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PLASMA LIPIDS AND LIPOPROTEINS OF SOME MEMBERS OF THE ORDER PERISSODACTYLA

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Abstract—1. The plasma lipoproteins of various members of the order Perissodactyla have been examined by electrophoresis and analytical ultracentrifugation.

2. In the Equidae, high density (α) lipoprotein was the major component (80–90%) and low density (β) lipoprotein (10–20%) the minor component.

3. In the Tapiridae represented by the Malayan tapir (*Tapirus indicus*), high density and low density lipoproteins were present in approximately equal amounts.

4. In the Rhinocerotidae, the high density lipoprotein characteristic of the Equidae and Tapiridae was absent, and the plasma lipoproteins consisted of a complex group having β mobility on electrophoresis and a flotation pattern usually associated with low density lipoprotein.

5. The fatty acid composition of plasma lipids was remarkably similar in all members of the Perissodactyla examined, with very high percentages of linoleic acid (> 70%) being found in the cholesteryl esters.

INTRODUCTION

In herbivorous animals the mode of digestion of dietary lipid is dependent on the anatomy and physiology of the digestive tract. In simple stomached animals such as the horse (*Equus caballus*) and pig (*Sus scrofa*), lipid is presumably digested in the small intestine in a manner similar to that seen in man (*Homo sapiens*) and the rat (*Rattus norvegicus*), whereas in herbivores with complex stomachs, such as the domestic ox (*Bos taurus*) and sheep (*Ovis aries*) extensive hydrolysis of dietary lipids occurs anterior to the small intestine in the rumen (see Garton, 1967). In addition, the size of lipid droplets absorbed into the lymphatics of ruminant animals appears to be smaller than that seen in non-ruminants, which could affect the subsequent metabolism of the absorbed lipids (see Harrison & Leat, 1975). To investigate whether differences between ruminant and non-ruminant animals in the digestion and absorption of lipids had any effect on the subsequent mode of transport of lipid in plasma, the distribution of plasma lipoproteins in domestic and non-domestic herbivores was examined. Preliminary observations (Leat *et al.*, 1975) indicated that there was no obvious relationship between the mode of lipid digestion and the profile of plasma lipoproteins, but interesting differences were noted between members of differing orders. The plasma lipoproteins of some members of the order Perissodactyla are now reported here in more detail.

MATERIALS AND METHODS

Animals

The non-domestic perissodactyl ungulates (see Fig. 1) were maintained by the Zoological Society of London either at Regents Park or Whipsnade Park. Blood samples were taken by venepuncture from animals which had been sedated for veterinary examination or movement to another enclosure. Blood was collected in tubes containing thiomersal (0.1 mg/ml blood) and EDTA (1 mg/ml blood), and centrifuged to obtain the plasma which was then stored at −20°C until analysis.

The Equidae examined were fed on a diet of meadow hay and commercial horse cubes. The white rhinoceros (*Ceratotherium simum*) were fed similarly, but with the

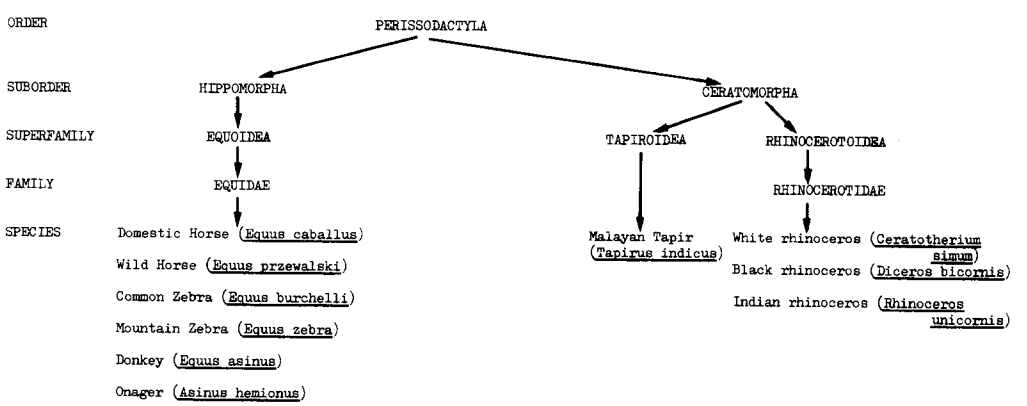

Fig. 1. Classification of the order Perissodactyla.

