Adsorptive pinocytosis of ^{125}I -labelled lactate dehydrogenase isoenzymes H4 and M4 by rat yolk sacs incubated *in vitro*

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The pinocytic uptake of ^{125}I -labelled porcine lactate dehydrogenase isoenzymes H4 and M4 by 17.5-day rat visceral yolk sac incubated *in vitro* was saturable and binding obeyed Michaelis–Menten kinetics. The uptake characteristics of the two isoenzymes were very similar. For the H4 and the M4 isoenzymes, the dissociation constants of the protein–plasma-membrane complex were $0.62\ \mu\text{M}$ and $0.84\ \mu\text{M}$ respectively, and the maximum rates of uptake 0.13 and 0.26 nmol/mg of yolk-sac protein per h respectively. These findings contrast with those from studies *in vivo*, which show the M4 form is taken up by rat liver sinusoidal cells at a much higher rate than the H4 form, and point to different recognition systems for the adsorptive pinocytosis of simple non-conjugate proteins in yolk-sac epithelial cells and liver sinusoidal cells. Competition experiments indicate that binding of the H4 isoenzyme to the yolk-sac cells is restricted to hydrophobic interactions, whereas the binding of the M4 isoenzyme involves hydrophobic as well as positively charged sites on the protein molecules.

Recent studies *in vitro* (see Livesey, 1979; Livesey & Williams, 1981), using ^{125}I -labelled forms of bovine pancreatic ribonuclease A, hen egg-white lysozyme and formaldehyde-denatured bovine serum albumin as probes, have enabled certain general conclusions to be drawn about the specificity of adsorptive pinocytosis of simple non-conjugated proteins by rat yolk-sac epithelial cells. The wide variation in the observed rates of uptake of these various proteins results from their differential adsorption to the external surface of the internalizing plasma membrane. Competition experiments indicate that formaldehyde-denatured albumin and ribonuclease A (and lysozyme) adsorb to different regions of the yolk-sac cell surface that possess different binding sites for hydrophobic and basic proteins (Livesey, 1979).

Several studies seem to indicate that the recognition sites for simple proteins on yolk-sac epithelial cells resemble those present on sinusoidal liver cells. Kooistra *et al.* (1979, 1980) showed that the positive charges on ribonuclease A and lysozyme are crucial for the uptake of these enzymes by sinusoidal rat liver cells *in vivo*. Likewise, Moore *et al.* (1977) found that there was a positive correlation between the rate of uptake of several modified forms of bovine serum albumin by rat yolk sacs incubated *in vitro* and the rate of clearance from the bloodstream by (sinusoidal) rat liver cells.

In the present paper we studied the pinocytosis of the isoenzymes H4 and M4 of porcine lactate dehydrogenase in the yolk-sac system. These proteins were chosen since they are naturally occurring molecules of identical size, but carry different charges. Studies *in vivo* have shown that the more positively charged lactate dehydrogenase isoenzyme M4 is endocytosed much more rapidly by the sinusoidal liver cells than the negatively charged isoenzyme H4 (Sinke *et al.*, 1979; Wachsmuth & Klingmüller, 1978).

Dependence of uptake on concentration, and competition between ^{125}I -labelled forms of the isoenzymes and a number of different unlabelled proteins, characterized with respect to positive charge and hydrophobicity, were investigated. These experiments enabled us to test whether the findings of Livesey (1979) have general validity, and also permitted the specificity of pinocytosis of simple proteins by yolk-sac epithelial cells to be compared with that of sinusoidal liver cells.

Materials and methods

Materials

Suspensions of crystals of porcine lactate dehydrogenases (band 1 and band 5 respectively) were obtained from The Boehringer Corp., London, U.K.; Na^{125}I (IMS.30) and ^{125}I -labelled polyvinylpyrrolid-

done (1-vinylpyrrolidin-2-one polymers; IM.33P) were products of The Radiochemical Centre, Amersham, Bucks., U.K. Tissue-culture medium 199 (TC 20) and inactivated calf serum (CS 07) were obtained from Wellcome Reagents, Beckenham, Kent, U.K., CNBr-activated Sepharose 4B, lactoperoxidase (L 7129), bovine pancreatic ribonuclease A (Type XI-A), hen egg-white lysozyme (grade 1) and bovine serum albumin (A 7638) were purchased from Sigma (London) Chemical Co. (London S.W.6, U.K.). All other chemicals used were analytical grade.

