

THE COMPARATIVE HISTOLOGY OF THE BREAST AND LEG MUSCLES
OF THE LIZARD (CALOTES), BIRD (COLUMBA) AND BAT (ROUSETTUS)

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The structure of the vertebrate skeletal muscle has for long been a material for careful study by several workers. The presence of fat droplets and interstitial granules in the striated muscle has been known. That the fibres of the red muscle of the rabbit contain more interstitial granules than are present in those of the white or pale muscles was noted by Krause (1864). Knoll (1891) concluded that the dark fibres are rich in intrafibrillar substance or sarcoplasm containing many interstitial granules while the light ones are poorer in this substance and also contain only very few granules. He also believed that in general the more active muscles contain more of the dark muscle fibres with greater number of these granules. This was later confirmed by Shaeffer (1893). Bell (1911) observed that the dark fibres contained strongly refractive 'Liposomes' (fat droplets) while the light fibres contained tiny faintly refractive liposomes. He also demonstrated that the fat content of muscle is largely dependant upon the nutritive condition of the animal. Bullard (1912) in his study of the interstitial granules of the striated muscle, distinguishes

two types, the true interstitial granules and fat droplets as Kolliker did. The true interstitial granules, according to him are not composed of fat, but consists of an albumino-lipoid. Bullard also observed that the fat droplets consists of neutral fat, and that there is no evidence to show that they contain any substance other than neutral fat least of all any non-lipid substance.

George in two contributions (1947 and 1952) showed the presence of an abnormal amount of fat in the breast muscle of the pigeon and in another, George and I (1953) pointed out the possibility of fat being utilized as fuel in long and sustained contraction of the flight muscle. It was also shown that a reduction of fat in the liver occurred as the contraction of the muscle was continued. The present study was undertaken to see if histological proof of the utilization of fat in muscle contraction could be found.

Materials and Methods:

The material for this investigation consisted of the breast and leg muscles of the lizard (*Calotes versicolor*), the pigeon (*Columba livia*), a local kite

(*Milvus migrans*), domestic fowl (*Gallus domesticus*) and a common local bat (*Rousettus lechinaulti*). The animal in each case was pithed and small pieces of the breast muscle (pectoralis major) of one side were cut out. Some of the pieces were placed in normal saline and others in Jackson's acetic carbol-sudan III stain prepared as given in Glick (1949). Pieces from a leg muscle were also cut out and put in both these solutions separately. The pectoral muscle of the other side in each case was electrically stimulated by a series of make and break shocks (about 50 per minute) with the current passed through the Du Bois induction coil from a battery cell of 4 volts, and adjusting the current at a minimum for a visible contraction and gradually increasing its intensity until the muscle was thoroughly fatigued. Then pieces were cut out from it and separately put in the fluids mentioned above.

To study the effect of fatigue on the structure of the muscle fibre, a pigeon was forced to fly repeatedly in a large hall till it was more or less fatigued. It was immediately pithed and a small piece of the breast muscle of one side was removed to study the muscle fibres. The breast muscle on the other side was then electrically stimulated to total fatigue. A piece of this muscle was

also examined. Some pigeons were starved for a week and pieces of their breast muscle were also treated in the manner mentioned above.

All the treated pieces of muscle were separately teased out and the fibres were mounted. Microphotographs of the fibres were also made.

Results:

On examining the stained as well as unstained preparations of muscle fibres, the following histological features were noted:

(1) The breast muscle of the lizard consists of uniformly broad well striated and clear fibres ($70\ \mu$ to $80\ \mu$ in diameter) with hardly any fat globules within (Plate, 1).

(2) The breast muscle of the pigeon is red and is made up of two types of fibres. One type consists of narrow fibres, the sarcoplasm of which is interspersed with translucent fat globules as well as opaque interstitial granules. The granules appear to be of lipoprotein nature. In this fibre the striations

characteristic of the skeletal muscle are obscured by these inclusions. The other type of fibre is broader, clearer in microscopic vision and the striations are well-marked on it. In this fibre the granules and globules characteristic of the first type are markedly fewer and the latter are minute. In the construction of the muscle the small fibres are surrounded by a number of broad ones, so much so that the former are much more numerous than the latter. Diameter of the narrow fibre ranges from 20 μ to 35 μ and that of the broad one from 40 μ to 60 μ (Plate, 3).

(3) The breast muscle of the kite is also red and is made up of only the broad type of fibres unlike as in the pigeon.

(4) The breast muscle of the fowl is pale unlike the red-tinted ones of the pigeon. and kite. The fibres are all of the broad type. Diameter: 40 μ to 60 μ (Plate, 2).

