Relative specific activity (RSA) and phosphate ester content of cells after 4 h incubation in control or phloridzin media

	Control		Phloridzin 2·5 *mM*		Phloridzin 20 *mM*	
	RSA	Quantity μgP/ml cells	RSA	Quantity μgP/ml cells	RSA	Quantity μgP/ml cells
Diphospho-glycerate	214, 250	135, 165	110, 94	150, 143	71	165
Inorganic phosphate	125, 143	35, 27	47, 36	42, 32	27	59
Adenosine triphosphate	195, 204	45, 58	56, 71	51, 46	17	36

tents of the fractions were little affected by the phloridzin the amount of P 32 incorporated was reduced, even in the 'inorganic' fraction. From these results we conclude that the red cell phosphate is in a dynamic state and that the turnover is sensitive to phloridzin and phloretin.

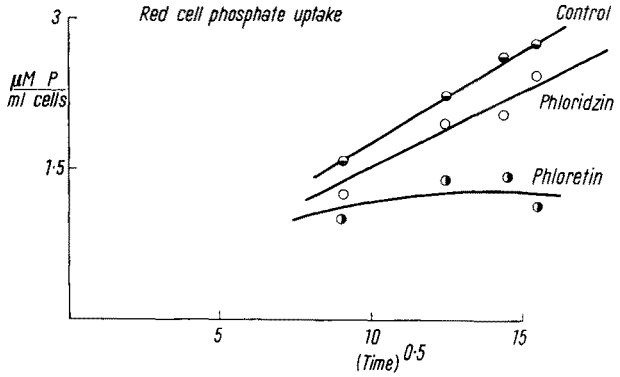

Fig. 2.—The uptake of P 32 by red cells from a large volume of medium containing 1·2 *mM* P 32 labelled phosphate. The test media contained either 6 *mM* phloridzin or 0·03% w/v phloretin. The P 32 concentration in the medium remained nearly constant.

(Time measured in minutes)

In the same experiments analyses for Na and K were carried out. These showed that no changes of Na or K content took place during 4 h exposure to phloridzin or phloretin. One sample of cells was cold stored overnight so that the Na content was 36 meq/l cells and the K content 72·5 meq/l. During subsequent incubation the Na content fell to 26 meq/l and the K content rose to 79 meq/l in both control, phloridzin, and phloretin media showing that net cation movement is unaffected. From this it appears that sufficient sugar still enters the poisoned cells to maintain that part of the metabolism which energises conversion from the low K-high Na to the high K-low Na condition.

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Résumé

L'addition de phlorizine ou de phlorétine à une suspension d'hématies humaines ralentit l'allure d'incorporation du radio-phosphate dans les cellules. Celles-ci contiennent les mêmes quantités d'esters phosphoriques après l'action de la phlorizine. Les deux drogues ne modifient pas non plus les concentrations en Na et en K des cellules.

Lipase Activity in Insect Flight Muscle

GEORGE and JYOTI[1] showed that flying birds and bats utilize fat as the chief fuel for long and sustained muscular activity. WEIS-FOGH[2] established that in the locust, during sustained flight, about two-thirds of the total energy expended is derived from fat. The importance of lipase in such muscles which utilize fat as fuel for their activity has been demonstrated by GEORGE and SCARIA[3]. They have shown that the flight muscles of birds indulging in sustained flight and the heart muscle of all vertebrates possess a high concentration of lipase. They correlated the lipase concentration of the muscle with the extent of fat utilization depending on the activity of the muscle. It was therefore thought desirable to study how far this correlation could be extended to the flying insects which exhibit various degrees of muscular activity during flight.

Aqueous extracts of the flight muscles of the following insects were used for the study: the desert locust (*Schistocerca gregaria*), the dragon fly (*Pentala flavescens*) and the bumble bee (*Xylocopa* sp.). The enzyme was prepared in the following manner: Weighed quantities of the flight muscles were extracted in known quantities of distilled water in cold for 1 h by scrubbing with sand in a test tube; it was then centrifuged at 2500 r.p.m. for 5 min and the supernatant used for the study. The method used for the assay was a manometric method adopted from MARTIN and PEERS[4] using the Warburg apparatus in a bicarbonate carbon dioxide buffer system of pH 7·4 at 37°C using tributyrin as substrate. The reaction flask contained 1·5 ml 0·025 *M* bicarbonate solution, 0·5 ml distilled water and 0·5 ml of the enzyme solution in the main chamber and 0·5 ml 4% (v/v) tributyrin in 0·0148 *M* bicarbonate (emulsified by shaking with a drop of Tween 80) in the side arm, thus making up a total volume of 3 ml. The manometers and flasks were gassed for 3 min with a mixture of 95% nitrogen and 5% carbon dioxide from a cylinder. After equilibration for 10 min the substrate was tipped in and the readings taken twice at intervals of 15 min. The manometers were shaken at about 100 oscillations/min allowing an amplitude of 4–5 cm per oscillation.

Lipase activity was calculated on the basis of wet weight and also the protein concentration of the enzyme solution used, and is expressed as the number of μl of CO_2 produced/mg/30 min (Table). Protein was estimated according to the colorimetric method for total proteins[5].

[1] J. C. GEORGE and D. JYOTI, J. Anim. Morph. Physiol. *2*, 1 (1955); *4*, 2 (1957).

[2] T. WEIS-FOGH, Proc. roy. Soc. [B] *237*, 640 (1952).

[3] J. C. GEORGE and K. S. SCARIA, J. Anim. Morph. Physiol. *3*, 2 (1956); *4*, 2 (1957).