Protein labelling

Lactate dehydrogenase isoenzymes H4 and M4 were iodinated by the lactoperoxidase method as described in detail by Sinke *et al.* (1979). The labelled protein was precipitated by $(\text{NH}_4)_2\text{SO}_4$ and stored at 4°C. It was freshly dialysed against the appropriate buffer and shortly before use was centrifuged at 40000 g-min to remove any insoluble material.

Uptake (and digestion) of radiolabelled substrates by 17.5-day rat yolk sacs incubated in vitro

The rates of uptake of ^{125}I -labelled polyvinylpyrrolidone were determined by the method described by Williams *et al.* (1975a). The rates of uptake and digestion of ^{125}I -labelled lactate dehydrogenase isoenzymes H4 and M4 were determined by the method of Ibbotson & Williams (1979). Each rate of uptake was expressed as an Endocytic Index as defined by Williams *et al.* (1975a,b).

Assays of radioactivity

Measurements of radioactivity were performed as described by Ibbotson & Williams (1979).

Determination of protein

The protein content of each yolk sac was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. The lactate dehydrogenase concentration was determined spectrophotometrically at 280nm using an absorbance factor of 1.34 for a solution containing 1 mg of the H4 isoenzyme/ml and a factor of 1.4 for the M4 isoenzyme (Holbrook *et al.*, 1975).

Preparation of formaldehyde-denatured bovine serum albumin

Bovine serum albumin was treated with formaldehyde as described by Buys *et al.* (1973).

Sulphopropyl-Sephadex C-25 and phenyl-Sepharose CL-4B chromatography

Sulphopropyl-Sephadex C-25 and phenyl-Sepharose CL-4B chromatography were performed as described previously (Kooistra *et al.*, 1979, 1980).

Results

Fig. 1 shows a typical experiment in which yolk sacs were incubated with ^{125}I -labelled lactate dehydrogenase isoenzyme M4 in serum-free medium 199. The amount of radioactivity found in the tissue, the amount of acid-soluble radioactivity released back into the incubation medium and the sum of these two quantities (i.e. the total amount of substrate ingested by the tissue) are all shown. The tissue-associated radioactivity rose initially, but became virtually constant after 2 h of incubation, by which time acid-soluble radioactivity was released into the medium at a constant rate. This pattern was seen in all the experiments reported here, and is in agreement with previous observations on the uptake of other simple proteins by yolk sacs (Williams *et al.*, 1975b; Moore *et al.*, 1977; Livesey, 1979; Ibbotson & Williams, 1979). Such findings indicate

Fig. 1. Accumulation of radioactivity by yolk-sac tissue and appearance of trichloroacetic acid-soluble radioactivity in the incubation medium when yolk sacs are incubated in the presence of ^{125}I -labelled lactate dehydrogenase isoenzyme M4

Points at each time interval correspond to data derived from eight single yolk sacs incubated separately for up to 5.0 h in serum-free medium 199 in the presence of lactate dehydrogenase isoenzyme M4 (mean concentration during the incubation was $2.5 \mu\text{g/ml}$). The ordinate shows the volume of incubation medium whose contained trichloroacetic acid-insoluble radioactivity is found associated with the tissue in either acid-soluble or -insoluble form (●), or is found in the medium as acid-soluble radioactivity (○). These two quantities are summed for each yolk sac to give the total volume of incubation medium whose contained radiolabelled isoenzyme M4 has been ingested by the tissue (■).

Table 1. *Effects of removing calf serum from the incubation medium on the rates of uptake by yolk sacs of ^{125}I -labelled polyvinylpyrrolidone and ^{125}I -labelled lactate dehydrogenase isoenzymes H4 and M4*

Each value of the Endocytic Index was obtained from a separate experiment (see Williams *et al.*, 1975a; Ibbotson & Williams, 1979) in which the mean concentration of the substrates in the medium was $2.5\text{ }\mu\text{g/ml}$. For the proteins, the amounts of radioactivity associated with the tissue in the steady-state period (i.e. beyond 2.0 h of incubation) are also shown, and expressed in the same units as uptake. Results are means $\pm$ S.D. for the numbers of experiments given in parentheses.

