under conditions in which the gut appears relatively quiescent viz: In vivo, in animals under deep ether anaesthesia, when the gut appears to be relaxed; and in vitro, in buffer at 25 °C when the gut appears partially contracted. The following experiment was done to determine the variability and relation of lengths measured in vivo and in vitro by comparing these to the lengths of the same segments when relaxed completely by inhibitors.

Methods. 20 male Sprague-Dawley rats (175–200 g) were anaesthetized with an O_2 : ether vapour mixture to deep surgical anaesthesia and the abdomens opened. By means of a piece of flexible, fine, rubber tubing, 8.0 cm lengths of the duodenum, proximal jejunum, middle of the small gut, distal ileum and colon were measured off and excised. The gut contents were flushed out and the mesentery was dissected away. The segments were put into Krebs-Henseleit buffer[3] containing 0.3% glucose and gassed with O_2:CO_2 (95:5), at 25 °C. After 5 min the lengths of the gut segments were measured in the buffer while stretching them just sufficiently to straighten out any curves. The buffer was then warmed to 37 °C and after 10 min the lengths were taken again. The segments were then relaxed by adding to the buffer firstly a mixture containing the following inhibitors: Methysergide 20 µg, Hyoscine 10 µg, Promethazine 10 µg, Hexamethonium 2 mg and Lignocaine 200 µg; and secondly, after 10 min, EDTA pH 7.4, 5 m*M* (all final concentrations/l). After a further 10 min the gut segments were measured again. The ratios of the lengths of each segment under the first 3 conditions to the relaxed length were individually worked out and the geometric means and S.D. for the various gut regions and for each experimental condition were determined from these.

Ratios of gut lengths measured in vivo and in vitro to relaxed lengths

	In vivo length (= 8.0 cm) Relaxed length	25 °C length Relaxed length	37 °C length Relaxed length
Duodenum	0.97 ± 0.02	0.78 ± 0.02	0.72 ± 0.06
Jejunum	0.93 ± 0.04	0.81 ± 0.02	0.74 ± 0.05
Mid gut	0.92 ± 0.03	0.79 ± 0.04	0.80 ± 0.09
Ileum	0.91 ± 0.04	0.79 ± 0.03	0.84 ± 0.07
Colon	0.98 ± 0.02	0.82 ± 0.04	0.68 ± 0.07
Overall ratios	0.94 ± 0.03	0.80 ± 0.03	0.75 ± 0.06

Values for each gut region are geometric means ± S.D. for segments from 20 rats.

The results shown in the Table indicate that as far as the relaxed lengths may be considered as an absolute measure of gut lengths then the lengths measured at 25 °C were fairly constantly about 0.80 or 80% of this length, and the in vivo lengths (i.e. 8.0 cm), though varying slightly more between gut regions, were about 0.94 or 94% of the relaxed lengths. Length measurement under one or other of these conditions would appear to be constant enough for standardization in most transport studies. The length variation of gut segments in vitro at 37 °C however was much greater and suggests that such measurements would be less useful[4].

Résume. La longueur des segments d'intestin de rat, mesurée in vivo sous une anesthésie profonde à l'ether et in vitro, dans une solution tampon à 25 °C, atteint environ le 94% et le 80% de la longueur des segments totalement relâchés. L'une ou l'autre de ces mesures semble assez constante pour être employée dans la standardisation des méthodes en usage dans la plupart des études sur le transport mucosal.

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[1] E. Y. Berger, in *The Transfer of Calcium and Strontium Across Biological Membranes* (Ed. R. H. Wasserman; Academic Press, New York and London 1963), p. 57.
[2] E. Urban and H. P. Schedl, Am. J. Physiol. *217*, 126 (1969).
[3] H. A. Krebs and K. Henseleit, Hoppe-Seyler's Z. physiol. Chem. *210*, 33 (1932).
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A Histochemical Study of the Pectoralis Muscle of the South Indian Flying Lizard, *Draco dussumieri*

The vertebrate skeletal muscle has been the material for careful investigation by several workers. These muscles in different vertebrates, apart from having morphological differences, vary also in the physiological and biochemical characteristics. They are often referred to as the 'red' and 'white' muscles. The existence of a mixed type of muscle composed of narrow, red and broad, white fibres is well-known among vertebrates. These 2 morphologically different fibres vary in their metabolite utilization as well. George et al.[1] attributed the difference in muscle fibres to their functional adaptation at the molecular level. Grinyer and George[2] suggested that the red fibres are 'slow twitch' fibres and the white 'fast twitch' fibres. The histochemical studies on the pectoralis muscle of the South Indian Flying Lizard, *Draco dussumieri*, revealed a number of interesting peculiarities and the results of that study are reported in this short communication.

