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D-GLUCOSE UPTAKE BY A RAT LIVER PLASMA MEMBRANE PREPARATION

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SUMMARY

1. Plasma membranes isolated from rat liver according to a modification of the method of Neville, D.M. ((1960) J. Biophys. Biochem. Cytol. 8, 413–422) were used as a model to test current hypotheses on the mode of glucose uptake into the liver cell. Glucose uptake studies were performed by a filtration technique using labeled glucose analogues.

2. D-Glucose uptake by rat liver plasma membranes is characterized by features of simple diffusion, i.e. linearity of uptake, lack of stereospecificity, and by facilitated diffusion, i.e. temperature dependence, counterflow phenomenon and inhibition by phloretin. These findings confirm earlier studies on liver slices and perfused liver.

3. Binding studies on sonicated membranes provide evidence for a specific binding site or protein for D-glucose at the plasma membrane by isolating Tris-soluble membrane proteins which reveal a higher binding capacity than the unsonicated membrane.

4. These findings are interpreted as showing the presence of a "carrier"-mediated transport system for D-glucose superimposed by free diffusion due to artificial disruption of the plasma membranes.

INTRODUCTION

Cahill et al. [1] suggested that the liver is freely permeable to glucose. Their experiments were performed in vitro with rat liver slices and in vivo on dogs. Similar results were reported by Crawhall and Segal [2] who also used rat liver slices. In contrast, Gorsky [3], Williams et al. [4] and Gorsky and Naeau [5] reported that sugar uptake by the liver is via a relatively stereospecific transport system having the properties of facilitated transport. Although they presented strong evidence for a saturable hepatic transport system for glucose, the existence of additional free diffu-

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sion as a second uptake mechanism could not have been excluded by the latter authors. Common to all these investigations is the rapidity and the high capacity of the hepatic glucose transport system. It is of interest that the different methods led to different characterization of glucose uptake.

In the present study an attempt was made to test these current hypotheses on glucose translocation with a plasma membrane preparation in analogy to similar model systems from other tissues [15]. Although the model is complex due to the heterogeneity of membrane structures, the data provide evidence for simple diffusion as well as for carrier-mediated transport. A short report of this work has appeared [6].

MATERIALS AND METHODS

D-[1-^{14}C]glucose (specific activity 57 Ci/mol), D-[1-^{14}C]glucose-6-phosphate (150 Ci/mol) and 3-*O*-methyl-D-[^{14}C]glucose (24 Ci/mol) were obtained from Amersham/Searle Corporation, Arlington Heights, Ill.; L-[1-^{14}C]glucose (51.6 Ci/mol) and D-[1-^{3}H]glucose (5.15 Ci/mmol) from New England Nuclear, Boston, Mass. PCS-solubilizer was also from Amersham/Searle Corporation.

Adenosine 5'-monophosphate disodium salt, adenosine 5'-triphosphate disodium salt and glucose-6-phosphate were purchased from Sigma Chemical Company, St. Louis, Miss.; $MgCl_2$ from Matheson Coleman and Bell, Norwood, Ohio; $HgCl_2$ and *N*-ethylmaleimide from J. T. Baker Chemical Co., Phillipsburg, N.Y. and phloretin from K and K Laboratories, Inc., Plainview, N.Y.; insulin from Eli Lilly and Comp., Indianapolis, Ind. Precoated thin-layer chromatography cellulose sheets were from Merck AG, Darmstadt, G.F.R., and Sephadex G-100 from Pharmacia Fine Chemicals Inc., Piscataway, N.Y. All other chemicals were reagent grade and purchased from Merck AG, Darmstadt, G.F.R.

Male Wistar rats weighing 140–160 g were used throughout the experiments. The animals were allowed food ad libitum until killed. The isolation of the plasma membranes was generally started at 8 a.m. The rats were anesthetized with 10 mg/rat Nembutal intraperitoneally.

Preparation of plasma membranes

Plasma membrane isolation was performed according to the method of Neville [7] in the modification of Ray [8] with the only difference being that the membranes were collected in the sucrose gradient at the interface d 1.153/1.176. The purity and yield of the plasma membranes was routinely followed by measuring the protein content of the membrane fraction and the activities of the marker enzymes for plasma membranes (5'-nucleotidase [9], Mg^{2+} ATPase [10]), endoplasmatic reticulum (glucose-6-phosphatase [11]) and mitochondria (succinate-2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride reductase [12]). Table I gives a summary of the activities of these enzymes. The protein concentration was determined by the method of Lowry et al. [13] with crystallized bovine serum albumin as standard. The yield was 1.2–1.5 mg membrane protein per g liver. The electron microscopic appearance of the plasma membranes was that of a highly purified membrane fraction consisting mainly of small and large vesicles as well as large strips of membranes containing junctional complexes and bile canaliculi.

