'Real V_{max}' for glycine, valine and methionine transport in vivo in jejunum and ileum in domestic fowls

	Gly		Val		Meth	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Per unit area (pmoles cm^{-2} sec^{-1})	36.6± 5.3 (6)	55.2± 6.1* (6)	37.5± 7.0 (6)	82.3± 12.7** (6)	147.2± 14.4 (7)	148.0± 5.4 (6)
Per region (nmoles sec^{-1})	26.5± 3.9 (6)	20.5± 2.5 (6)	27.2± 5.1 (6)	31.0± 4.5 (6)	105.9± 10.3 (7)	55.2± 2.0*** (6)

The values are given as the mean± 1 SE of the mean. The results are calculated a) per unit surface area and b) per region. The figures in parenthesis indicate the number of estimates. Jejunal-ileal comparisons * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

tion data depends on the structural basis used for calculation. When V_{max} is calculated per unit surface area of intestine it represents an index of enterocyte function whereas when V_{max} is calculated for a whole region of intestine it represents the functional capacity of the region. Using this interpretation our results indicate clearly functional differences between jejunal and ileal enterocytes with respect to the absorption of the amino acids. The V_{max}'s calculated per cm^2 suggest that the ileal enterocytes have a greater absorbing capacity than jejunal enterocytes for glycine and valine but not for methionine which has the same high V_{max} in both regions. Because the whole jejunum has a greater number of enterocytes than the whole ileum the functional maximum absorptive capacities of the 2 regions exhibit another pattern. On this basis, there are no differences for glycine and valine absorption but the maximal rate of methionine absorption is 1.9 times greater in the jejunum than in the ileum.

Previous studies have produced conflicting results. A higher rate of neutral amino acid absorption was found in jejunum than in the ileum per unit length in rat[4] and in man[5]. In chickens lysine absorption was higher in the jejunum per unit weight[6]. Under in vitro conditions, however, the transport of lysine by chicken intestine[7] was greatest in the ileum regardless of the basis used. None of these studies employed a range of concentrations or kinetic analysis to characterize the transfer mechanisms. The only in vitro kinetic study using chick intestine[8] reported a constant transport rate of methionine throughout the small bowel but only 1 week-old birds were used and, like all the previous in vivo studies, no correction for unstirred layers was applied.

Our results reveal functional differences in the capacities for absorption of the amino acids when comparing the jejunal and ileal enterocytes. Nutritionally, however, the functional maximum absorptive capacity of the whole jejunum or ileum is probably more important. From this point of view the maximum absorptive capacities for glycine and valine are the same in the 2 regions but that for methionine is much higher in the jejunum.

Further analysis of the importance of the concept of the functional maximum absorptive capacity to the nutrition of animals must await measurements of residence/contact times of nutrients in the respective regions.

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Lipid transport in the migrating Monarch butterfly, *Danaus p. plexippus*[1]

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Summary. The transport of lipid in the haemolymph of the Monarch butterfly during its fall migration was examined. Diglyceride was the major lipid class of 2 electrophoretically distinct lipoprotein fractions in both males and females. Triglycerides, hydrocarbons, free fatty acids, phosphatidyl cholines and phosphatidyl ethanolamines were minor components of these lipoproteins. Differences in lipid transport attributable to sex were not detected.

Although the ecology of the long distance migration of the Monarch butterfly, *Danaus plexippus plexippus,* has received considerable study[4,5], little is known about the butterfly's regulation of its available energy sources[6-9]. During the autumn Monarch butterflies migrate from their breeding grounds in the Great Lakes Region and Northern Plains of North America to overwintering locations in the Gulf Coast, southern California, and Mexico. Some individuals undergo a return migration in the spring to recolonize northern breeding grounds. During their fall migration adults utilize their nutrient reserves, mainly triglycerides, supplemented by consumed nectar, whereas during their spring migration they draw primarily upon their nutrient reserves of lipid[10,11].