^{125}I -labelled substrate	Medium 199 + 10% (v/v) calf serum		Medium 199 alone	
	Endocytic Index ($\mu\text{l/mg}$ of yolk-sac protein per h)	Tissue-associated radioactivity ($\mu\text{l/mg}$ of yolk-sac protein)	Endocytic Index ($\mu\text{l/mg}$ of yolk-sac protein per h)	Tissue-associated radioactivity ($\mu\text{l/mg}$ of yolk-sac protein)
Polyvinylpyrrolidone	2.39 ± 0.19 (8)	—	3.62 ± 0.54 (9)	—
Lactate dehydrogenase isoenzyme H4	40.5 ± 4.1 (3)	39.6 ± 2.7 (3)	236.0 ± 29.8 (6)	100.4 ± 17.0 (6)
Lactate dehydrogenase isoenzyme M4	42.2 ± 4.7 (3)	27.4 ± 6.4 (3)	311.5 ± 46.9 (4)	74.0 ± 5.3 (4)

that pinocytic capture of ^{125}I -labelled proteins is the rate-limiting step in the overall process of pinocytic capture and intracellular hydrolysis.

A summary of the experiments in which yolk sacs were incubated with the radiolabelled isoenzymes H4 and M4 in either medium 199 alone or in medium 199 containing 10% (v/v) calf serum is shown in Table 1. Both isoenzymes were ingested at higher rates than ^{125}I -labelled polyvinylpyrrolidone (a marker for non-adsorptive pinocytosis) as a result of their adsorbing to the plasma-membrane surface of the pinocytic cells of the yolk-sac tissue. Absence of serum from the medium caused a small but significant increase in the rate of pinosome formation, as determined by the rate of capture of ^{125}I -labelled polyvinylpyrrolidone. The 6–7-fold rise in the Endocytic Indices of both isoenzymes on removing calf serum from the incubation medium compared with the 50% rise in the rate of uptake of ^{125}I -labelled polyvinylpyrrolidone indicates that some components of serum compete for the same binding sites on the plasma membrane of yolk-sac epithelial cells that are used by the adsorbing lactate dehydrogenase molecules. To permit a more detailed study of the binding of the protein substrates to the plasma membrane, all subsequent incubations were performed in serum-free medium 199.

The results of experiments, in which the concentration of the unlabelled form of the isoenzyme in the medium was increased, while the initial concentration of the ^{125}I -labelled form of the same isoenzyme was held constant (Table 2), show the Endocytic Indices to fall with increasing total isoenzyme concentration. Such increases in protein concentration were without effect on the Endocytic Index of ^{125}I -labelled polyvinylpyrrolidone (results not shown), which dismisses the possibility that the decrease resulted from a pharmacological effect of

these concentrations of the unlabelled isoenzymes on the rate of pinosome formation in serum-free medium. The data in Table 2 also indicate that, for each isoenzyme, the quantity of radiolabelled protein associated with the tissue is a constant fraction of the Endocytic Index (0.43–0.54 for lactate dehydrogenase isoenzyme H4, and 0.29–0.36 for the M4 isoenzyme), and shows no tendency to rise with increasing total protein concentration in the medium. These findings are in agreement with the results shown in Fig. 1, which indicate that pinocytic capture rather than lysosomal breakdown is the rate-limiting step in the overall process of pinocytosis and intracellular degradation of simple proteins.

The data of Table 2 were analysed in a manner equivalent to that used in simple enzyme kinetics by using the general approach described in detail by Ibbotson & Williams (1979) for the analysis of the adsorptive uptake of formaldehyde-denatured ^{125}I -labelled bovine serum albumin by yolk sacs. This method assumes a rapid and complete equilibration of the substrate with the binding sites on the plasma membrane. Fig. 2 shows the Lineweaver–Burk plots for adsorptive pinocytosis of the radiolabelled isoenzymes H4 and M4. These plots indicate that binding of both isoenzymes obeys Michaelis–Menten kinetics. From these plots dissociation constants of the isoenzyme–plasma-membrane complex and maximum rates of uptake (when all available binding sites are occupied) can be calculated. By using a molecular weight of 140 000 for the isoenzymes (Holbrook *et al.*, 1975), the dissociation constants of the protein–plasma-membrane complex were $0.62\text{ }\mu\text{M}$ and $0.84\text{ }\mu\text{M}$ respectively, and the maximum rates of uptake 0.13 and 0.26 nmol/mg of yolk-sac protein per h for the H4 and M4 isoenzymes respectively. These results indicate that the higher

