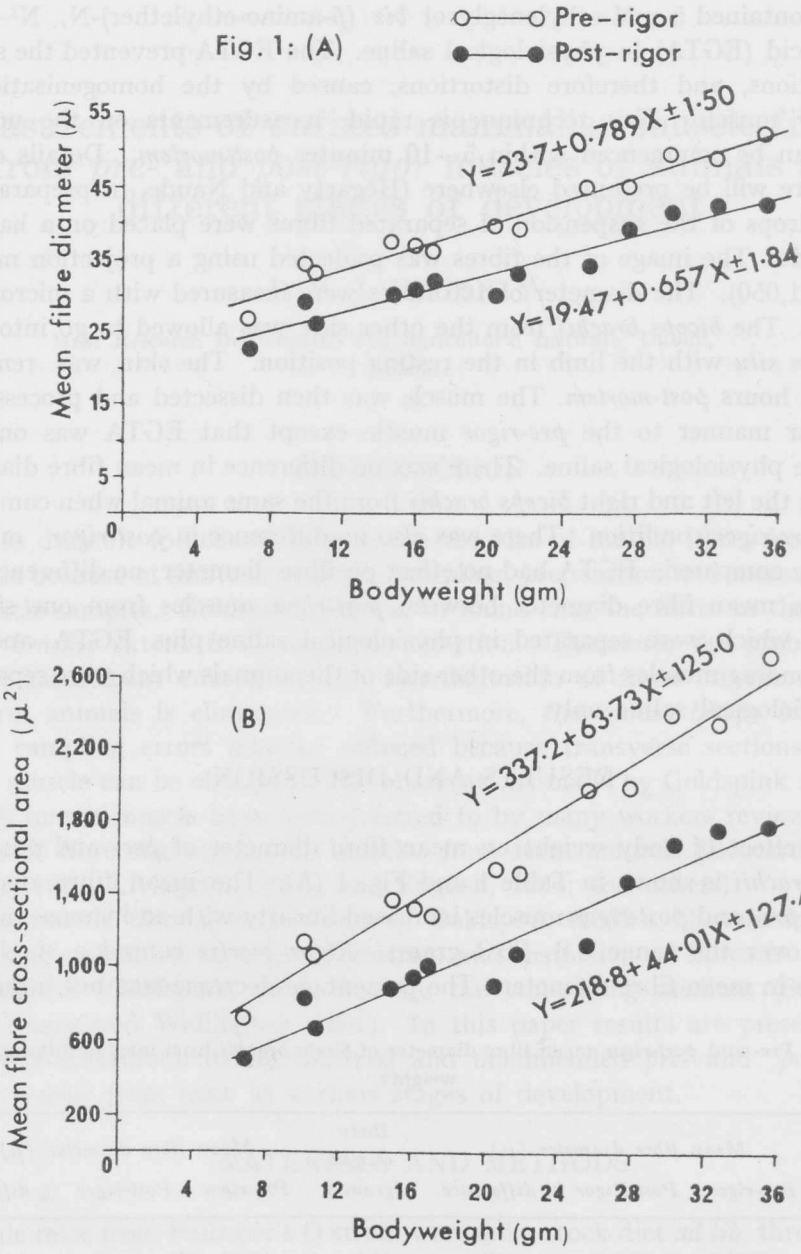


Fig. 1: (A)

by the size of animal (Table 1). The equations for regression of fibre diameter on body weight for the *pre-* and *post-rigor* sub-sets were: —

Pre-rigor: $Y = 23.7 + 0.789\ X \pm 1.50$
Post-rigor: $Y = 19.47 + 0.657\ X \pm 1.84$
where X = body weight and Y = mean fibre diameter.