(5) The breast muscle of the bat is red and is made up solely of the narrow type of fibres, the broad type being absent. Moreover, the inclusions are numerically more than in the breast muscle fibres of the

pigeon, and present therefore a prominent crowded appearance . Diameter: 20 μ to 35 μ (Plate, 4).

(6) The leg muscles of all the above-mentioned animals consists of only the broad type of fibres. The fibres of the leg muscle of the fowl, however, are slightly reddish and possesses comparatively prominent striations. Diameter, lizard: 70 μ to 80 μ ; fowl: 45 μ to 65 μ ; pigeon: 40 μ to 60 μ ; bat: 30 μ to 35 μ (Plates, 9,10,11).

(7) The narrow fat-loaded fibres of the breast muscle of the pigeon on forced, exhausting flight or electric stimulation gradually lose their fat globules and consequently their striations appear rather very prominent after prolonged stimulation (Plates 5,6,7).

(8) The narrow fat-loaded breast muscle fibres from starved pigeons are also found to be depleted of their fat inclusions (Plate, 8).

Discussion:

It was Bullard who gave us for the first time a clear picture of the muscle fibre inclusions. The

present study has shown that two types of muscle fibres occur in the higher vertebrates, those richly laden with large fat globules in their sarcoplasm and others with only a sparse distribution of tiny globules. The presence of the fat-loaded fibres in the breast muscle of the pigeon in large numbers and the disappearance of the fat globules on stimulation and also in flight, give demonstrable support to ^{my}earlier findings (1953) on the utilization of fat in sustained muscular activity. This view gets support from the work of Weis-Fogh (1952) who maintained that fat constitutes the principal source of energy in the locust muscle and suggested that they make use of it in their migration. In this connection it may be mentioned that Kenney J.W. and Glenn Richards in a recent publication (1955) has noted the presence of only the small variety of fibres (75 μ) in the flight muscle and the large ones (350 - 400 μ) alone in the leg muscle respectively in the giant water bug Lethoceros americanus. So a comparative study of the histology of the leg and flight muscle in the various types of insects should be quite enlightening and in the words of the above two authors, "The possibility of finding physiological differences correlated with morphological differences between the muscle systems for any one species seems worthy of consideration". Bullard

who noted the disappearance of fat globules in starvation came to the conclusion that fat in muscle fibres served as reserve food material. The present finding is that globules in the muscle fibres are first and foremost material for prolonged flight and their utilization during starvation is a secondary purpose. Otherwise, there is no reason why the domestic fowl should not have a store of fat in its flight or leg muscle.

It appears that the broad, less-fatty fibres which surround the fat-loaded ones in the breast muscle of the pigeon, are the first to contract in flight, and as the muscle gets warmed up, the others also contract and thus gradually the fat in the fibres is utilized. For short flights therefore the glycogen load of the muscle might be readily used and that must also be the case in walking and running by the domestic fowl.

The soaring bird, kite, has also a fairly high percentage of fat in its breast muscle, but the fat-loaded narrow fibres are absent. The soaring flight is less taxing and goes on for long and the glycogen loaded fibres alone might meet the needs of soaring flight in the kite. Moreover, the sharp movements

involved in the soaring flight are better met by the glycogen-loaded fibres.

The bats are for most of the time on their wings during their foraging at nights. During such flights they have to expend a large amount of energy and that is supplied by the fat-loaded breast muscle fibres.

There is no need of fat-loaded fibres in the leg muscle. Even the domestic fowl which is a runner can never stand persistent pursuit, which means that its leg muscle gets easily fatigued. In the case of the wing muscles of the domestic fowl, the fat-loaded fibres are absent and that explains the limited flight propensities of the domestic fowl.

The over-all outcome of the present investigation is the light it throws on the evolution of the muscle fibre in vertebrates. The ancestral and embryonic muscle fibre is the glycogen-deposited white one. The amphibian and reptilian muscles are generally composed of white fibres only. But in some species in addition to the white muscle, streaks of red muscle are also met with. Alexander (1955) showed that the red muscle of fishes is metabolically superior to the white one, as it

contains more proteins and fat and less water. Moreover, the myoglobin present in it supplies it with ready oxygen to facilitate oxidation. The red muscle is met with extensively in birds and mammals. The emergence of the red fibre is the first stride in the physiological evolution of the muscle fibre. The next evolutionary stride is the fat-loaded narrow red fibre. The breast muscle of the pigeon consists mostly of the thin fibres while that of the bat exclusively of these.