Table 2. Effect of increasing the concentration of unlabelled isoenzyme on the rate of pinocytic capture of the ^{125}I -labelled form of the same isoenzyme by yolk sacs incubated in serum-free medium 199

Each value of the Endocytic Index was obtained from a separate experiment (see Ibbotson & Williams, 1979), performed in serum-free medium 199, with ^{125}I -labelled lactate dehydrogenase isoenzymes H4 or M4 as substrate and in the presence of differing concentrations of the corresponding unlabelled protein preparation. The amount of radioactivity associated with the tissue is also reported, expressed in the same units as uptake. Results for both lactate dehydrogenase isoenzyme H4 (LDH H4) and isoenzyme M4 (LDH M4) are means $\pm$ s.d. for the numbers of experiments given in parentheses. Protein concentrations are the mean total concentrations of test protein (labelled and unlabelled) in the medium during the incubation period.

Mean concn. of LDH H4 ($\mu\text{g/ml}$ of medium)	Uptake of LDH H4		Concn. of LDH M4 ($\mu\text{g/ml}$ of medium)	Uptake of LDH M4	
	Endocytic Index ($\mu\text{l/mg}$ of yolk-sac protein per h)	Tissue-associated radioactivity ($\mu\text{l/mg}$ of yolk-sac protein)		Endocytic Index ($\mu\text{l/mg}$ of yolk-sac protein per h)	Tissue-associated radioactivity ($\mu\text{l/mg}$ of yolk-sac protein)
7.39	195.2 $\pm$ 39.2 (6)	83.1 $\pm$ 8.3 (6)	8.21	290.3 $\pm$ 34.9 (4)	83.1 $\pm$ 15.8 (4)
11.9	184.9 $\pm$ 31.8 (3)	91.4 $\pm$ 28.2 (3)	12.5	278.2 $\pm$ 22.3 (4)	89.8 $\pm$ 14.3 (4)
18.0	158.4 $\pm$ 13.3 (5)	81.3 $\pm$ 11.1 (5)	17.9	252.5 $\pm$ 8.4 (4)	74.5 $\pm$ 3.0 (4)
35.6	158.1 $\pm$ 33.3 (5)	68.8 $\pm$ 10.1 (5)	35.1	241.3 $\pm$ 22.5 (4)	71.8 $\pm$ 4.9 (4)
46.1	147.1 $\pm$ 19.0 (3)	80.3 $\pm$ 16.6 (3)	43.2	241.8 $\pm$ 38.3 (3)	87.9 $\pm$ 10.4 (3)

Fig. 2. Lineweaver-Burk plots for adsorptive-phase pinocytosis of ^{125}I -labelled lactate dehydrogenase isoenzymes H4 and M4

The ordinate is the reciprocal of v , the rate at which radiolabelled isoenzyme H4 (●) or M4 (○) is ingested via the adsorptive route by yolk sacs incubated at 37°C in serum-free medium 199 (for details of methods of calculation, see Ibbotson & Williams, 1979). The abscissa is the reciprocal of c , the mean concentration of radiolabelled substrate present in the culture medium during the incubation period.

rate of uptake of the M4 isoenzyme by yolk sac, compared with the uptake of the H4 isoenzyme, arises from the larger number of binding sites available to the former isoenzyme rather than from its higher affinity for the plasma membrane.

To establish whether these results can be inter-

preted in terms of positive charge and hydrophobic interactions, as postulated by Livesey (1979), we carried out competition experiments between the two isoenzymes of lactate dehydrogenase. Similar experiments were also performed using the ^{125}I -labelled form of each of the isoenzymes and a number of other unlabelled proteins that had been characterized with respect to charge and hydrophobicity. Table 3 shows the molecular features of the proteins with respect to binding to sulphopropyl-Sephadex C-25 (cation exchanger), and binding to phenyl-Sepharose CL-4B (hydrophobic chromatography). The data indicate that formaldehyde-denatured bovine serum albumin possesses high affinity for phenyl-Sepharose CL-4B and that the isoenzymes H4 and M4, and, to a lesser extent bovine serum albumin, are tightly bound, but that lysozyme and ribonuclease have only weak affinities for the hydrophobic matrix. At a physiological pH, lysozyme and ribonuclease were shown to be very basic proteins; lactate dehydrogenase M4 appeared to be weakly basic, and lactate dehydrogenase H4, bovine serum albumin and formaldehyde-denatured albumin were acidic.