addition of clover or lucerne hay in the winter. The other species sampled received a higher crude protein intake in the form of a high protein horse cube or a dairy cube with clover hay. Most of the animals kept at Whipsnade have access to grass in the summer. Those at London receive fresh vegetables. All animals are basically fed a diet suitable for maintenance only. Blood samples from the domestic horse and donkey (*Equus asinus*) were obtained through local veterinary practice.

Only samples from adult animals which were in good health or with minor ailments were analysed. Most of the samples were from non lactating females.

Analytical

The plasma lipids were extracted into chloroform–methanol (2:1, v/v) and after separation by thin layer chromatography the individual lipids were estimated by methods described by Leat *et al.* (1976). The fatty acid compositions of the individual plasma lipids were determined as described by Bowyer *et al.* (1964).

Electrophoresis of the plasma lipoproteins was carried out on cellulose acetate strips basically as described by Magnani & Howard (1972). The strips were divided longitudinally, one half being stained for protein with amido black and the other half for lipid by the ozone/Schiff's reaction. The electrophoretographs were scanned in a densitometer with an automatic integrator (Vitatron Scientific Instruments) and figures for areas representing the various lipoproteins were recorded. Estimates of the percentage distribution of the individual lipoproteins were obtained by multiplying the area printout of the β (low density) lipoprotein by 1.26 and the α (high density) lipoprotein by 1.83, factors which reflect the relative content of lipid in ovine plasma lipoproteins (Leat *et al.*, 1976).

The total lipoproteins of plasma were separated by centrifugation at density 1.21 g/ml for 40 hr at 40,000 rev/min using a 40.3 rotor in a Beckman preparative ultracentrifuge (Model L2 65B). The lipoproteins were removed with a fine Pasteur pipette (De Lalla & Gofman, 1954) and dialysed against buffer of density 1.21 g/ml.

Analytical ultracentrifugation was carried out in a Beckman Model E Analytical Ultracentrifuge at density 1.21 g/ml using Schlieren optics. Pairs of samples were examined simultaneously using double sector standard and wedge cells. Samples were centrifuged at 20°C, first at 36,000 rpm with photographs being taken every 8 min for 40 min. The speed was then increased to 56,000 rpm and a similar photographic procedure repeated.

A second estimate of the percentage composition of the plasma lipoproteins was obtained by projecting suitable frames of the Schlieren negatives at a 5-fold magnification onto good quality paper. The peaks were marked on the paper, cut out, weighed and expressed as a percentage of the total. The frames were selected such that the peaks measured were at similar distances from the base of the cell to minimize errors due to radial concentration.

RESULTS

Cellulose acetate electrophoresis

Some representative tracings of plasma lipoproteins superimposed on the protein separation are shown in Fig. 2. In the family Equidae illustrated by the common zebra (*Equus burchelli*) and horse (Fig. 2a and b) the α lipoprotein* migrating in the α-globulin region just behind the albumen band is the major component comprising 80–90% of the total lipoproteins. The lipoprotein migrating in the β globulin

* The term α-lipoprotein is used in electrophoresis for the lipoprotein migrating in the α-globulin region and corresponds to the high density lipoprotein (HDL) fraction separated by ultracentrifugation. The β-lipoprotein migrates with the β-globulin and corresponds to the low density lipoprotein (LDL). The pre-β band corresponds to very low density lipoprotein (VLDL).