"Sonicated plasma membranes" were prepared by sonicating the final plasma

TABLE I

ENZYME ACTIVITIES IN PLASMA MEMBRANES FROM RAT LIVER

Plasma membranes were isolated according to the procedure of Neville [7] as described in Methods. Enzyme activities are expressed as μmol phosphate/h per mg protein at 37 °C, except for succinate INT-reductase which is given in nmol reduced INT/h per mg protein at 37 °C. Means ±standard deviations.

Enzyme	Specific activity	
	Homogenate	Plasma membranes
5'-Nucleotidase ($n = 25$)	5.81 ±0.90	73.81 ±13.13
Mg^{2+}ATPase ($n = 16$)	5.77 ±1.24	30.37 ± 5.29
Glucose 6-phosphatase ($n = 13$)	4.10 ±0.61	0.63 ± 0.28
Succinate INT-reductase ($n = 16$)	2.03 ±0.48	0.036 ± 0.015

TABLE II

ENZYME ACTIVITIES OF SONICATED PLASMA MEMBRANES FROM RAT LIVER

After isolation plasma membranes were sonicated for 60 s at 90 watts. The protein content of the membrane fractions did not alter by sonication. Enzyme activities are expressed as μmol phosphate/h per mg protein at 37 °C. Means ±standard deviations.

Enzyme	Specific activity	
	Before sonication	After sonication
5'-Nucleotidase ($n = 3$)	70.60 ±8.91	70.00 ±7.32
Mg^{2+}ATPase ($n = 3$)	29.60 ±4.23	33.10 ±3.89

membrane suspension for 15–120 s in periods of 15 s at 90 watts in an ice-bath with a sonifier cell disruptor (Heat System Co., Melville, L.I.N.Y.). After 15 and 60 seconds of sonication no change in the morphological features shown by electron microscopy, was seen. Also the specific activities of the two measured plasma membrane marker enzymes remained unchanged (Table II).

Assay for uptake of labeled sugars

All incubations were carried out in 50 mM Tris · HCl, pH 7.4. All plasma membranes were first incubated in 0.2 ml of the Tris-buffer for 15 min. The reaction was started by adding the labeled sugar. The final volume of the incubation mixture was 0.25 ml, except where mentioned otherwise. During the incubation the tubes were gently shaken in a constant temperature bath. The incubation was stopped by putting 0.2 ml of the mixture on the middle of a previously chilled and wetted H-A-Millipore filter (pore size 45 μm). The filters were washed immediately with 30 ml of ice-cold

50 mM Tris, pH 7.4, containing the same unlabeled final sugar concentration used in the incubation. After washing, the filters were removed and put in glass scintillation counting vials which contained 2 ml of 10 % sodium dodecyl sulfate. After shaking at room temperature for 30 min, 10 ml of PCS-solubilizer were added and counted in a Nuclear Chicago Mark II liquid scintillation counter. All samples including control samples without plasma membranes, were performed in quadruplicate. Neither binding nor retention of the sugars by the filter could be detected. Each experiment was repeated with at least three different membrane preparations. The standard deviation was less than ± 8 %.

In order to measure the rate of release of bound sugars, membranes and labeled D- or L-glucose were first incubated for 5 min at 20 °C in a total volume of 0.25 ml. At zero time 2.5 ml of Tris-buffer was added. At varying times after the dilution, 2.5 ml of the incubation mixture was put on the filter. Washing and counting were performed as mentioned above.

In the counterflow experiment the membranes were preincubated with D-[^{14}C]glucose in a final concentration of 100 mM. Then D-[^{3}H]glucose was added at a concentration of 1 mM to dilute D-[^{14}C]glucose to 10 mM. The final volume was 0.25 ml. The reaction was stopped by filtering and washing with ice-cold buffer as described in the uptake experiments.