As the breast muscle of the pigeon with its majority of fat-loaded fibres represents an extreme of the red muscle evolution in birds, the breast muscle of the domestic fowl represents an extreme of the glycogen-loaded white fibre muscle. I have found that the amount of glycogen in the breast muscle of the fowl is nearly double that of the breast muscle of the pigeon. Again, the broad fibre of the pigeon breast muscle have been isolated and their glycogen content estimated spectrophotometrically in this department. So also of the narrow ones. It is found that the former contains nearly five times the amount of glycogen than the latter (unpublished work of George and Naik¹⁹⁵⁶).

In addition to the light the present investigations

have thrown on the evolution of the vertebrate muscle, they have also indicated the possible lines along which the evolution of flight in birds took place. With a better understanding of the origin and evolution of flight in birds we shall also be in a position to understand the interrelationship of the various orders of birds. A discussion on this aspect is reserved for the concluding chapter of the thesis.

Plate No. 1 ... Breast muscle fibres of the lizard (Calotes)

Plate No. 2 ... Breast muscle fibres of the fowl

Plate No. 3 ... Breast muscle fibres of the pigeon

Plate No. 4 ... Breast muscle fibres of the bat

B.F. Broad fibres

F.G. Fat globule

I.G. Interstitial granule

N.F. Narrow fibre

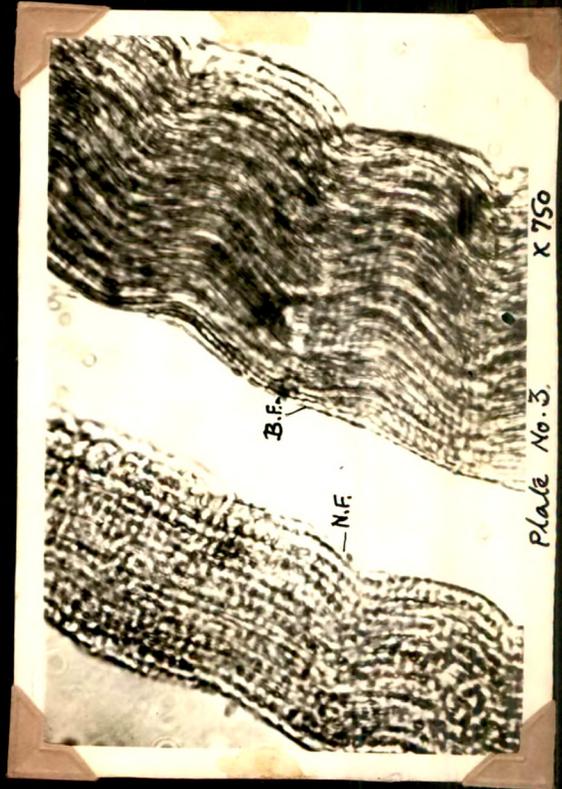
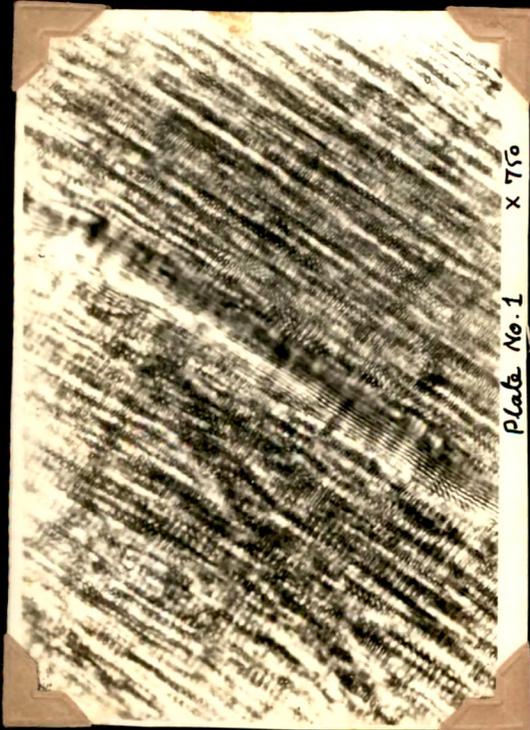
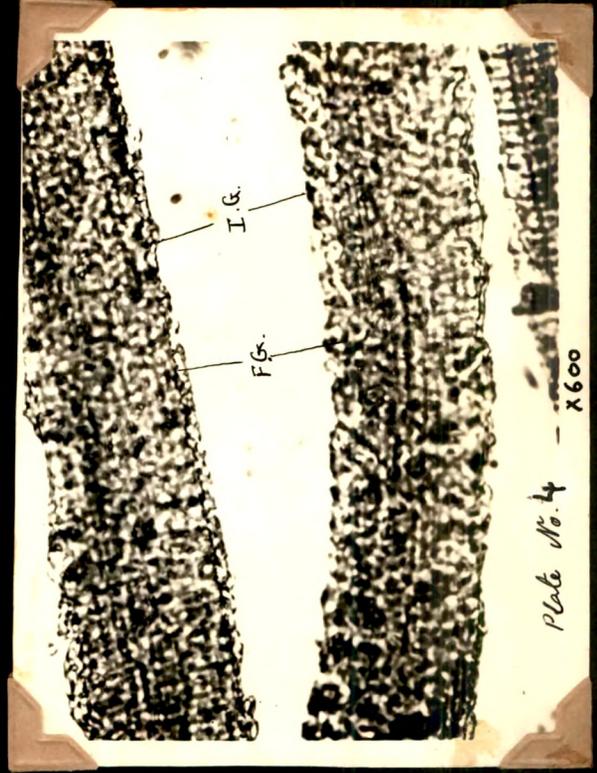
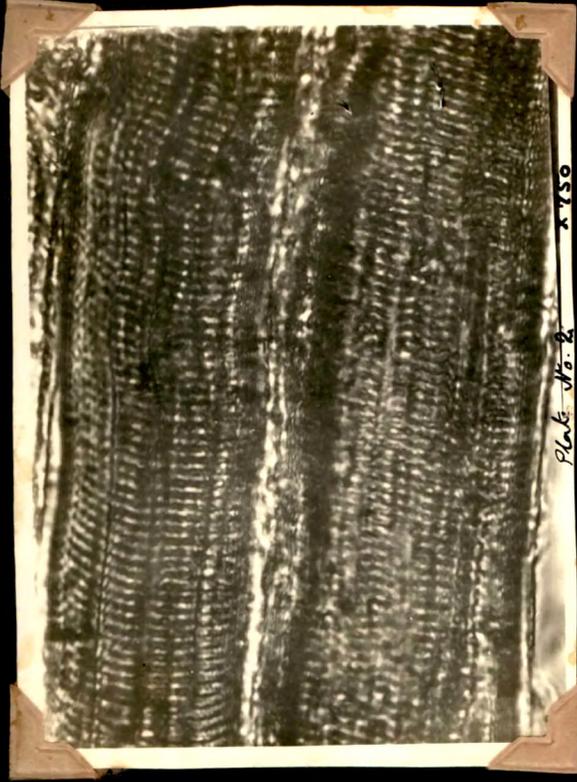