The analysis of variance of the regression parameters indicated a significant difference ($P<0.001$) in the elevations (a). There was no significant differences in the slopes (b) of the regression lines, therefore the following parallel equations could be computed: —

Pre-rigor: $Y = 25.11 + 0.723\ X \pm 1.75$
Post-rigor: $Y = 18.10 + 0.723\ X \pm 1.75$

Goldspink (1962 a) expressed fibre size in terms of mean fibre cross-sectional area $(\mu)^2$. Results of the present investigation are presented in this form in Fig. 1 (B) for comparative purposes. Goldspink (1962 a) found that the mean fibre cross-sectional area increased in direct proportion to body weight up to approximately 34 grams. Similar findings were obtained in this investigation, where the maximum weight of mouse studied was 35.3 grams. The equations for regression of mean fibre cross-sectional area on body weight for the *pre-* and *post-rigor* sub-sets were: —

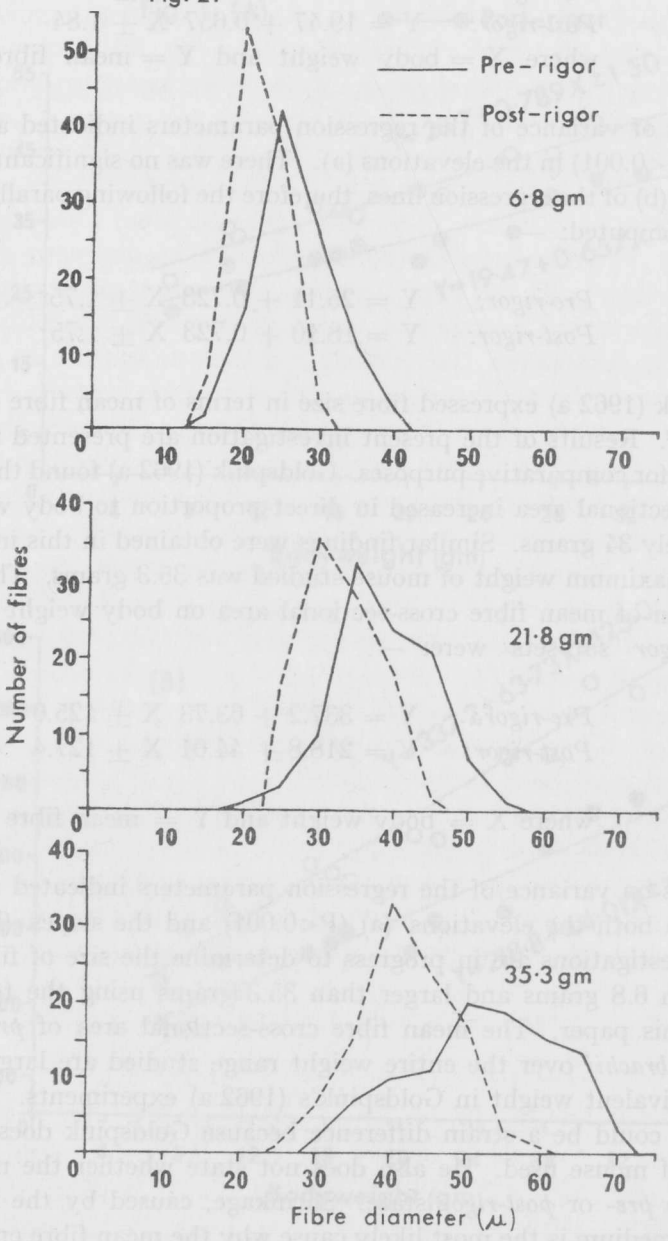
Pre-rigor: $Y = 337.2 + 63.73\ X \pm 125.0$
Post-rigor: $Y = 218.8 + 44.01\ X \pm 127.4$

where X = body weight and Y = mean fibre diameter.

The analysis on variance of the regression parameters indicated a significant difference in both the elevations (a) ($P<0.001$) and the slopes (b) ($P<0.01$). Further investigations are in progress to determine the size of fibres in mice smaller than 6.8 grams and larger than 35.3 grams using the technique reported in this paper. The mean fibre cross-sectional area of *pre-* and *post-rigor biceps brachii* over the entire weight range studied are larger than animals of equivalent weight in Goldspink's (1962 a) experiments. Some of the discrepancy could be a strain difference because Goldspink does not specify the strain of mouse used. He also does not state whether the muscles were fixed in the *pre-* or *post-rigor* state. Shrinkage, caused by the fixative and embedding medium is the most likely cause why the mean fibre cross-sectional area calculated by Goldspink was lower than the *pre-* or *post-rigor* values calculated in th experiment reported here (Fig. 1, B).