Identification of radioactive compounds in plasma membranes

To identify the radioactive compounds taken up or later released by the membranes into the incubation medium, thin-layer chromatography was performed as described by Clark [14]. D-[1-^{14}C]glucose (3 Ci/mol) and D-[1-^{3}H]glucose (160 Ci/mol) were used in these experiments. Incubations for 5 and 20 min were performed as described in the uptake experiments. 100 μl of each of the following samples were applied on precoated thin-layer chromatography cellulose sheets: (a) whole incubation mixture, (b) eluate gained from the filters after the washing procedure by shaking them in 0.5 or 1.0 ml of distilled water at room temperature for 15 min. From each of these samples an aliquot was counted directly in order to measure total radioactivity. For determination of the residual, nonextractable radioactivity the filters were counted after the extraction procedure. D-[1-^{14}C]glucose, D-[1-^{3}H]glucose and D-[1-^{14}C]glucose-phosphate were applied to each plate as separate standards as well as superimposed on the unknown samples. The plates were developed in two different solvent systems: (a) isopropanol/pyridine/acetate/water (8 : 8 : 1 : 4, by vol.) and (b) *n*-butanol/formic acid/water (75 : 15 : 15, by vol.). The dry plates were cut in 1 cm segments which were counted in 10 ml of a Triton scintillation mixture.

Chromatography on Sephadex G-100

40 to 60 mg of plasma membrane protein was suspended in 3.0 ml 50 mM Tris, pH 7.4 and sonicated for 15 or 60 s with 90 watts. Then the sample was either filtered through a H-A-Millipore filter or centrifuged at 30 000 $\times g$. The filtrate (6.0 ml) or the supernatant (3.0 ml) containing approximately 10 % of the original membrane protein was incubated for 5 or 20 min with a final concentration of D-[^{3}H]-glucose (spec. act. 40 Ci/mol) as described in the uptake experiments.

Immediately after the incubation the sample was applied to a Sephadex G-100 column (1 cm $\times$ 38 cm or 1.7 cm $\times$ 27 cm) equilibrated with 2 column volumes of

50 mM Tris buffer containing 1 mM D-glucose. The column was eluted with 50 mM Tris+1 mM D-glucose. The flow rate was 0.5–1.0 ml/min.

RESULTS

Uptake of labeled D- *and* L-*glucose*

The time course of D-[^{3}H]glucose uptake at 20 °C shows that equilibrium was reached within 5 min (Fig. 1). Similar uptake curves were seen for L-[^{14}C]glucose and *O*-methyl-D-[^{14}C]glucose, a nonmetabolisable sugar. Glucose uptake was at a concentration of 1 mM and 20 °C so fast that it proved impossible to measure initial rates with the Millipore filtration technique. The time for half maximal uptake for D- and L-glucose was estimated to be between 20 and 30 s. As shown in Fig. 2 the rate of uptake of D-[^{3}H]glucose was dependent on the temperature of the incubation medium.

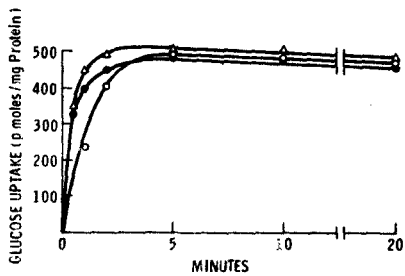

Fig. 1. Time course of the uptake of D-[^{3}H]glucose, *O*-methyl-D-[^{14}C]glucose and L-[^{14}C]glucose by rat liver plasma membranes. The specific activity of D-[^{3}H]glucose was 40 Ci/mol, of *O*-methyl-D-[^{14}C]glucose 5 Ci/mol and of L-[^{14}C]glucose 7.5 Ci/mol. Plasma membranes (98 μg/sample) were first incubated for 15 min at 20 °C. At zero time the sugar was added (1 mM final concentration) and at varying times later samples were removed and processed as indicated in Materials and Methods. Each point is a mean value of four determinations. D-[^{3}H]glucose, (●–●); *O*-methyl-D-[^{14}C]-glucose, (○–○); L-[^{14}C]glucose, (△–△).

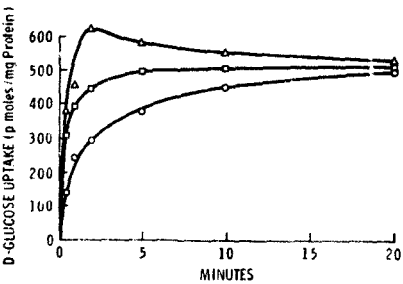

Fig. 2. Uptake of D-[^{3}H]glucose by rat liver plasma membranes at 4, 20 and 37 °C as a function of time. The membrane suspension (72 μg/sample) was first incubated in 50 mM Tris buffer at the indicated temperatures for 15 min. At zero time D-[^{3}H]glucose (spec. act. 40 Ci/mol) was added to reach a final concentration of 1 mM. At varying times the incubation was stopped as described in Materials and Methods. Each point is a mean value of four determinations. 4 °C, (○–○); 20 °C, (□–□); 37 °C, (△–△).

