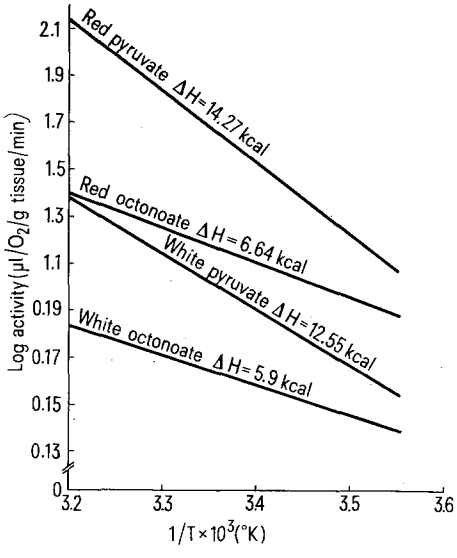
Oxidation of Pyruvate and Octonoate by Red and White Myotomal Muscles of the Crucian Carp (*Carassius carassius*)

In the myotomal musculature of the fish, two main muscle fibre types, red and white, are recognisable. These two may be differentiated by the relatively high concentrations of mitochondria and the greater oxidative capacity of the red fibres compared to the white fibres[1,2]. The ability of these two muscles to oxidize substrates belonging to different metabolic pathways had received little attention in the past. Hence, in this present study the rate of oxidation of pyruvate and octonoate has been measured and their enthalapies of activation determined.

Materials and methods. Crucian carp (*Carassius carassius*) between 12 and 16 cm in length were maintained in a fresh water aquarium (water temperature 11.8 ± 0.1 °C) and fed on a daily diet of proprietary pond fish food. The animals were killed by a sharp blow on the head and red and white muscle samples then quickly dissected out from a region immediately posterior to the dorsal fin. Samples for incubation were weighed and then finely minced with a sharp scalpel.

Incubations were carried out over a range of temperatures (6–30 °C) in a medium the same as that used by BILINSKI[3], except that a 0.025 *M tris* buffer (pH 7.3) was used. The rates of oxidation of the 2 substrates, 0.16 *M* pyruvate with 0.016 *M* malate and 0.16 *M* octonoate with 0.016 *M* ketoglutarate, were determined using the Gilson oxygen electrode apparatus. Malate and ketoglutarate were incorporated with the substrates in order to 'spark' the reactions. The tissue was allowed to use up its own endogenous substrates before the experimental substrate was added. The point at which endogenous substrates had been exhausted was indicated by a rapid fall off in the rate of oxygen consumption.



Arrhenius plots of oxygen uptake. Each regression line calculated from 40 data points.

Results. The results of the experiments are given in the Table. The enthalapies of activation, ΔH, were calculated from the regression plots given in the Figure using the Arrhenius equation[4]. The red and white muscles of the crucian carp have been demonstrated to be similar with respect to their enthalapies of activation of octonoate and pyruvate. It has been shown that both muscles oxidise pyruvate more rapidly than octonoate although red muscle utilised the 2 substrates at several times the rate of the white muscle. The ratio of the rate of oxygen consumption with pyruvate to the rate of oxygen consumption with octonoate was also found to be similar for both muscles at all temperatures (see Table).

Discussion. The precise locomotory and metabolic role of the two types of myotomal muscle in fish is uncertain. Nevertheless, there is a large body of evidence to suggest that red muscle plays an important role during long periods of slow sustained swimming whilst white muscle provides most of the mechanical energy required for short bursts of speed[5–8]. Octonoate was found to be oxidised at a low rate compared with pyruvate (see Table). Therefore if these experimental substrates reflect the in situ condition regarding the relative rates of fat and pyruvate oxidation, then it would appear that synthesis of ATP required for muscular contraction will not be as rapid when fat is the substrate. P/O values of 2.7 and 2.4 for pyruvate and palmitoylcarnitine respectively have been determined in mammalian muscles[9]. Thus fats are a more suitable substrate for muscles working at a low intensity, such as the red muscles of the fish, as under these conditions there is plenty of time to oxidise the lipids and relatively more oxygen available.

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Oxygen uptake of red and white muscle using pyruvate and octonoate as substrates expressed as $\mu l/O_2/g$ wet weight/min. Each result represents the mean of 10 determinations

Temperature (°C)	Red muscle			White muscle		
	Pyruvate	Octonoate	Ratio	Pyruvate	Octonoate	Ratio
6	10.29 ± 0.80	5.45 ± 1.00	1.89	1.83 ± 0.26	0.63 ± 0.09	2.9
14	21.87 ± 1.55	11.15 ± 1.40	1.96	4.37 ± 0.27	2.28 ± 0.22	1.92
22	32.29 ± 3.48	14.51 ± 1.31	2.23	5.00 ± 0.57	2.36 ± 0.36	2.12
30	78.82 ± 8.64	15.73 ± 2.23	5.01	18.40 ± 1.98	3.98 ± 0.78	4.62

Observations of fat utilization during prolonged low speed swimming would tend to support this view[5,10].