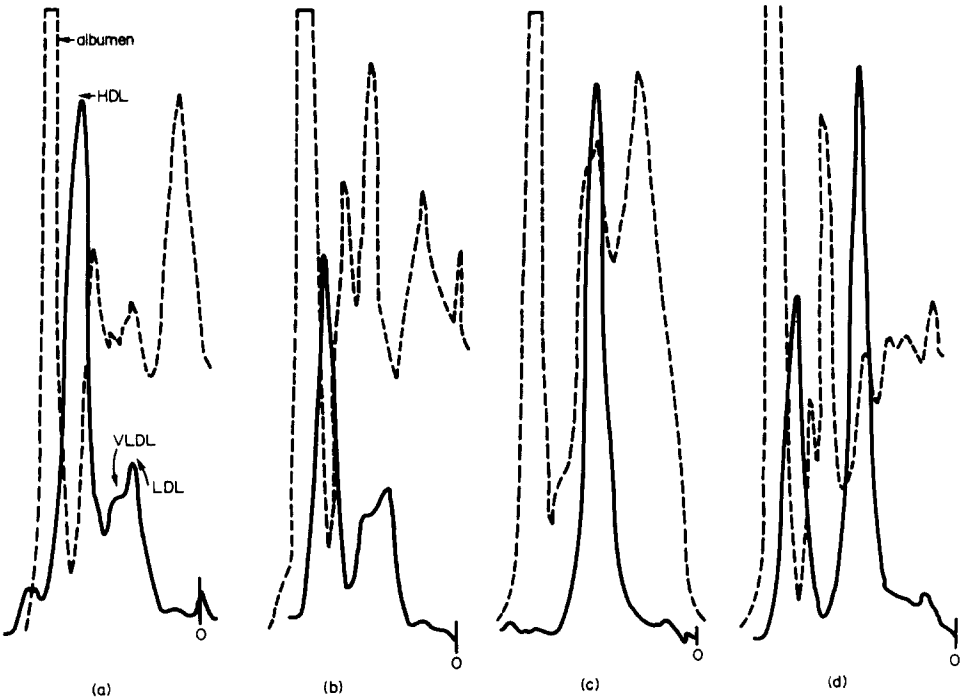

Fig. 2. Cellulose acetate electrophoresis of the plasma of (a) common zebra, (b) domestic horse, (c) white rhinoceros, (d) Malayan tapir. Protein (-----); lipid (——); O = origin.