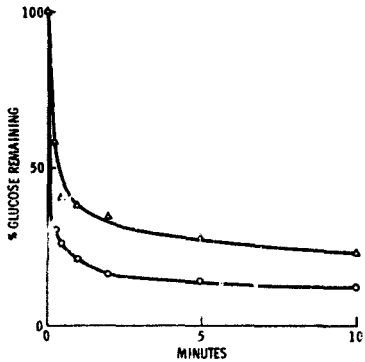

Fig. 3. Glucose release from rat liver plasma membranes. Plasma membranes (86 μg/sample) were loaded with 1 mM D-[^{3}H]glucose (spec. act. 40 Ci/mol) or 1 mM L-[^{14}C]glucose (spec. act. 7.5 Ci/mol) by incubating for 5 min at 20 °C. At zero time the incubation mixture was diluted 10-fold with buffer and the rate of release measured as described in Materials and Methods. The results are expressed as percentage of the sugar remaining in the membranes. The amount of the labeled sugars taken up before dilution was 100 %. Each point is the mean value of four determinations. D-[^{3}H]-glucose, (○–○); L-[^{14}C]glucose, (△–△).

Protein loss during filtration was tested by measuring protein in the filtrate directly or after precipitation with 10 % (w/v) trichloroacetic acid. In addition, the protein was eluted from the filter with 2 % sodium dodecyl sulphate for 30 min and measured again in the eluate. Less than 1 % of protein applied to the filter was lost by filtration. 5′-nucleotidase was absent in the filtrate, but could be recovered in the eluate.

When membranes, preloaded with 1 mM D- or L-glucose, which had accumulated the sugar up to the plateau level, were diluted in a 10-fold volume of 50 mM Tris buffer, both compounds were released rapidly (Fig. 3). A new equilibrium was reached after 1 to 2 min. The release of D-glucose was more pronounced than that of L-glucose, to 12.5 % and 25 % of the control, respectively.

D-Glucose uptake by the membrane preparation was dependent on D-glucose concentration in the incubation medium. At an incubation time of 1 min the uptake of D-glucose was linear up to a concentration of 500 mM. Higher glucose concentrations were found to slow filtration time to more than 30 seconds which was considered to be the upper time limit for the washing with 30 ml.

After 1 and 5 min incubations the rate of D-glucose uptake into the plasma membranes was linearly related to the membrane concentration up to 300 μg of membrane protein. Higher amounts of membrane proteins were found to clog the filter resulting in a decrease in the rate of filtration of reaction mixture and washing buffer.

The uptake was little affected by pH over the range of 5.4 to 9.4. The uptake of D-glucose by rat liver plasma membranes was effected by phloretin, a relatively selective inhibitor of glucose transport. At 8 mM, phloretin decreased D-glucose uptake by 68 % (Table III). $HgCl_2$ and *N*-ethylmaleimide, two relatively specific reagents for sulfhydryl groups also inhibited the uptake of D-glucose by plasma membranes (Table III).

TABLE III

D-[^{3}H]GLUCOSE UPTAKE OF RAT LIVER PLASMA MEMBRANES IN THE PRESENCE OF PHLORETIN OR SULFHYDRYL-REACTING COMPOUNDS

The final D-[^{3}H]glucose concentration (spec. act. 40 Ci/mol) was 1 mM, the concentrations of phloretin and sulfhydryl-reacting compounds as indicated. Phloretin was solved in acetone. Control samples for the phloretin-investigations had the same acetone concentration as the sample with phloretin. Plasma membranes (82 μg/sample) were incubated with D-glucose and phloretin or sulfhydryl-reacting compounds for 5 min at 20 °C. The mean ± S.E. for four different membrane preparations are given.

Addition	Concentration	pmol/mg protein	Percentage inhibition
None	-	357 ± 5.8	-
Phloretin	1 mM	235 ± 8.6 $P < 0.001$	33
Phloretin	8 mM	125 ± 11.6 $P < 0.001$	72
None	-	383 ± 8.5	-
$HgCl_2$	10 mM	205 ± 18.1 $P < 0.001$	47
N-ethylmaleimide	10 mM	343 ± 9.6 $P < 0.02$	14

The effect of insulin on the uptake capacity of plasma membranes for D-glucose is shown in Table IV. Preincubation of plasma membranes with different concentrations of insulin led to a small increase of the D-glucose uptake capacity by the membranes. The increase of 25–35 % at a time when equilibrium is reached, is comparable with results shown for fat cell membranes [15].

