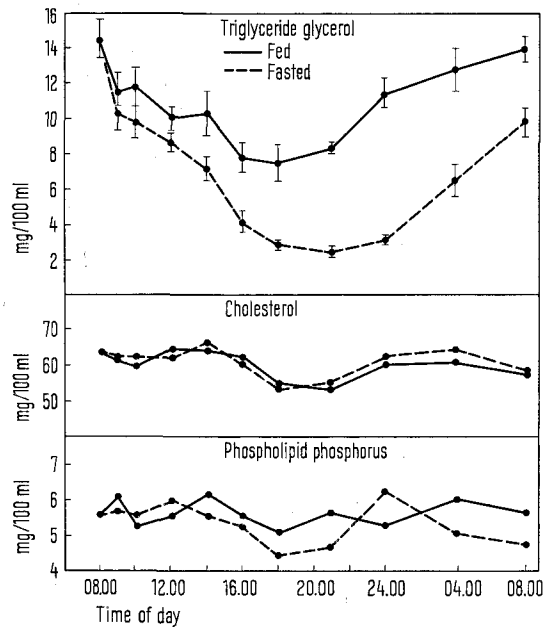
recovering to 9–10 mg/100 ml by 08.00 the following day. The difference between fed and fasting triglyceride levels became statistically significant as early as 14.00 h. Although rats consume most of their daily food intake at night, they do tend to 'nibble' during the day[8]. Comparison of our results with those on the diurnal rhythm of free fatty acids[3] indicates an inverse relationship between rat serum triglycerides and free fatty acids throughout the day.



Effect of fasting and time of day on serum lipids in male rats. Food (but not water) was withdrawn from the fasted animals at 08.00 h. Standard errors are given for serum triglycerides only. Each point represents the mean of 10 rats.

Serum cholesterol and phospholipid levels in both fed and fasted rats remained virtually unchanged during the 24 h test period. No standard errors are presented in the Figure for cholesterol and phospholipids because no statistical difference was found between the fed and fasted rats. As discussed previously[9], much has been reported on the effects of fasting on lipid levels (especially sterols), but literature comparisons are difficult due to such variations as species, strain, sex, age and duration of fast.

The results show that, in contrast to cholesterol and phospholipids, serum triglycerides in both fed and fasted rats exhibit a marked diurnal rhythm and that the effect is more pronounced in fasted animals. Thus, the time of killing is a factor of particular importance in studies of agents affecting serum triglyceride levels.

Résumé. Chez le rat albinos, le niveau des triglycérides sériques varie au cours de la journée, avec un maximum à 08.00 h et un minimum au début de la soirée. Cette variation est plus marquée chez les rats à jeun. Par contre, aucune variation n'a été observée dans les niveaux de cholestérol et de phospholipides.

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The Cholinesterase Activity of Myoneural Junctions from Frog Twitch and Tonic Muscles

At least two different types of extrafusal muscle fibre have been described in the skeletal muscles of frogs. The fibres have been classified as twitch or tonic on the basis of their physiological[1,2], structural[3–5], and biochemical properties[6]. Also the myoneural junctions on these fibres differ in their form and distribution[7]. Günther[8] has shown by staining nerve terminals with silver that 'en-grappe' motor nerve endings occur on the muscle fibres of the tonus bundle of the frog iliofibularis muscle (described by Sommerkamp[3]) and not on fibres in other parts of the iliofibularis or in the sartorius. Other authors[4,9] have demonstrated that the twitch muscles, stained for cholinesterase, have myoneural junctions, 'Endbüschel', which are relatively extensive and which run lengthwise along the muscle fibre with a few oblique branches connecting the main longitudinal terminations. This type of ending occurs in a band of innervation so that nerve terminals on adjacent fibres are more or less at the same level. The en-grappe endings appear irregularly scattered along the muscle fibre and do not occur on the same level on adjacent tonic fibres.

The question posed in this investigation is – can differences in cholinesterase (ChE) activity of myoneural junctions be related to the morphological and functional differences of frog muscles? In order to answer this question a radiometric method[10] was used to give a direct measure of the ChE activities of populations of single myoneural junctions from the different muscles.

The muscles investigated were the iliofibularis (twitch) and its tonus bundle (tonic), sartorius (twitch) and rectus abdominus (mixed) of *Rana tempororia*. The muscles were pinned at their resting length on hard paraffin and incubated in a thiocholine medium[10] for 30 min to make the

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myoneural junctions visible for dissection. Only myoneural junctions from multiply innervated fibres were dissected from the tonus bundle. The iliofibularis had one innervation band and the sartorius had two. 'Endbüschel' were dissected from these bands. Both 'Endbüschel' and engrappe endings were dissected from the multiply innervated fibres of the rectus abdominus. These were treated as one population. BUCKLEY and HEATON[10] have described the technique for dissection of single myoneural junctions. The ChE activity of the myoneural junctions was assayed by enzymatic hydrolysis of ^{14}C-acetylcholine (ACh) followed by solvent extraction of the reaction product ^{14}C-acetate which was then measured by liquid scintillation spectrometry[10]. ChE activity was expressed as the number of pico-moles of ACh hydrolyzed per myoneural junction per h (p. mol/j/h).