Although diglycerides are known to predominate in the haemolymph of several insects, including the Monarch butterfly[9], where they are conjugated to proteins and are the primary lipid transport molecule[12-14], the details of lipid transport in the Monarch butterfly are lacking. For example, the mechanism by which this insect regulates its energy supplies to carry out its annual cycles of migration and reproduction is just beginning to receive study. Juvenile hormone and an adipokinetic neurohormone appear to be control factors[9,11]. A low titre of juvenile hormone may promote migratory flight, whereas a high titre of the hormone may be required to initiate ovary development. The titre of juvenile hormone may, therefore, be involved in regulating whether migration or reproduction is initiat-

ed, and whether lipid reserves are diverted to flight metabolism or yolk production. Preliminary information is available that the Monarch butterfly contains an adipokinetic hormone, which regulates lipid mobilization depending upon environmental cues, migratory stage, and flight activity level[9].

The present study examined the involvement of lipoproteins present in the haemolymph of the Monarch butterfly in lipid transport. Our results show that up to 70% of the non-polar lipids are present in the haemolymph of the migrating butterfly as diglycerides. The triglyceride reserves of the fat body, therefore, appear to be carried primarily as protein-bound diglyceride, although some protein-bound triglyceride is also transported.

Monarch butterflies were collected from *Aster pilosus* in Boone County, Missouri, on October 2 and 8, 1979. The flowers of this plant provide an important source of nectar for the butterfly during its fall migration. Haemolymph, usually < 10 µl/insect was drawn from an abdominal incision from 25 males and 20 females into a calibrated capillary tube. Plasma was prepared by centrifuging the haemolymph at 22,000 × g for 10 min at 4 °C to remove hemocytes. Lipids, isolated by the procedure of Folch et al.[15], were separated by TLC on prewashed silicic acid plates in hexane:diethyl ether:acetic acid (90:10:1) (non-polar lipids), and chloroform:methanol:water (65:25:4) (polar lipids). Iodine-stained plates were photocopied. Scraped lipid spots were eluted, dried, and weighed on a Cahn Electrobalance. Lipoproteins were separated from clear plasma using disc electrophoresis in 5% acrylamide gels[16]. Unstained gel sections containing the major lipoproteins [relative mobility (Rm) 0 to 0.03, and 0.11] were homogenized and extracted with chloroform:methanol (2:1) (v/v). The constituent lipids of these lipoproteins were examined using the techniques described above.

TLC followed by the determination of lipid classes by weight showed that non-polar lipids accounted for about 78% of the total lipids of the haemolymph. It has been reported previously that pre-migratory and overwintering Monarch butterflies contain mean total lipid titres in their haemolymph of 43 mg/ml and 26 mg/ml, respectively[9]. The principal non-polar lipid class was diglyceride [1,2-diglyceride (65%) and 1,3-diglyceride (5%)]. Other non-polar lipid classes detected were triglyceride (10%), hydrocarbon (5 to 10%), and free fatty acid (5%). Small amounts of sterol ester, and sterol were also present. A previous study which also employed TLC reported that diglyceride was the major non-polar lipid class, along with small amounts of free fatty acid, sterol ester, and triglyceride[9]. The present study showed that the principal phospholipids present in the haemolymph were phosphatidyl cholines (50–55%) and phosphatidyl ethanolamines (30–35%). Differences attributable to sex were not detected in either the non-polar lipid or phospholipid profiles of the haemolymph.

Disc electrophoresis of plasma proteins revealed an almost identical profile for males and females (figure 1). Analytical isoelectric focusing in an acrylamide gel (Pharmacia Flat Bed, pH gradient 4.0–6.5) also revealed that the haemolymph of males and females had similar protein profiles. These results suggest that a vitellogenin is absent from the haemolymph of fall migrating females, and is consistent with the view that they are in a reproductive dormancy[17–19]. 2 low mobility lipoproteins with Rms of 0–0.03 and 0.11 were detected with Sudan black stain (figure 1, B). The first fraction (Rm 0–0.03) consisted of at least 2 bands that stained with Sudan black. Both fractions were yellow due to the presence of non-polar and polar biochromes, including lutein[7]. A polar blue biochrome was associated with the protein at Rm 0.47 (figure 1).