The liver plasma membranes took up D-glucose against a concentration gradient as shown in Fig. 4. Plasma membranes were first equilibrated with 100 mM D-[^{14}C]glucose, then rapidly diluted with 9 vol. of buffer containing 1 mM D-[^{3}H]glucose. During the first minute, while a rapid release of D-[^{14}C]glucose occurred, an uptake of D-[^{3}H]glucose was measured. After 1–2 min a new equilibrium was reached for both isotopes.

As shown in Fig. 5 the uptake of D-[^{3}H]glucose by rat liver plasma membranes was enhanced when membranes were first incubated with high concentrations of D-glucose. If previous incubation with high concentrations of D-glucose has not resulted

TABLE IV

EFFECT OF VARYING INSULIN CONCENTRATIONS ON D-GLUCOSE UPTAKE BY RAT LIVER PLASMA MEMBRANES

The plasma membranes (64 μg/sample) were first incubated with insulin for 5 min at 20 °C. After addition of D-[^{3}H]glucose (spec. act. 40 Ci/mol, final concentration 1 mM) the final incubation time was 5 min. Insulin was dissolved in distilled water, pH 3.0. To the control samples the same volume of distilled water, pH 3.0, without insulin was added. The mean ± S.E. for four different membrane preparations are given.

Concentration of insulin	pmol/mg protein	Percentage of control
None	323 ± 12.5	100
1 munits/ml	340 ± 16.0 $P > 0.40$	105
10 munits/ml	434 ± 1.8 $P < 0.001$	134
100 munits/ml	407 ± 39.9 $P > 0.10$	126

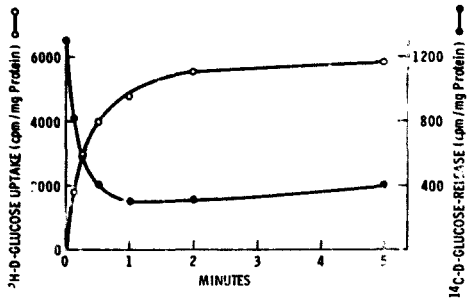

Fig. 4. Countertransport of D-[^{3}H]glucose in rat liver plasma membranes on addition of D-[^{14}C]-glucose to the incubation medium. Liver plasma membranes (57 μg/sample) were previously incubated with 100 mM D-[^{14}C]glucose for 20 min at 4 °C to reach equilibrium. Then the 9-fold volume of 1 mM D-[^{3}H]glucose was added and the suspension was incubated for varying times at 4 °C. At the indicated times the reactions were stopped and filtered as described in Materials and Methods. The results are expressed as the counts remaining in the membranes. Each point is the mean value of four determinations. D-[^{14}C]glucose, (● – ●); D-[^{3}H]glucose, (○ – ○).

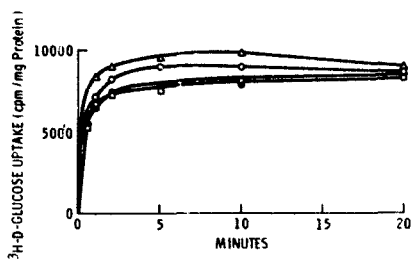

Fig. 5. Time course of D-[^{3}H]glucose uptake by rat liver plasma membranes after preincubation with different concentrations of unlabeled D-glucose. Plasma membranes (62 μg/sample) were preincubated for 10 min at 20 °C with different concentrations of D-glucose and 1000 mM sucrose. The incubation volume was 0.2 ml. At zero time 0.05 ml of 5 mM D-[^{3}H]glucose (spec. act. 40 Ci/mol) were added and at varying times later samples were removed and processed as indicated in Materials and Methods. Each point is the mean value of four determinations. 1 mM D-glucose, (● – ●); 100 mM D-glucose, (○ – ○); 1000 mM D-glucose, (△ – △); 1000 mM sucrose, (□ – □).

in a change of the uptake of tracer amounts of D-[^{3}H]glucose in the same incubation volume, the conclusion would be that equal distribution of labeled and unlabeled sugars occurred between the three possible spaces, i.e. incubation medium, internal vesicular spaces and membrane binding sites. If, however, the later uptake of D-[^{3}H]-glucose is enhanced by high concentrations of D-glucose, as shown here, the conclusion can only be that additional specific binding of D-[^{3}H]glucose to the membranes has occurred. Previous incubation with 1 M sucrose has no effect upon the uptake of D-[^{3}H]glucose as compared to the curve obtained with 1 mM D-glucose which indicates that higher osmolarity did not affect the uptake of D-[^{3}H]glucose under these conditions (Fig. 5).