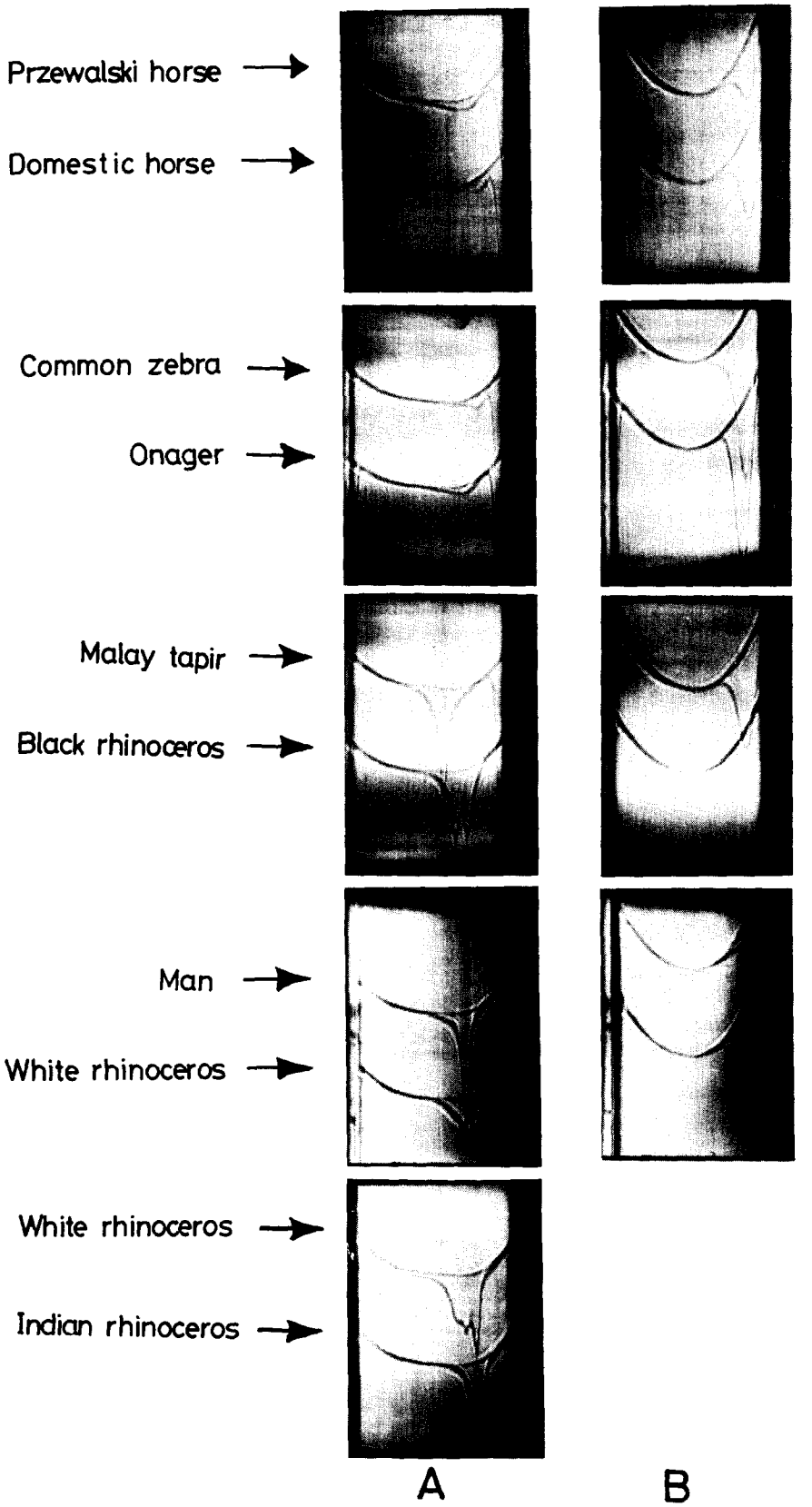

Fig. 3. Selected Schlieren patterns from the analytical ultracentrifugation of total plasma lipoproteins of various perissodactyls. Flotation is from right to left at density 1.21 g/ml (see text for further details). A, 24 min at 36,000 rev/min showing separation of low density lipoprotein from the high density lipoprotein, which remains near the base of the cell (at right). B, 40 min at 56,000 rev/min showing flotation of high density lipoprotein. The low density lipoproteins are now concentrated at the meniscus (left).

Table 1. Percentage low density (β) and high density (α) lipoproteins in the plasma of various perissodactyls (number of animals in brackets)

Species	Cellulose acetate electrophoresis		Analytical Ultracentrifuge	
	β lipoprotein	α lipoprotein	LDL	HDL
Domestic Horse (2)	29.3	70.7	(2) 18.9	81.1
Przewalski horse (1)	15.7	84.3	(1) 21.9	78.1
Onager (1)	21.5	78.5	(2) 15.8	84.2
Mountain Zebra (2)	24.1	75.9	(2) 8.1	91.9
Common Zebra (2)	21.3	78.7	(2) 9.5	90.5
White Rhinoceros (5)	100	-	(3) 100	-
Black Rhinoceros (1)	100	-	(1) 100	-
Indian Rhinoceros (1)	100	-	(1) 100	-
Malayan Tapir (2)	42.7	57.3	(2) 41.3	58.7

region is obviously of multiple composition and probably comprises a β and pre-β (very low density) lipoprotein. A basically similar pattern was found in other members of the Equidae family although samples from the mountain zebra (*Equus zebra*) had a large free fatty acid band which tended to distort the α-lipoproteins. In the single donkey examined there was poor separation between the α and β lipoproteins suggesting a high content of pre-β lipoprotein. The appearance of a large amount of very low density lipoprotein (VLDL) on analytical ultracentrifugation was consistent with this interpretation.