Plate No. 5 ... Narrow muscle fibres of the pigeon breast
muscle before exercise

Plate No. 6 ... Narrow muscle fibres of the pigeon breast
muscle after exercise (forced flight)

Plate No. 7 ... Narrow muscle fibre of the pigeon breast
muscle after exercise (forced flight and
electrical stimulation)

Plate No. 8 ... Narrow muscle fibres of the pigeon breast
muscle after starvation

F.G. Fat globule

I.G. Interstitial granule

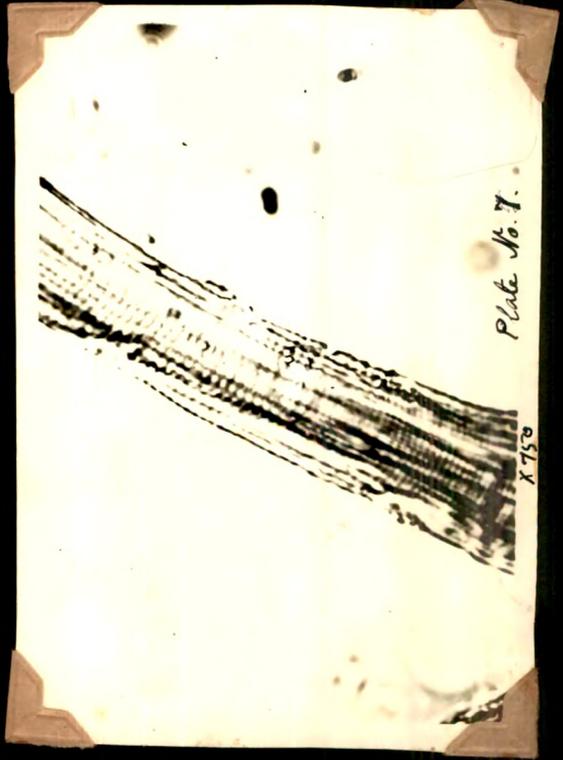
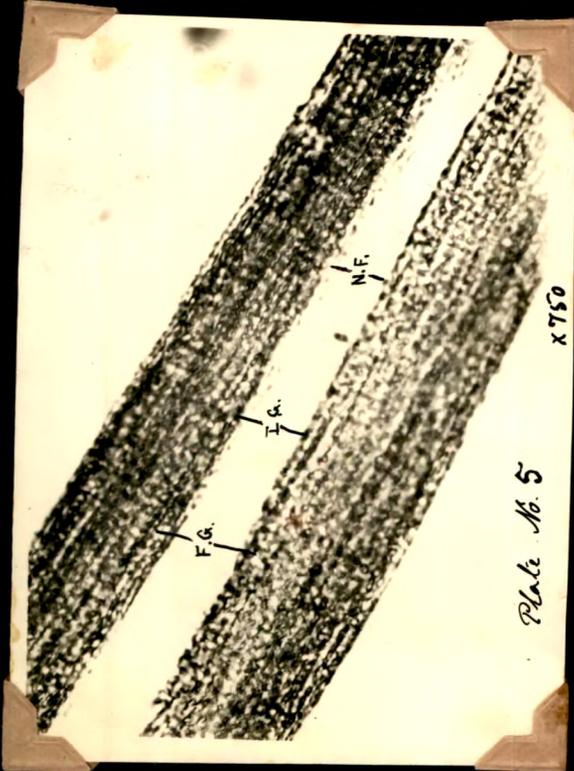
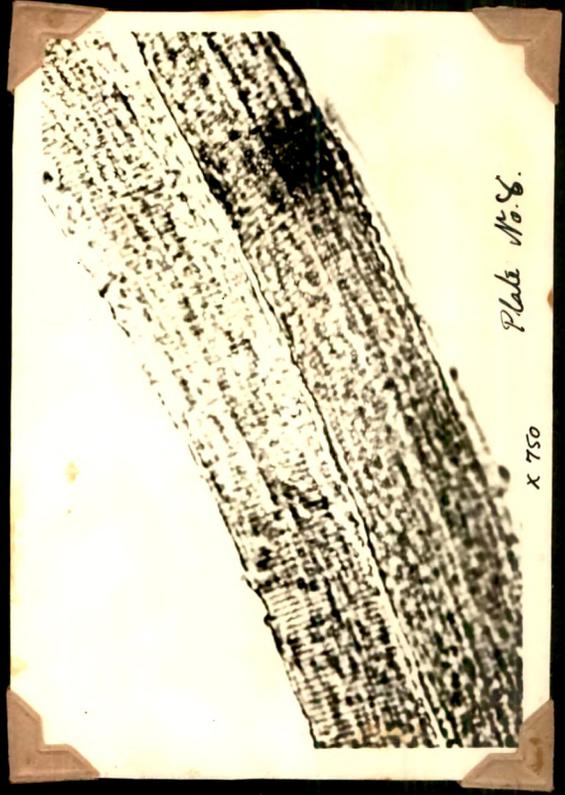
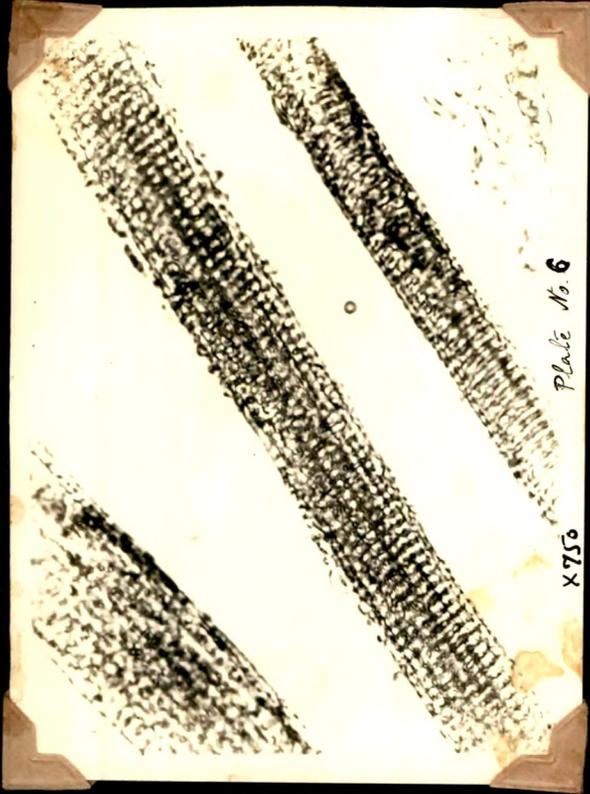


Plate No. 9 ... Leg muscle fibres of the pigeon

Plate No.10 ... Leg muscle fibres of the fowl

Plate No.11 ... Leg muscle fibres of the bat