Identification of radioactive compounds after D-[^3H]- *and* [^{14}C]*glucose uptake*

The uptake of labeled D-glucose (under the conditions shown in Fig. 1) amounted to less than 1 % of the labeled D-glucose present. It was therefore necessary to identify the radioactive compounds retained by the membrane. The radioactive sugar taken up by the membranes was quantitatively eluted with distilled water and was recovered entirely as the unphosphorylated glucose. The extracted labeled material behaved on thin-layer chromatography as one compound with a R_F identical with that of glucose. Furthermore in the incubation medium, too, only glucose could be detected. Therefore, no metabolic products of the ^3H- or ^{14}C labeled D-glucose studied were found in the membranes or in the incubation medium after 5 and 20 min of incubation at 20 °C.

Displacement of accumulated D-[^3H]*glucose*

The addition of excess nonradioactive D-glucose (500 mM final concentration) to a membrane suspension which had accumulated D-[^{3}H]glucose (100 mM) up to steady-state resulted in a rapid, but only limited decline of radioactivity (30 %) in the membranes. After 5 min a new steady-state was reached.

Specificity of D-*glucose uptake*

When the plasma membranes were first incubated with 1 mM, 100 mM or 500 mM of D-glucose no effect on the subsequent uptake of L-[^{14}C]glucose was observed. When D-[^{3}H]glucose and L-[^{14}C]glucose were added together in a double labeling experiment identical uptake curves for both isotopes were observed. Preincubation of plasma membranes with 10 mM *O*-methyl-D-glucose also did not inhibit the subsequent uptake of D-[^{3}H]glucose at a concentration of 1 mM.

Uptake of D-*glucose by sonicated plasma membranes*

Sonication of plasma membranes reduced the velocity and capacity of glucose uptake. Sonication for 15–120 s resulted in the similar effect for uptake. Fig. 6 shows the time-dependent uptake of D-[^{3}H]glucose and L-[^{14}C]glucose, both 1 mM final concentration, and D-[^{3}H]glucose in a final concentration of 100 mM at 20 °C. The diminished uptake of D-[^{3}H] and L-[^{14}C]glucose (about 50 %) by sonicated membranes was accompanied by a loss of 13–16 % of the membrane protein. When this lost protein was applied to a Sephadex-G-100 column, protein fractions with a much higher specific activity (up to 30 000 cpm/mg protein) than measured for unsonicated membranes (8000–9000 cpm/mg protein) were found (Fig. 7). This soluble protein fraction could be recovered in the supernatant after centrifugation of the sonicated membranes at 30 000 $\times g$ for 20 min.

DISCUSSION

Liver plasma membranes isolated by the method of Neville [7] exhibit mixed morphological features by electron microscopy, which consist mainly of small and large vesicles. Except for the double membrane strips it is not possible to distinguish between the inside and outside of the membrane. Therefore with this preparation it is not possible to distinguish between processes located on the inside or outside of the membrane. In the present case, only the uptake of glucose by the plasma membrane