In the Rhinocerotidae only one peak was seen on cellulose acetate electrophoresis, the mobility of which would characterize it as a β lipoprotein. An example of a white rhinoceros shown in Fig. 2c has been confirmed in six other animals, and very similar patterns were obtained with single samples from a black (*Diceros bicornis*) and Indian rhinoceros (*Rhinoceros unicornis*).

In the Tapiridae two major bands were noted in the two Malayan tapirs examined (Fig. 2d) one migrating with α mobility and the other with β mobility which were present in roughly equal amounts.

Analytical ultracentrifugation

The analytical ultracentrifugation of plasma lipoproteins of various species is shown in Fig. 3. Frames are selected to show best the separation of low density lipoprotein (A) and high density lipoprotein (B). Very low density lipoprotein (VLDL) when present appeared only on the first exposure when the centrifuge had reached 36,000 rev/min. Since the presence of VLDL was variable and was difficult to quantitate it was omitted from calculations.

In the Equidae the high density lipoprotein (HDL) was the most abundant lipoprotein accounting for over 90% of the total area (Table 1). This value is somewhat higher than that obtained by electrophoresis where however the value for β lipoprotein may be increased by the inclusion of the pre-β band.

In the Rhinocerotidae there was a complete absence of the HDL characteristic of the Equidae. The LDL was of complex composition usually composed of two peaks one having a flotation rate similar, and one slower, than that of human LDL. The Indian and black rhinoceros had very similar patterns to the white rhinoceros, although in the Indian rhinoceros there was more of the slower moving band.

In the Malayan tapir, the two major bands noted on electrophoresis were reflected in the ultracentrifuge patterns, where an LDL peak having a somewhat faster flotation rate than human LDL was present, together with an HDL peak similar in flotation characteristics to that of equine HDL.

Plasma lipids and fatty acids

The highest concentrations of plasma lipids in Perissodactyla were found in the Equidae particularly so in the common zebra (Table 2). The ratio of cholesteryl esters (CE): phospholipids (PL) was generally below 1.0. In the Rhinocerotidae plasma phospholipid concentrations were much lower than in the Equidae and generally less than 100 mg%. The ratio of CE:PL exceeds 2.0 except for the single sample from the Indian rhinoceros.

Although there were marked differences in the distribution of plasma lipoproteins the fatty acid composition of the plasma lipids of Equidae, Rhinocerotidae and Tapiridae were very similar (Table 3). Of note, was the consistently high percentage of linoleic acid (> 70%) in the cholesteryl ester fraction.

DISCUSSION

In an examination of eighteen species of animals of various orders Mills & Taylaur (1971) concluded that whereas some mammals, such as man, have at least two classes of lipoproteins, others have only one. This variability is also apparent within the members of the order Perissodactyla examined here. In the plasma lipoproteins of the Equidae, the only family of the suborder Hippomorpha still extant, the high density lipoprotein (HDL) was the major fraction and

Table 2. Concentration of plasma lipids (mg/100 ml) in various perissodactyls (number of animals in brackets)

Species	Phospho-lipids (PL)	Cholesteryl esters (CE)	Free cholesterol	Tri-glycerides	$\frac{CE}{PL}$	Total plasma lipoproteins* (mg/100ml)
Domestic horse (2)	151.0	130.0	27.3	20.1	0.86	522
Przewalski horse (4)	134.1	135.3	27.2	15.0	1.01	464
Mountain Zebra (5)	190.8	152.9	29.8	17.4	0.80	660
Common Zebra (3)	261.8	206.0	34.7	9.6	0.79	905
Onager (3)	173.3	114.6	33.5	25.2	0.66	599
Donkey (3)	182.0	155.2	29.6	9.3	0.85	629
White rhinoceros (4)	63.1	154.7	27.3	15.7	2.45	218
Black rhinoceros (1)	36.8	106.6	10.7	5.9	2.89	127
Indian rhinoceros (1)	60.8	74.8	23.4	4.3	1.23	210
Malayan tapir (2)	188.1	251.2	49.1	27.3	1.33	651

*mg phospholipid x $(\frac{100-17}{24})$ (see text for further details).