[4] H. F. MARTIN and F. G. PEERS, Biochem. J. *55*, 523 (1953).

[5] P. B. HAWK, B. L. OSER, and W. H. SUMMERSON, *Practical Physiological Chemistry* (McGraw-Hill Book Company Inc., 1954).

Lipase activity of Insect Flight Muscle*

Insects	μl Co_2/mg protein/30 min (1)	μl Co_2/mg wet weight/30 min (2)
Locust	9·5	1·35
Dragon fly	50·0	8·10
Bumble bee	9·0	0·73

* The values given are the average of five experiments.

In the Table above, the values in column 2 have to be considered as more accurate for the purpose of comparison, since the quantity of nonenzymic protein dissolved may not be the same in all cases. Considering the lipase activity of the aqueous extract of the pectoralis major muscle of pigeon prepared in exactly the same manner as above and calculated on the wet weight basis (lipase activity = 0·8 μl CO_2/mg wet weight/30 min), it is seen that the flight muscles of the locust and dragon fly have a higher lipase activity. In the case of the locust, it is known that it utilizes fat as the chief fuel during flight.

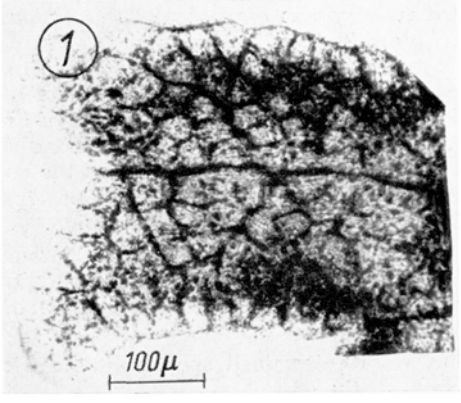

Fig. 1

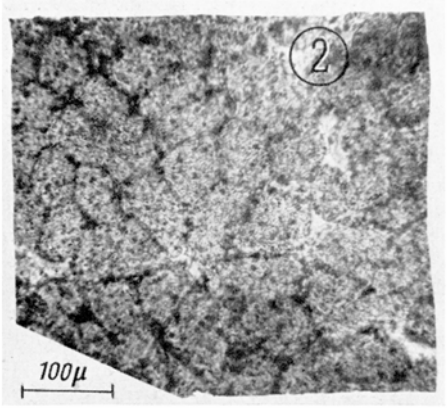

Fig. 2

Fig. 1 and 2.—Microphotographs of the T. S. of the flight muscle of the dragon fly and locust respectively, treated to show lipase activity.

But in the dragon fly the lipase concentration is 6 times that in the locust, which means that this animal also might be utilizing fat for the energy during flight. Nevertheless it is a fact that the dragon fly is a very good flier indulging in sustained flight for hours together with little rest. The low lipase concentration in the flight muscles of the locust as compared to that in the dragon fly may be explained as due to the fact that the locusts used in our study were laboratory bred animals and held in captivity throughout their life. Evidently, in such animals, the metabolic rate is lower and consequently the concentration of the various enzymes will also be at a lower level since enzymes are adaptive to a considerable extent. The presence of lipase in the flight muscle of the bumble bee also suggests the utilization of fat for muscular activity at least to a certain extent.

The lipase activity in the flight muscles of the locust and dragon fly was histochemically studied according to the Tween method of GOMORI[6]. The substrate used was 'Tween 80' which is specific for true lipase. The incubation medium contained 2 ml 10% $CaCl_2$, 2 ml 5% 'Tween 80' (Atlas), 5 ml bicarbonate buffer of pH 8·4, 40 ml distilled water and a crystal of thymol as preservative. The mixture was incubated at 40°C overnight to precipitate the free fatty acid, if any, and filtered to remove the precipitate[7]. Sections of the muscle, which were prepared according to the method described by GEORGE and SCARIA[8], were incubated for 8–10 h in the medium at 40°C. Boiled sections were used as control.

Figures 1 and 2 are the microphotographs of the flight muscle of the dragon fly and locust respectively, treated to demonstrate lipase activity. It could be seen that the lipase activity, as indicated by the deposition of precipitate, is higher in the muscle of the dragon fly as was also shown by the quantitative determination.

Grateful thanks are due to Dr. K. B. LAL, Plant Protection Advisor to the Government of India for arranging the supply of the locusts used in this study.

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Laboratories of Animal Physiology and Histochemistry, Department of Zoology, University of Baroda (India), February 28, 1958.

Zusammenfassung

Die Lipaseaktivität von wässerigen Extrakten der Libelle (*Pentala flavescens*), der Heuschrecke (*Schistocerca gregaria*) und der Hummel (*Xylocopa* sp.) wurde manometrisch festgestellt, unter Verwendung von Tributyrin als Substrat. Die höchste Lipaseaktivität wurde in der Libelle gefunden, die niedrigste in der Hummel. Der hohe Gehalt der Flugmuskulatur der Insekten an Lipase wird in Zusammenhang gebracht mit der Benutzung von Fett als Brennstoff bei dauernder Muskeltätigkeit. Die Lipaseaktivität in der Flugmuskulatur der Libelle und der Heuschrecke wird histochemisch nachgewiesen mit dem Verfahren nach GOMORI, unter Verwendung von «Tween 80» als Substrat.

[6] A. G. PEARSE EVERSON, *Histochemistry, Theoretical and Applied* (J. & A. Churchill, London 1954).

[7] J. C. GEORGE and K. S. SCARIA, Nature *181*, 783 (1958).

[8] J. C. GEORGE and K. S. SCARIA, Nature *181*, 783 (1958); J. Anim. Morph. Physiol. *5*, 1 (1958).