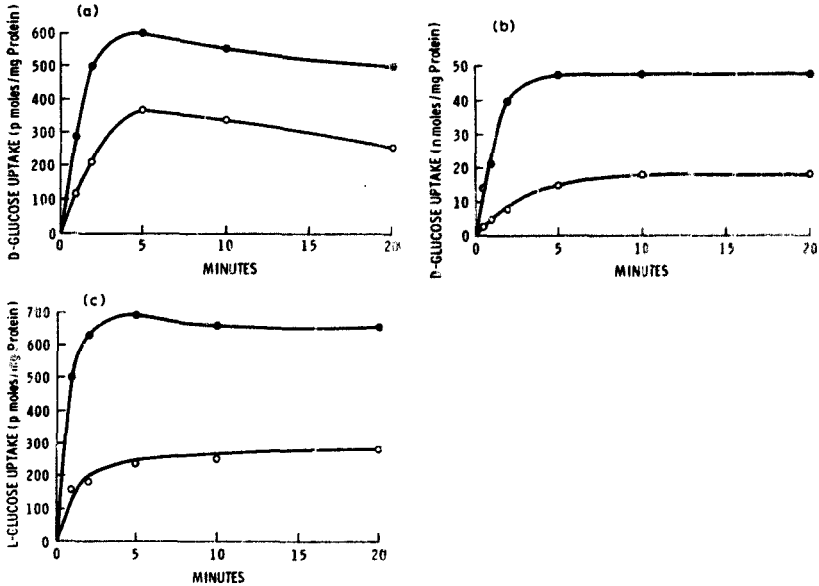

Fig. 6. D- or L-glucose uptake by sonicated rat liver plasma membranes. Plasma membranes were sonicated for 60 s at 90 watts. The membranes were first incubated for 15 min at 20 °C. At zero time the labeled sugar was added and the samples were removed at varying times as indicated. (a) The final concentration of D-[^{3}H]glucose was 1 mM, the specific activity 40 Ci/mol. (b) Final D-[^{3}H]glucose concentration 100 mM, spec. act. 0.8 Ci/mol. (c) Final L-[^{14}C]glucose concentration 1 mM, spec. act. 7.5 Ci/mol. Each point is the mean value of four determinations. Control with unsonicated membranes, (● — ●); sonicated plasma membranes, (○ — ○).

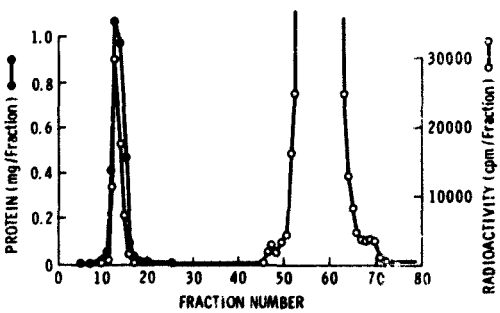

Fig. 7. Chromatographic separation of the binding protein from Sephadex G-100 chromatography. 50 mg of plasma membrane protein, suspended in 3.0 ml 50 mM Tris, pH 7.4, were sonicated for 15 s at 90 watts. Then the sample was filtered through a chilled, wetted HA-Millipore filter. The filter was washed with 3.0 ml of the buffer. 5.02 mg of the membrane protein were collected in the filtrate and incubated with D-[^{3}H]glucose (spec. act. 40 Ci/mol) in a final concentration of 1 mM for 10 min at 20 °C. Immediately after the incubation the sample was applied to the column (1 cm × 38 cm, 0.885 ml/min flow rate). The column was eluted with 50 mM Tris containing 1 mM D-glucose. 1.77 ml fractions were collected and measured for their protein and radioactive content. Peak 1 corresponds to membrane protein, peak 2 to D-glucose. The specific activities of the fraction numbers 12, 13, 14 and 15 were 20 800, 28 500, 19 000 and 15 700 cpm/mg protein, respectively.

could be measured without differentiation of the direction of the uptake. Both sides of the plasma membrane may be involved in the uptake measured.

Given the morphological features of the plasma membranes two possible mechanisms have to be discussed. (1) Uptake into the internal space of the vesicles. (2) Binding to either surface of the plasma membrane.

Uptake into the internal space of the vesicles is suggested by the following observations: (a) the linear relationship between medium concentration of D-glucose and uptake of D-glucose by the plasma membrane up to a concentration of 500 mM, (b) the lack of difference in the uptake curve between D- and L-glucose (Fig. 1), and (c) the minimal influence of pH-changes on D-glucose uptake by the membranes. Binding to the membranes may be concluded from: (a) the inhibition of uptake by sulfhydryl-blocking compounds (Table III), (b) the stimulation of D-glucose uptake by insulin (Table IV), and (c) the faster release of D-glucose compared with L-glucose after dilution with buffer (Fig. 3). Since there is evidence that both mechanisms may be operative, it can be assumed that the total amount of D-glucose taken up by the membranes is the sum of uptake into intravesicular spaces and into the plasma membrane. Since the intravesicular volume is larger than the intramembranal space, most of the measured glucose taken up should be collected within these vesicles. The transport into the membrane vesicles may occur (1) by simple diffusion, perhaps through pores or artificial holes of the plasma membranes, which were produced by hypotonic shock or disruption during the isolation procedure and (2) by a specific glucose transport system located in the plasma membrane. The latter implies binding to a specific site or protein of the plasma membrane. Simple diffusion has to be assumed from the following results: (a) linearity of D-glucose uptake up to 500 mM, (b) lack of differences in the uptake curves between D-, L- and *O*-methyl D-glucose (Fig. 1), (c) absence of inhibition of D-glucose uptake by L-glucose, (d) failure of high concentrations of unlabeled D-glucose to reduce significantly D-[^{3}H]glucose taken up and (e) minimal effects of pH-changes on uptake capacity. These points are similar to those which supported the preceding argument for transport into an intravesicular volume. Therefore the uptake of D-glucose into these vesicles takes place partially by free diffusion. This mechanism is certainly possible, since the vesicles should be formed artificially by disrupted membranes. However, the studies with the inhibitor phloretin suggest that up to 70 % of the total uptake can be specifically inhibited, a result which indicates at least an equal participation of mechanisms involving a specific binding site.