The pectoralis muscle of *Draco* is a mixed muscle consisting of narrow, intermediate and broad fibres. Diameter ranges from 35–45 µm in the narrow, 50–65 µm in the intermediate and 75–100 µm in the broad fibres. There is a small band of narrow fibres towards the centre of the muscle, surrounded by broad and intermediate ones. Some narrow fibres are found scattered at random towards the periphery. The number of narrow fibres is considerably less per unit area.

Since myoglobin content was very poor, distinction of fibres into red and white was difficult. The central narrow fibres possessed lesser amount of fat than the

[1] J. C. George and A. J. Berger, *Avian Myology* (Academic Press, New York 1966).
[2] I. Grinyer and J. C. George, Can. J. Zool. *47*, 517 (1969).

broad ones. The overall activity of succinic dehydrogenase was rather poor. The central group of narrow fibres did not show any appreciable enzyme activity; whereas it was higher in the broad followed by the intermediate (Figure 1). These narrow fibres, on the other hand, possessed a higher glycogen content than the broad and intermediate ones. Similarly they showed maximum phosphorylase and uridine diphosphate glucose glycogen transglucosylase activities, contrary to the condition in the avian pectoralis (Figures 2 and 3). But a few narrow fibres did not show any activity at all. The peripheral broad fibres in the majority of cases stained yellow; a few, however, stained blue, exhibiting a heterogeneity in the activity of these enzymes.

The present study revealed the presence of 2 types of fibres with different metabolism in the pectoralis of *Draco*. The narrow fibres showed higher glycogen content and enzymes concerned with its metabolism (glycogen synthetase and phosphorylase), but lesser fat, succinic dehydrogenase and myoglobin. Broad ones, on the other hand, showed the opposite results. Thus indicating that the narrow fibres are adapted for a glycolytic and broad ones for a low lipolytic metabolism, contrary to what has been observed in the mixed pectoral muscle of other vertebrates, especially of birds, where the narrow red fibres utilize fat and the broad white ones utilize glycogen as the energy source[1].

The biochemical differentiation of the pectoralis muscle of *Draco* could be correlated with its function. Due to the change in the trunk musculature caused by the gliding adaptation, the respiratory function of the abdominal muscles is shifted to the pectoralis. Therefore, the pectoralis is subjected to greater activity and consequently it has undergone this peculiar metabolic adaptation.

Since the narrow fibres of the pectoralis of *Draco* possess higher glycogen, phosphorylase and UDPGGT, they could be broadly compared histochemically with the red fibres of the appendicular muscle of pigeon and fowl, and the broad fibres of the pigeon pectoralis. The presence of both phosphorylase and UDPGGT in the narrow fibres indicate the occurrence of the complete glycolytic machinery with its enzymatic pathways for the synthesis as well as the break-down of glycogen.

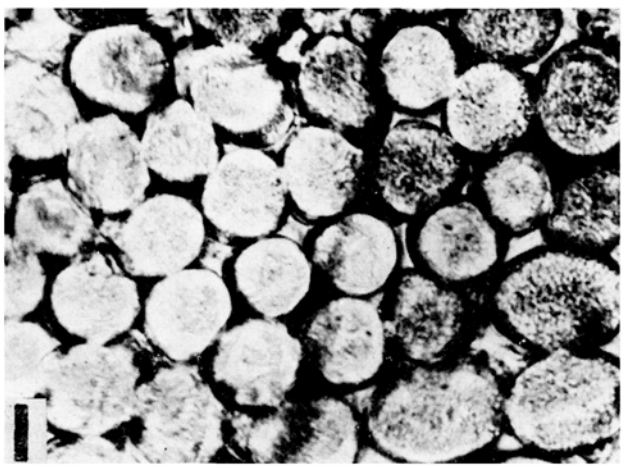

Fig. 1. Photomicrograph of the transverse section of the pectoralis muscle showing mitochondrial localization of succinic dehydrogenase activity by the improved method of GEORGE and TALESARA[6]. Narrow fibres show less of the enzyme while broad ones show more of the enzyme. ×184.

Fig. 3. Photomicrograph of the transverse section of the pectoralis muscle to demonstrate UDPGGT activity by the method of TAKEUCHI[8]. Narrow fibres show maximum activity. ×306.

Fig. 2. Photomicrograph of the transverse section of the pectoralis muscle to demonstrate phosphorylase activity by the method of ERANKO and PALKAMA[7]. Narrow fibres with greater activity and broad ones with less activity. ×306.

It is generally supposed that muscle fibres which lie towards the periphery are more active than those in the interior. Consequently, they increase in diameter. NENE and GEORGE[3] suggested that the large diameter of the fibres of the appendicular muscles in the fowl was due to hypertrophy as a result of tetanic activity of the fibres at a low oxidative metabolism. This seems to be the case in the pectoralis of *Draco* also. The larger fibres in its pectoralis are like the white fibres of the pigeon pectoralis as far as only the diameter is concerned. But as regards the metabolite utilization, they are similar to the narrow red fibres. Therefore, these broad fibres in the pectoralis of *Draco* could be considered as hyper-

[3] R. V. NENE and J. C. GEORGE, Pavo *3*, 35 (1965).

trophied fibres due to the greater tetanic contractions accompanied by greater utilization of fat than glycogen.