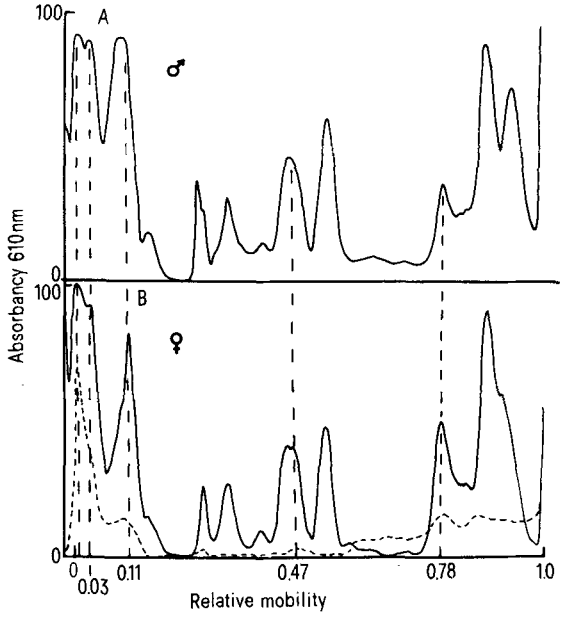

Fig. 1. Densitometric scans of electropherograms showing proteins and lipoproteins present in the haemolymph of the Monarch butterfly collected during its fall migration. *A* Male; *B* Female. Plasma (1 µl/gel for proteins, 6 µl/gel for lipoproteins) was electrophoresed in 5% acrylamide gels. Proteins were stained with Coomassie Blue (——) and lipoproteins were stained with Sudan Black (- - - -). Gels were scanned at 610 nm.

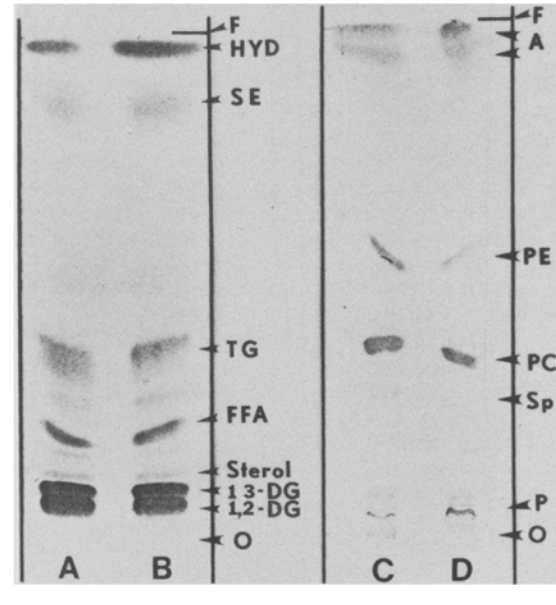

Fig. 2. Non-polar lipids (A, B) and polar lipids (C, D) of 2 major lipoproteins present in the plasma (100 µl) of male and female Monarch butterflies collected during their fall migration. A and C, lipoprotein Rm 0–0.03; B and D, lipoprotein Rm 0.11. Photocopies of iodine-stained plates, DG, diglycerides; FAA, free fatty acids; TG, triglycerides; SE, sterol esters; HYD, hydrocarbons; Sph, sphingomyelins; PC, phosphatidyl cholines; PE, phosphatidyl ethanolamines; P and A, unidentified polar and apolar lipids; O, origin; F, front.