Table 3. Fatty acid composition (percentage by weight) of the plasma lipids of various perissodactyls (mean of two animals)

	16:0	16:1	18:0	18:1	18:2	18:3	20:4	Others
(a) Phospholipids								
Domestic horse	15.6	0.7	27.5	11.9	39.5	0.8	tr	4.0
Przewalski horse	10.5	0.7	30.4	10.2	39.3	4.6	2.0	2.3
Mountain Zebra	13.3	1.1	27.6	12.9	42.7	0.7	0.5	1.2
Common Zebra	14.4	0.9	25.9	10.7	39.4	3.0	0.9	4.8
Donkey	18.1	0.8	29.4	11.2	33.0	1.2	1.7	4.6
Onager	20.3	1.0	25.8	12.6	34.4	2.4	1.4	2.1
White rhinoceros	17.7	0.8	27.8	12.2	31.7	1.8	4.6	3.4
Black rhinoceros (1)	10.5	0.6	30.2	12.6	24.9	10.1	4.2	6.9
Malayan tapir	12.1	tr	34.5	10.2	33.1	0.5	5.0	4.6
(b) Cholesteryl esters								
Domestic horse	7.1	3.0	0.8	10.8	72.6	1.0	tr	4.7
Przewalski horse	9.0	2.8	1.0	13.9	67.0	3.2	0.7	2.4
Mountain Zebra	6.7	3.7	1.0	11.5	74.0	1.3	tr	1.8
Common Zebra	6.4	3.8	1.5	12.0	71.0	4.3	tr	1.0
Donkey	5.6	3.1	0.7	14.2	65.9	3.0	tr	7.5
Onager	7.6	3.9	0.9	13.6	68.5	3.4	tr	2.1
White rhinoceros	7.3	2.1	0.8	13.4	69.1	4.2	1.2	1.9
Black rhinoceros (1)	6.8	2.0	1.0	8.7	71.6	2.7	0.7	6.5
Malayan tapir	5.9	1.5	0.9	16.5	71.5	1.2	1.2	1.3
(c) Triglycerides								
Domestic horse	28.0	6.3	8.1	37.6	8.5	7.2	tr	4.3
Przewalski horse	27.5	5.5	5.0	27.5	9.1	22.3	tr	3.1
Mountain Zebra	34.5	4.8	4.8	28.0	10.4	11.8	tr	5.7
Common Zebra	29.0	6.8	5.3	32.5	8.2	10.8	tr	7.4
Donkey	29.6	4.9	5.2	31.5	11.9	10.3	tr	6.6
Onager	39.1	6.4	5.0	34.0	4.6	5.3	tr	5.6
White rhinoceros	40.7	4.3	5.4	32.3	7.5	1.8	tr	8.0
Black rhinoceros (1)	32.8	4.6	4.2	33.9	9.0	2.5	tr	13.0
Malayan tapir	28.6	5.2	7.8	38.5	9.7	3.5	tr	2.41

the low density lipoprotein (LDL) was a minor component. The percentage HDL, as estimated by ultracentrifugation, appeared to be higher in the plasma of the common and mountain zebra (>90%) when compared with the domestic and wild horse (80%) but a larger number of samples would have to be examined to determine if this difference is real. In the Malayan tapir, a family of the suborder Ceratomorpha, plasma LDL and HDL were present in approximately equal amounts. It might be expected that the other members of the suborder Ceratomorpha, the Rhinocerotidae, would have a distribution of plasma lipoproteins similar to that of the Malayan tapir. However in all three species of rhinoceroses examined here, namely, black, white and Indian, there was a complete absence of the HDL characteristic of tapirs and of Equidae; and the only lipoprotein detected had electrophoretic and flotation properties which were characteristic of an LDL. Leat *et al.* (1975) concluded that the distribution of plasma lipoproteins was independent of whether the animal has a ruminant or non ruminant digestion. From the results reported here it is also apparent that even among members of the same order and which have the same type of digestion, i.e. non-ruminant with a predominant caecal fermentation, there is no uniformity in the distribution of plasma lipoproteins.