JOHN[4,5] found that both the specific acetylcholinesterase and the non-specific butyrylcholinesterase are present in the narrow and broad fibres of the pectoralis of *Draco*, and that acetylcholinesterase is slightly higher than butyrylcholinesterase. It was also observed that each fibre contains numerous closely spaced 'en plaque' type nerve endings, suggesting that pectoralis of this lizard is physiologically more active and a fast muscle[9].

Résumé. Le muscle pectoral du *Draco dussumieri* comprend des fibres de trois sortes: minces, intermédiaires et épaisses. Les fibres minces sont adaptées à un métabolisme glycolytique et les fibres épaisses au lipolytique. L'activité des fibres minces révèle la présence de phosphorylase et de synthétase glycogénique.

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[8] T. TAKEUCHI, Proc. 2nd Ann. Gen. Meeting., Jap. Histochem. Ass. (1960), p. 98.
[9] Grateful acknowledgment is made to Prof. J. C. GEORGE, University of Guelph, Ontario (Canada), and to Prof. A. P. MATHEW, Mar Ivanios College, Trivandrum, for guidance and encouragement.

Influence of Extra Sucrose, Fats, Protein and of Cyclamate in the Daily Food on the Life-Span of Rats

Studying the life-span of Wistar strain albino rats, it has been found that male animals with 30 Cal/100 Cal sugar in their food lived shorter than controls receiving 14.5 Cal/100 Cal, whereas the life-span of the females was not affected[1]. The study has been repeated with weanling male animals, and groups were added receiving butter, sunflower oil or dried lean meat extra, whereas a 6th group received 0.43/100 g cyclamate. A human type diet was given, composed according to the actual composition data of the Dutch population in 1961, in dried and ground form[2-4]. The variations of this Ran-1961 diet were made by substituting bread and potato starch in proportionate amounts isocalorically by the various compounds. The protein lost by replacing potato and bread was resupplied by cooked potato protein and wheat gluten. Sodium cyclamate was given instead of $^2/_3$ of the sugar in the original ration and in such a way that the sweetening effect was the same, which means $^1/_{30}$ of the weight of the sugar. The sugar content of the Ran-1961 ration is 14.5 Cal/100 Cal. The composition of the rations is given in Table I. The animals were kept – in individual cages – until 'spontaneous' death. Body weights were recorded every 2 weeks. Food and water were always given ad libitum. Kidneys, liver, adrenals, pancreas and testes were examined histologically. Urinary bladders of the control and cyclamate groups have been examined after the first rumours came about cyclamate producing bladder tumors[5-7]. Routine staining was done with haematoxilin-eosin and with sudan (III+IV)-Ehrlich. WILCOXON's test[8] has been used for the statistical evaluation of the data.

Effect of the diets. The animals receiving cyclamate showed a significant higher weight gain and had a better food efficiency than the controls, already in the first weeks. The other groups did not show distinct differences, except for a relative low food consumption and

Table I. Protein and fat content of the diets and fat composition[a]

	I Control Ran-1961	II Sugar extra	III Butter extra	IV Sunflower oil extra	V Dried meat extra	VI Sodium cyclamate food
Protein of dry matter (%)	12.8	12.6	13.7	13.7	23.4	14.7
Fat of dry matter (%)	21.6	21.2	30.6	31.2	23.8	24.9
Fatty acids of total fatty acids (%)						
C12	4.2	4.75	3.6	3.8	4.0	4.35
C 14	6.0	5.6	7.05	4.5	5.2	5.8
C 16	21.5	21.2	22.6	17.8	21.65	21.85
C 16:1	2.5	2.25	2.2	1.75	2.45	2.2
C 17	0.7	0.7	0.75	0.5	0.75	0.7
C 17:1	0.3	0.3	0.35	0.2	0.3	0.3
C 18	12.2	11.95	12.8	9.5	13.15	12.05
C 18:1	29.85	30.55	30.6	28.0	30.0	29.85
C 18:2	14.0	14.35	12.05	27.25	14.4	14.1
C 18:3/20	0.65	0.65	0.55	0.5	0.65	0.65
C 20:1	3.0	2.9	2.65	2.25	2.85	2.9
C 22	0.45	0.35	0.55	0.5	0.35	0.4
C 22:1	1.6	1.45	1.2	1.4	1.5	1.55

[a] Averages of duplicate measurements in each of samples taken with an interval of one year; protein and fat content were controlled in twice as many batches.