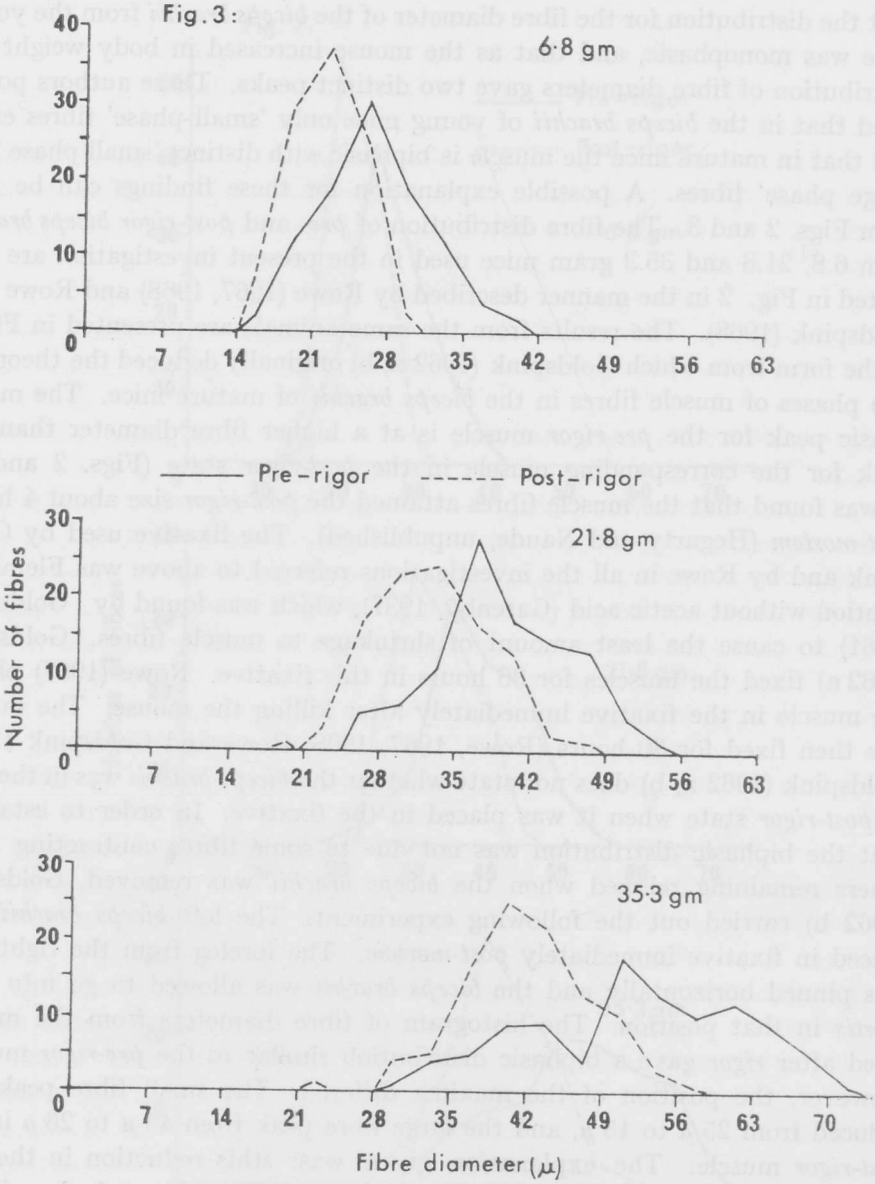
In the present investigation the fibre diameter distributions of *pre-* and *post-rigor biceps brachii* from mice at different stages of development are given in Fig. 2. The measurements of the individual fibres were grouped in