The tapir is probably the most primitive form of the Perissodactyla and is often termed a "living fossil". Morphologically, modern tapirs appear to show little change from the perissodactyl stock of about 40 million years ago, and have retained many of the characteristics of the ancestors of all Perissodactyla (Young, 1962). It is therefore feasible that the pattern of plasma lipoproteins in modern tapirs might also reflect the distribution found in the primitive Perissodactyla. It is not possible to assess if there was any evolutionary advantage in the Equidae possessing predominantly HDL and the Rhinocerotidae not possessing any of the HDL characteristic of Equidae and tapirs.

The distribution of plasma lipoproteins of the Malayan tapir in the analytical ultracentrifuge was similar to that of man (Fig. 3) although tapir LDL had a higher flotation rate than human LDL. In the Rhinocerotidae, the plasma lipoprotein having LDL characteristics was of multiple composition, having at least two components, one of similar flotation rate to human LDL and the other of slower rate. The single sample from an Indian rhinoceros appeared to have more of the slower component compared to the white and black rhinoceros.

Whether a lipoprotein is classified as LDL or HDL depends on a number of factors such as electrophoretic mobility and flotation characteristics. However, in some animals HDL can overlap in density with LDL (Calvert, 1976) and the densities of the lipoproteins can vary depending on the amount of fat in the diet, e.g. guinea pig (Mills *et al.*, 1972). Precipitation by dextran sulphate and calcium is a characteristic of LDL whereas HDL remains in solution (Cornwell & Kruger, 1961). Addition of dextran sulphate

to horse and tapir plasma resulted in the precipitation of the lipoproteins classified here as LDL. Analysis of the fractions by electrophoresis and ultracentrifugation showed that there was no cross contamination between LDL and HDL. In the white rhinoceros, however, the complex lipoprotein characterized as LDL was only partly precipitated by dextran sulphate suggesting that not all of this lipoprotein can be regarded as a true LDL.

Plasma lipid concentrations varied considerably between the various species with the Equidae having the highest levels and the Rhinocerotidae the lowest. The distribution of plasma lipids in the Malayan tapir resembled that of man. The ratio of CE:PL was below 1.0 for most of the Equidae, reflecting the preponderance of HDL which usually contains more phospholipid than cholesteryl esters. In the Rhinocerotidae however the ratio CE:PL was greater than 1.0 which is consistent with the ratio of these lipids in LDL.

Although the concentrations of plasma lipoproteins were not directly determined in this investigation, an estimate can be obtained from the concentration of plasma phospholipid. In the plasma lipoproteins of man (Magnani & Howard, 1971), sheep (Leat *et al.*, 1976) and other mammals (Mills & Taylaur, 1971) phospholipid accounts for 20–25% of both LDL and HDL. In the sheep 17% of plasma phospholipid is not associated with plasma lipoproteins, and a corrected value for total plasma lipoproteins of this species can be obtained by multiplying the concentration of plasma phospholipid by (100–17)/24 where 24 = mean percentage of phospholipid in LDL and HDL. Applying this same factor (3.46) to the species examined here an estimate can be made of the total plasma lipoproteins (Table 2) and the content of LDL and HDL can then be calculated using the percentage values recorded in Table 1.

Although the distribution of plasma lipoproteins varied considerably between perissodactyl species, there was a remarkable similarity between species in the fatty acid composition of the individual plasma lipids. This is consistent with the non ruminant digestion found in these mammals, where, unlike ruminant animals, dietary fatty acids are absorbed from the intestinal tract unchanged. The appreciable quantities of α-linolenic acid found in triglycerides would be expected when animals had access to grass which is rich in this fatty acid. However linoleic acid is the major fatty acid present, particularly in the cholesteryl ester fraction where this acid frequently accounted for more than 70% of the total fatty acids. The high content of linoleic acid seems to be a characteristic of the horse (Leat & Baker, 1970) and other members of the Perissodactyla.

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