The rates of oxidation by the white muscle were found to be relatively low. Thus it is doubtful whether oxidative processes can play a major role in supplying energy during intensive bursts of activity by this muscle.

The role of glycogen in red muscle metabolism is uncertain. Higher resting levels are found in the red than in the white muscle, and during sustained swimming glycogen has been observed to fall in the red muscle[7,8]. It has been demonstrated (see Table) that red muscle has a high oxidative capacity for pyruvate, the product of glycolysis that enters the citric acid cycle. It may well be that both fats and glycogen are used as substrates by this muscle. An alternative hypothesis is that red muscle supplements the role of the liver in supplying metabolites for glycolysis to the white muscle and in oxidising its lactate[11]. The high rate of pyruvate oxidation observed in the red muscle is compatible with the view that this tissue could play a significant role in the oxidation of lactate from the white muscle.

Zusammenfassung. Sowohl in roten als auch in weissen Muskelfasern ist die Oxydationsgeschwindigkeit des Pyruvats grösser als die des Octonoats. Für rote Muskelfasern sind die Werte des ΔH: Pyruvat, 14.27 kcal; Octonoat, 6.64 kcal; und für weisse Muskelfasern: Pyruvat, 12.55 kcal; Octonoat 5.90 kcal.

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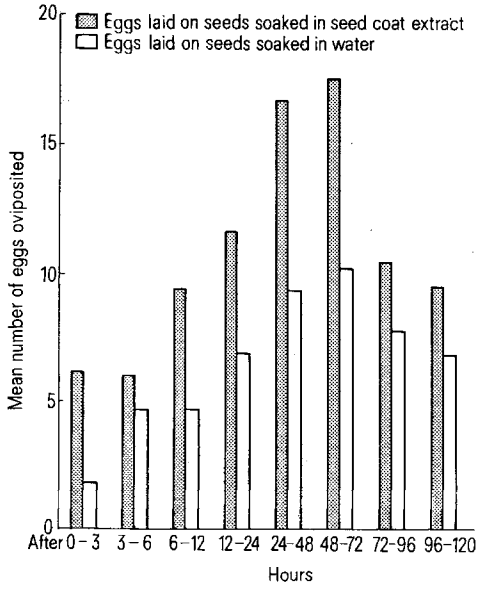
French Bean Seed Coat as an Ovipositional Attractant for the Pulse Beetle, *Callosobruchus maculatus* (Fabricius)

Several species of beetles belonging to the family Bruchidae are serious insect-pests of stored pulses and beans. At generic and specific level, these insects exhibit a high degree of specificity for their growth and development towards the various seed species of legumes. In most of the known species of bruchids, it is the ovipositing adult which performs the actual selection of the host by cementing her eggs on the seed surface during oviposition. In a mixture of many seed species of legumes, at least 4 species of bruchids are known to exhibit a distinct preference for oviposition on some seeds rather than on others[1-4]. No correlation, however, exists between the preferential oviposition by the adult for different seed species and the subsequent larval development. Some of the physical characteristics of the legume seeds, like curvature of the seed for *C. chinensis* (L.), texture of the seed coat for *C. maculatus*, C. *chinensis* and *C. analis* (F.), have been shown to be possible guiding stimuli for preferential oviposition[5,6]. The ovipositional attractant activity of soybean saponin extract for *C. chinensis* was not considered to be of any biological significance[7]. The present work for the first time reports the ovipositional attractant activity of French bean seed coat for *C. maculatus*.



Ovipositional response of *Callosobruchus maculatus* on chick pea seeds soaked in the acqueous extract of French bean seed coat and water.

Methods and results. A culture of *C. maculatus* was maintained in glass jars covered with muslin containing green gram seeds at 30 °C ± 1 °C and 55–60% relative humidity. All the experiments were also conducted at the aforesaid temperature and relative humidity.

Since the seed coat of different legume seeds comes in direct contact with the ovipositing females; these were gently removed without damaging the internal surface. For each legume, 10 seeds without seed coat and 10 with seed coat were placed together in a petridish wherein 2 pairs of newly emerged adults were released for oviposition for 96 h. The results of the experiment have been shown in Table I. It appeared that the seed coat of some legumes may contain substance(s) which induce the gravid females to lay more eggs, since the mean number of eggs laid were significantly more on the seeds having seed coat than on those without seed coat. Chickpea, however, was an exception, where the absence of seed coat did not

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