The lipids present in these 2 lipoprotein fractions were found to be virtually identical (figure 2). Diglyceride was the major non-polar lipid class (60-70%). Other non-polar lipids present were triglycerides (8-10%), hydrocarbons (10-15%), and free fatty acids (5%). Small amounts of sterol esters and free sterols were also present (figure 2, A and B). The major phospholipids present included phosphatidyl cholines and phosphatidyl ethanolamines (figure 2, C and D), along with sphingomyelins, and unidentified polar (figure 2, 'P') and apolar lipids, including biochromes (figure 2, 'A'). The lipid profile of the 2 major lipoprotein fractions was similar to that of whole plasma, suggesting that haemolymph lipids are transported as lipoproteins. Since more 1,3-diglyceride than 1,2-diglyceride was found in the lipoproteins than in whole plasma, some 1,2-diglyceride may be converted to 1,3-diglyceride during electrophoresis.

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Differential sensitivity of the two phases of ear artery contraction to intimal and adventitial norepinephrine[1]

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Summary. The biphasic contraction of the rabbit ear artery to norepinephrine (NE) was investigated in the normal (adventitial stimulation) and the everted (intimal stimulation) segment of ear artery. The 2nd phase response showed an intimal ED_{50} of 8.2×10^{-8} M which was significantly ($p < 0.05$) lower than the adventitial ED_{50} of 42.6×10^{-8} M. This difference was abolished by inhibition of neuronal and extraneuronal uptake for NE. The 1st phase response also showed an ED_{50} for the intimal stimulation (6.9×10^{-8} M) which was significantly ($p < 0.05$) lower than adventitial (65.5×10^{-8} M). This difference was reduced but not abolished by NE uptake inhibition. This suggsets that some feature of the adrenergic neuroeffector apparatus is asymmetrically arranged to favor fast responses to blood borne NE.

The response of vascular smooth muscle to norepinephrine (NE) is biphasic[2-4]. The 1st phase is due to release of calcium from a bound or intracellular store while the final contraction is due to influx of calcium from the external medium[5]. Norepinephrine when applied to the intimal surface of the arteries elicits the steady-state final contraction with a greater efficacy than when applied to the adventitial surface and this difference is abolished by blockade of neuronal and extraneuronal uptake mechanisms[6,7]. Recent work suggests that the 1st phase of the NE contraction shows a similar asymmetric difference which is not due to uptake mechanisms[7-9]. The present study investigated this suggestion over the complete concentration-response curve for NE.

A 15-mm segment of the central ear artery was removed from New Zealand white rabbits which had been stunned and killed by exsanguination. This was transferred to a dissection bath containing Krebs bicarbonate solution gassed with 95% O_2 and 5% CO_2. Two 4-mm segments were prepared for in vitro tension recording as previously described[6]. One of the segments was prepared in the normal configuration with the adventitia outward. The lumen was filled with petroleum jelly and this segment was used to investigate the effects of drugs applied only to the adventitial surface. The 2nd segment was everted in the manner previously described[6]. The lumen of the everted segment was filled with the waterproof petroleum jelly and this preparation was used to investigate the effects of drugs applied only to the intimal surface of the vessel.

The responses to the adventitial and intimal stimulation with NE were investigated. NE was added to the tissue bath to give final concentrations of between 10^{-9} M and 10^{-5} M.

The mean ED_{50} concentration (M) for the 1st and 2nd phases of NE contractions are shown in control and in uptake blockaded arteries. The range in brackets defines the 95% confidence interval. The asterisk denotes a significant difference between intimal and adventitial values

	Control	DMI+DOC
Intimal NE		
1st phase	6.9×10^{-8} (6.3–7.7)	7.1×10^{-8} (5.8–9.2)
2nd phase	8.2×10^{-8} (7.2–9.4)	6.5×10^{-8} (5.1–8.3)
Adventitial NE		
1st phase	65.5×10^{-8}* (47.8–125.1)	13.0×10^{-8}* (9.5–20.9)
2nd phase	42.6×10^{-8}* (29.6–76.1)	4.4×10^{-8} (3.8–5.1)