Figure 1 illustrates the range of ChE activities found for moyneural junctions in the iliofibularis. The median ChE activity of myoneural junctions ($N = 21$) from multiply innervated fibres in the tonus bundle was 18 p. mol/j/h. The median ChE activity of junctions ($N = 40$) from singly innervated fibres outside the tonus bundle was 76.0 p. mol/j/h. The range of activities were significantly different ($P > 99\%$) as determined by a ranking procedure[11].

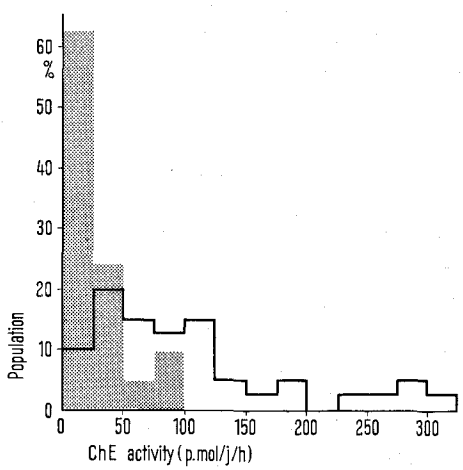

Fig. 1. The distribution of ChE activities in myoneural junctions from muscle fibres in the frog iliofibularis. ▦, Myoneural junctions ($N = 21$) from multiply innervated fibres inside the tonus bundle. □, Myoneural junctions ($N = 40$) from singly innervated fibres outside the tonus bundle.

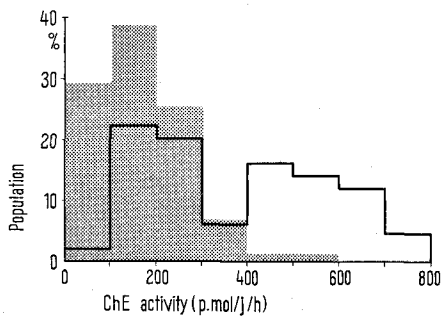

Fig. 2. The distribution of ChE activities in myoneural junctions from muscle fibres in the frog rectus abdominus and sartorius. ▦, Mixed population of myoneural junctions ($N = 100$) from the rectus abdominus. □, Myoneural junctions ($N = 47$) from the sartorius.

The median ChE activity of myoneural junctions (mixed population) from the rectus abdominus was 140 p. mol/j/h and the value for the sartorius was 320 p. mol/j/h. The range of activities for the two muscles is illustrated in Figure 2.

The conclusion from these measurements is that the myoneural junctions from tonic muscles of the frog have a lower ChE activity than myoneural junctions from twitch fibres. This is in agreement with the results of studies on mammalian[10] and avian[12] muscles. The weaker ChE activity of nerve-endings of tonic fibres is likely to be a consequence of the lesser degree of folding of their postsynaptic membranes[13]. The subneural apparatus of the 'Endbüschel' consists of a palisade-like arrangement of synaptic gutters parallel to each other and standing normal to the terminal brach of the terminal axon[14]. Nerve endings of frog tonic fibres consist of rings or dots made up into a grape-like structure without any sign of subunits[15,16].

Attempts other than the present one have been made to quantify the impression that the ChE activity of grape-like apparatuses is weaker than that of palisade-like apparatuses. CSILLIK[9] has expressed ChE activity as a quotient (Q value) of staining intensity, measured by photometry, and an estimate of the reactive area. By this method the ratio of Q value for 'Endbüschel' and engrappe subneural apparatuses was 2 or 3:1. The more direct measurements of the present investigation give a ratio of 4:1 using the results from the iliofibularis.

The functional differences between twitch and tonic fibres require that transmitter action at myoneural junctions of twitch fibres be more abruptly terminated than at junctions of tonic fibres. The differences in ChE activity of junctions from the two types of fibre could be important in fulfilling this requirement.

Résumé. La distribution de l'activité cholinestérasique dans des faisceaux de jonctions myoneurales séparés des fibres cloniques et toniques de muscles de grenouille a été étudiée par une méthode radiochimique. Les jonctions myoneurales de fibres cloniques ont montré une plus forte activité cholinestérasique que celles de fibres toniques. Il est suggéré que la différence d'activité enzymatique est liée à la différence fonctionnelle des deux types de fibres.

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