Fig. 2:



the manner described by Rowe (1967) and Rowe and Goldspink (1968). A single peak was obtained for *pre-* and for *post-rigor biceps brachii* from young and mature mice. This is contrary to the findings of Goldspink (1962 a, b), Rowe (1967, 1968) and Rowe and Goldspink (1968). These authors found

that the distribution for the fibre diameter of the *biceps brachii* from the young mice was monophasic, and that as the mouse increased in body weight the distribution of fibre diameters gave two distinct peaks. These authors postulated that in the *biceps brachii* of young mice only 'small phase' fibres exist, and that in mature mice the muscle is biphasic with distinct 'small phase 'and 'large phase' fibres. A possible explanation for these findings can be seen from Figs. 2 and 3. The fibre distribution of *pre-* and *post-rigor biceps brachii* from 6.8, 21.8 and 35.3 gram mice used in the present investigation are presented in Fig. 2 in the manner described by Rowe (1967, 1968) and Rowe and Goldspink (1968). The results from the same animals are presented in Fig. 3 in the form from which Goldspink (1962 a, b) originally deduced the theory of two phases of muscle fibres in the *biceps brachii* of mature mice. The monophasic peak for the *pre-rigor* muscle is at a higher fibre diameter than the peak for the corresponding muscle in the *post-rigor* state (Figs. 2 and 3). It was found that the muscle fibres attained the *post-rigor* size about 4 hours *post-mortem* (Hegarty and Naude, unpublished). The fixative used by Goldspink and by Rowe in all the investigations referred to above was Fleming's solution without acetic acid (Gatenby, 1937), which was found by Goldspink (1961) to cause the least amount of shrinkage to muscle fibres. Goldspink (1962 a) fixed the muscles for 36 hours in this fixative. Rowe (1967) placed the muscle in the fixative immediately after killing the mouse. The muscle was then fixed for 30 hours (Rowe, 1967, 1968; Rowe and Goldspink 1968). Goldspink (1962 a, b) does not state whether the *biceps brachii* was in the *pre-* or *post-rigor* state when it was placed in the fixative. In order to establish that the biphasic distribution was not due to some fibres contracting and others remaining relaxed when the *biceps brachii* was removed, Goldspink (1962 b) carried out the following experiment. The left *biceps brachii* was placed in fixative immediately *post-mortem*. The foreleg from the right side was pinned horizontally and the *biceps brachii* was allowed to go into *rigor mortis* in that position. The histogram of fibre diameters from the muscle fixed after *rigor* gave a biphasic distribution similar to the *pre-rigor* muscle. However, the position of the maxima differed. The small fibre peak was reduced from 25 μ to 15 μ, and the large fibre peak from 40 μ to 26 μ in the *post-rigor* muscle. The explanation given was: »this reduction in the diameter is to be expected as the right muscle was fixed in a stretched position». (Goldspink 1962 b). This observation was made on one mouse. The theory of biphasic fibre distribution in mature mouse *biceps brachii* (Goldspik 1962 a) was based on the same dimensions as the *pre-rigor* muscle quoted above. Therefore, it is assumed that Goldspink (1962 a, b) fixed the *biceps brachii* in the *pre-rigor* state. But Fleming's solution without acetic acid contains osmium tetroxide ('osmic acid') which has poor penetration properties and fixation is liable to be uneven (Drury and Wallington, 1967). Because of the



slow penetration of the fixative and the rapid speed *post mortem* in which fibre diameter decreases (approximately 4 hours), it is reasonable to assume that when a muscle is fixed *pre-rigor* the fibres on the periphery are fixed in the *pre-rigor* state while the fibres in the centre of the muscle are probably in a *post-rigor* state before being fixed. Therefore, the observations of Goldspink (1962 a, b); Rowe (1967, 1968) and Rowe and Goldspink (1968) were made on

muscles that may have contained some fibres in the *pre-* and some in the *post-rigor* state. The number of fibres which would be fixed in a *pre-* or *post-rigor* condition would depend on the size of the muscle. Two peaks for the *biceps brachii* can be obtained from the results of the present investigation if the *pre-* and *post-rigor* distributions are superimposed (Figs. 2 and 3) to give a condition resembling that obtained by Goldspink and by Rowe. The histograms of *pre-* and *post-rigor biceps brachii* from a 6.8 gram mouse almost overlap. There is a divergence in the position of the peaks in the 21.8 gram animal, which becomes more pronounced in a 35.3 gram mouse. Histograms of the fibre diameter of the other mice reported on in Table 1 gave similar results. These observations may account for the reason why Goldspink (1962 a, b); Rowe (1967, 1968) and Rowe and Goldspink (1968) found the *biceps brachii* of young mice to be monophasic and the muscle from mature mice to biphasic.

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