## PHYSIOLOGY

### Changes in Haemolymph Lipoproteins during Locust Flight

MUCH of the energy for long distance flight in locusts is derived by oxidation of lipids[1], and the lipid is transported from the fat body (the principal site of lipid storage) to flight muscle through the haemolymph. During flight the lipid content of the haemolymph of *Locusta migratoria* increases to four times the resting value[2] and this is accounted for by an increase in the glyceride fraction. Chino and Gilbert[3] found that diglyceride is released from fat body during *in vitro* incubations with haemolymph, and suggested that diglyceride is important in lipid transport in the several insect species they studied. In mammals lipids are transported chiefly in the form of lipoproteins, and this is also so in a number of insect species[3–5]. We have therefore examined the nature of the increase in glyceride which occurs during locust flight, and have attempted to relate this to changes in haemolymph lipoproteins.

Adult male *Schistocerca gregaria* were used 14–18 days after the final moult, and samples of haemolymph were obtained from the cervical region by puncture with a glass capillary. Electrophoresis of haemolymph was carried out on cellulose acetate strips using 0·05 molar sodium glycinate buffer, *p*H 9·8; proteins were detected by staining with nigrosine and lipids were detected on separate strips by oxidation with hydrogen peroxide followed by staining with Schiff reagent. Lipids were estimated by direct densitometry of the stained strips.

Electrophoresis of haemolymph from resting locusts showed eight protein components (Fig. 1), only two of which (group *A*) usually contained lipid. When insects were flown[1] for 2 h the total lipid content of the haemolymph increased to between three and four times the resting value (as estimated by densitometry of electrophoretograms). Part of this increase was accounted for by an increase in the lipid content of the group *A* lipoproteins to 2·5 times the resting value. In addition lipid appeared in a second pair of proteins (group *B*, Fig. 1). After a flight lasting 2 h, 70 per cent of the total haemolymph lipid was associated with group *A* lipoproteins and 30 per cent with group *B* lipoproteins. Three hours after flight had stopped the haemolymph lipoprotein pattern had returned to the resting state.

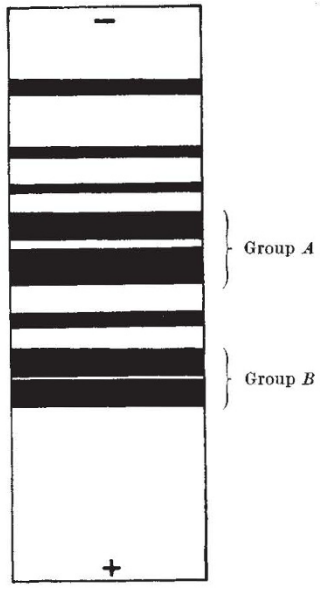


Fig. 1. Electrophoresis of locust haemolymph proteins. (Cellulose acetate; *p*H 9·8.)

These results were confirmed by the results of other experiments in which locusts were fed from the time of the final moult on a diet containing [U − $^{14}$C] palmitate. The fat bodies of such insects was found to be labelled principally in the lipid fraction; less than 1 per cent of the radioactivity was present as carbohydrate. Electrophoresis of haemolymph from resting animals showed that almost all the radioactivity was present in the group *A* lipoproteins. After a flight of 2 h the radioactivity of group *A* lipoproteins had increased, and an additional radioactive area was present coincident with group *B* lipoproteins. More than 95 per cent of the total radioactivity of the haemolymph was accounted for in these two fractions.

The radioactive lipids were further examined by extraction of haemolymph with chloroform/methanol[6] followed by thin-layer chromatography[7] of the extract.

Table 1. DISTRIBUTION OF RADIOACTIVITY IN HAEMOLYMPH LIPIDS BEFORE AND AFTER FLIGHT

| | C.p.m. in 0·01 ml. haemolymph | |
|---|---|---|
| | Before flight | After flight |
| Total haemolymph radioactivity | 180 | 680 |
| Fatty acids | 5 | 6 |
| Diglycerides | 26 | 540 |
| Triglycerides | 140 | 110 |

Table 1 shows that the main increase in lipid radioactivity after a flight of 2 h was accounted for by an increase in the diglyceride fraction; the triglyceride and free fatty acid fractions remained fairly constant.

The lipids of lipoproteins which had been electrophoretically separated from haemolymph from flown insects were examined by thin-layer chromatography. Group *A* lipoproteins contained both triglyceride and diglyceride, whereas group *B* lipoproteins contained chiefly diglyceride.

These results show that the extra demand for lipid substrates during locust flight is reflected by an increased concentration of lipid bound to protein in the haemolymph, and that one group of proteins (group *B*) carries lipid only when the concentration of lipid is high. Haemolymph from resting locusts at different stages of development has been examined qualitatively. Lipid in the group *B* fraction was present at two stages: fifth instar hoppers just before moulting and female adults during egg production. At both these stages demand for fat body lipid is likely to be high so the presence of lipid in the group *B* fraction seems to be related to extensive lipid mobilization from the fat body.

High concentrations of fuels in insect haemolymph are an adaptation to the rapid rates of fuel utilization of active flight muscle[8]. The considerable increase in haemolymph diglyceride during flight may be a response to an increased requirement by flight muscle for lipid in this form. It is noteworthy that flight muscle lipase of the Cecropia moth hydrolyses diglycerides at five times the rate of triglycerides[9]. Until the turnover rates of the haemolymph lipids during flight are known, however, it is difficult to assess the relative contributions of the different forms of lipid to flight metabolism

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R. J. MAYER
D. J. CANDY

Department of Biochemistry,
University of Birmingham.

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