The effects of the presence of different, unlabelled proteins at a set initial concentration ($50\mu\text{g/ml}$ of medium) on the rate of uptake of the radiolabelled isoenzymes H4 and M4 are shown in Fig. 3. None of the competing proteins had a significant effect on the rate of uptake of ^{125}I -labelled polyvinylpyrrolidone (results not shown), when added to the incubation medium at a concentration of $50\mu\text{g/ml}$, indicating that none of these proteins has the ability to modulate the rate of pinosome formation.

Discussion

The omission of serum from the incubation medium permits a detailed study of the adsorptive

Table 3. Charge and hydrophobicity characteristics of various proteins shown to be taken up by adsorptive pinocytosis in yolk sacs

Ion-exchange chromatography on sulphopropyl-Sephadex C-25, and hydrophobic interaction chromatography on phenyl-Sepharose CL-4B were performed as described by Kooistra *et al.* (1980). The elution data are given as phosphate concentrations and ethyleneglycol concentrations respectively, present in the peak fractions. Abbreviation used: n.b., no binding.

	Phosphate buffer concn. on sulphopropyl-Sephadex (M)	Ethyleneglycol concn. on phenyl-Sepharose (%)
Lysozyme	0.42*	9.7*
Ribonuclease A	0.11	14.0
Lactate dehydrogenase isoenzyme M4	0.04	36.4
Lactate dehydrogenase isoenzyme H4	n.b.	33.1
Serum albumin	n.b.	20.7
Formaldehyde-denatured serum albumin	n.b.	49.1

* Data from Kooistra *et al.* (1980).

pinocytosis of the radiolabelled isoenzymes H4 and M4 of lactate dehydrogenase by avoiding the presence of unwanted, competing serum proteins. Although the elimination of such competition greatly simplifies the quantitative analysis of the binding process, the absence of serum from the medium also causes some fundamental changes in the process of pinocytosis in the rat yolk sac (Ibbotson & Williams, 1979). If the rate of uptake of ^{125}I -labelled polyvinylpyrrolidone is taken as a measure of the rate of fluid-phase pinocytosis (Roberts *et al.*, 1977), the data in Table 1 show that removal of serum results in a 50% rise in the rate of pinosome formation (see also Livesey, 1979; Ibbotson & Williams, 1979). Moreover, the 6–7-fold increase in the Endocytic Indices of both isoenzymes on removing calf serum from the medium is accompanied by only a 2.5-fold rise in the amount of radioactivity associated with the tissue in the steady-state period, i.e. beyond 2.0 h of incubation (see Tables 1 and 2). Similar results were reported for other proteins (Ibbotson & Williams, 1979; Livesey, 1979). The faster rate of processing of internalized proteins in the absence of serum might arise from either a faster rate of fusion of pinosomes with lysosomes, or from a faster rate of intralysosomal breakdown, or from a combination of these two possibilities.

The results in Tables 1 and 2 indicate that, for each of the isoenzymes, the ratio of the quantity of radiolabelled protein associated with the tissue (in the steady-state period) to the Endocytic Index has a characteristic value. For the M4 form the ratio lies in the range 0.29–0.36 and for H4 isoenzyme the corresponding value is slightly higher, 0.43–0.54. Similar studies in our laboratory with other proteins (Livesey, 1979) showed that each protein is processed at a characteristic rate after being internalized, suggesting a different susceptibility of each protein to lysosomal proteinases. In contrast with findings by Dice & Goldberg (1976), which showed

a positive correlation between susceptibility of intracellular proteins and serum proteins to proteinases *in vitro* and their half lives *in vivo*, no correlation between rate of intracellular processing and rate of uptake of a protein by yolk-sac epithelial cells could be demonstrated.