The existence of a specific binding site or protein is suggested by the following observations: (a) inhibition of D-glucose uptake by phloretin (Table III). The phloretin concentration necessary was relatively high. This was also shown for phlorizin by liver perfusion [4, 5], (b) the enhanced uptake of D-glucose by plasma membranes preincubated with insulin (Table IV), (c) the more rapid release of D-glucose from preloaded membranes than for L-glucose (Fig. 3), (d) the evidence for a counterflow phenomenon with an apparent uptake against a concentration gradient (Figs. 4 and 5), (e) the enhanced uptake of a tracer amount of D-[^{3}H]glucose after preincubation of the plasma membranes with high concentrations of nonradioactive D-glucose (Fig. 5), (f) the temperature dependence of initial uptake velocity (Fig. 2), and (g) the isolation of a soluble plasma membrane constituent which exhibits a high binding capacity for D-glucose (Fig. 7) The nature of this fraction containing the glucose binding protein is as yet not clear. Sodium dodecyl sulphate polyacrylamide electro-

phoresis of the pooled fractions 12–15 of Fig. 7 revealed four major bands corresponding to a molecular weight of between 50 000 and 70 000.

It is likely that the preparation under study contains three functionally different plasma membrane sides of the hepatocyte, a fact which would limit the conclusions to be drawn from our data. It may well be possible that the 3 different sides, because of their different functions, handle the uptake or release of glucose in different ways which could be one explanation for the fact that different techniques for the study of glucose transport in the liver led to different results. (1) Studies on liver slices led to the conclusion that D-glucose was taken up by free diffusion [1, 2]. Here all three membrane surfaces would have been exposed for uptake. (2) Studies on perfused liver led to the conclusion that glucose uptake by the liver involved facilitated transport [3, 4, 5]. Here only the blood sinusoidal surface of the liver should be exposed for the uptake of D-glucose from the perfusate. In the present results with isolated plasma membranes, evidence for both mechanisms is shown. In common to all studies is the rapid uptake and an apparently unlimited uptake capacity.

An effect of insulin on glucose uptake by liver cells is denied by most authors [1, 4, 16, 17]. Only Bergman and Bucolo [18] have shown a stimulatory effect of insulin on hepatic glucose uptake. In their studies, performed with canine perfused liver, livers were first infused with insulin before the experimental increase of portal vein glucose concentrations. An uptake, 2-fold greater after that procedure was reported. In our experiments where a modest stimulation of the glucose uptake capacity was seen, the plasma membranes were first incubated with insulin and then glucose was added. Authors reporting no effect of insulin on glucose transport, used glucose and insulin together.

From the literature, the weight of current evidence suggests that the major portion of glucose uptake by liver cells is due to a facilitated transport system. The results presented here confirm these former studies on liver slices and perfused liver. Among other possible modes of glucose transport, facilitated diffusion has been identified as one mechanism of glucose uptake in many tissues investigated, i.e. fat cells [15, 19], red blood cells [20–22], small intestine [23, 24], muscle [25], kidney tubules [26], pancreatic β-cells [27] and brain [28]. In accordance a similar mechanism could be the physiological mode for glucose transport between blood sinus and liver cell. In this paper evidence for a specific binding site or protein for D-glucose in the rat liver plasma membrane is reported. This binding site or protein may well have the function of a "carrier", the existence of which is postulated to be necessary for facilitated transport. Free diffusion as a second uptake mechanism could not be excluded, since it has to be assumed that the artificial disruption of the plasma membranes as a result of the isolation procedure may have effected the studies.

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