The uptake of both radiolabelled isoenzymes was saturable and binding obeyed Michaelis–Menten kinetics. The dissociation constants of the isoenzyme–plasma-membrane complex were $0.62\ \mu\text{M}$ for the H4 form and $0.84\ \mu\text{M}$ for the M4 form. The binding constants of the lactate dehydrogenase isoenzymes for the plasma membrane are similar in magnitude to that of ^{125}I -labelled formaldehyde-denatured serum albumin ($1.27\ \mu\text{M}$; Ibbotson & Williams, 1979), but much higher than, for example, those of lysosomal enzymes for binding sites on human fibroblasts (approx. $1\ \text{nM}$; Fischer *et al.*, 1980). The maximum rates of uptake for the H4 and M4 isoenzymes were 0.13 and $0.26\ \text{nmol/mg}$ of yolk-sac protein per h respectively. The slightly higher rate of uptake of the M4 form by yolk sac therefore results from a larger number of binding sites for the isoenzyme rather than from a higher affinity for the plasma membrane.

Consideration of both the results obtained from the competition experiments (Fig. 3) and the characteristics of the proteins with respect to positive charge and hydrophobicity (Table 3) suggests that the binding of lactate dehydrogenase isoenzyme H4 is restricted to hydrophobic interactions, whereas the binding of lactate dehydrogenase isoenzyme M4 to the plasma membrane might involve hydrophobic interactions as well as interactions of positively charged sites on the molecules with negatively charged areas of the cell surface. The experiments thus suggest that the general features of basicity and hydrophobicity determine the type of site to which a protein binds.

The importance of positive charge on proteins for

Fig. 3. Rates of pinocytosis of ^{125}I -labelled lactate dehydrogenase isoenzymes H4 and M4 by rat yolk sacs incubated in serum-free medium 199, in the presence of various proteins

Values of the Endocytic Index of ^{125}I -labelled lactate dehydrogenase isoenzymes H4 (a) and M4 (b) in serum-free medium, and in the presence of various proteins at a set initial concentration ($50\text{ }\mu\text{g/ml}$), were determined as described by Ibbotson & Williams (1979). Each value is the mean $\pm$ S.D. (vertical bar) of results obtained using yolk sacs from three or four different animals. Abbreviations used: contr., control (no added protein); BSA, bovine serum albumin; RNase, bovine pancreatic ribonuclease A; lyso., hen egg-white lysozyme; LDH M4, porcine lactate dehydrogenase isoenzyme M4; LDH H4, porcine lactate dehydrogenase isoenzyme H4; f-BSA, formaldehyde-denatured bovine serum albumin.

adsorptive pinocytosis finds support from several studies. Kooistra *et al.* (1979, 1980) demonstrated that, *in vivo*, positive charges play a dominant role in the uptake of ribonuclease and lysozyme by rat liver sinusoidal cells. Cationized ferritin (pI 8.5) was taken

up by peritoneal macrophages (Skutelsky & Hardy, 1976) and dissociated anterior pituitary cells (Farquhar, 1978) in far greater amounts than unmodified ferritin (pI 4.6). Similarly, polycations enhanced lipoprotein uptake in fibroblasts (Basu *et al.*, 1977) and Ryser (1968) demonstrated for sarcoma S-180 cells that a number of basic proteins all had higher rates of uptake than albumin.

The selective pinocytosis of hydrophobic proteins parallels the findings of Bohley & Riemann (1977) for the rates of turnover of intracellular proteins, turnover being faster for those proteins that were more hydrophobic. In our laboratory, we have demonstrated that an increase in the hydrophobicity of bovine serum albumin, by denaturation with an alkaline formaldehyde solution, resulted in a 2.5-fold increase in the affinity of the protein for the plasma membrane of yolk-sac epithelial cells (T. Kooistra & K. E. Williams, unpublished work).

In contrast with studies *in vivo*, in which lactate dehydrogenase isoenzyme M4 is taken up by rat liver sinusoidal cells at a much higher rate than the H4 isoenzyme, the rate of uptake of the M4 isoenzyme by yolk-sac epithelial cells incubated *in vitro* is only slightly higher than that of the H4 isoenzyme. Although the surfaces of all vertebrate cells are negatively charged (Weiss, 1969), different cell types capture a given basic protein at greatly differing rates. Apparently in yolk sacs negatively charged groups are less concentrated in those regions of the plasma membrane from which pinocytic vesicles form than in equivalent regions of sinusoidal liver cells. In yolk-sac epithelial cells hydrophobic interactions are as important as cationic interactions.

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