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# THE HEPATOCELLULAR UPTAKE OF GLUCOSE, GALACTOSE AND FRUCTOSE IN CONSCIOUS SHEEP

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SUMMARY

1. Surgical techniques for chronic catheterization of hepatic and portal veins in the sheep are described. These catheters remained usable for 2–6 months and did not alter hepatic morphology.

2. Hepatocellular uptake of monosaccharides was estimated from their ability to pass the boundaries of the sucrose space in a double indicator dilution procedure in conscious fed sheep.

3. A large proportion (81 %) of D-glucose carried in the portal blood was found to enter an hepatic cellular compartment.

4. The radioactive label of D-glucose infused in the portal vein remained associated with D-glucose in hepatic venous blood samples during the experimental period.

5. A large proportion (74 %) of an infused trace of D-galactose, a smaller proportion (33 %) of D-fructose, and negligible amounts of L-glucose were taken up in a single passage through the liver.

6. Raised blood concentrations of sucrose or of methyl-α-D-glucoside (Me-α-DG) significantly diminished the proportional uptake of D-glucose. Raised blood concentrations of glucose, galactose or Me-α-DG diminished the proportional uptake of D-galactose. Raised blood concentrations of fructose diminished the proportional uptake of fructose.

7. Neither total hepatic blood flow changes nor competitive effects within the cell could account for these findings.

8. It is concluded that these monosaccharides enter the liver cell by facilitated diffusion, and share at least some of the membrane elements that mediate this process. It seems likely that only a proportion of the glucose-transporting apparatus is accessible to galactose.

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## INTRODUCTION

There have been studies of the mechanism of glucose transfer across the placenta (Widdas, 1952), the erythrocyte plasma membrane (LeFevre, 1948), and the luminal borders of the enterocyte (Crane, 1960), and of the renal tubular cell (Kleinzeller, Kolinska & Benes, 1967); the subject is extensively reviewed by LeFevre (1972). The liver's role in the control of plasma monosaccharide levels presupposes that the sugars pass freely across the plasma membrane of the hepatocyte, but there is a relative paucity of experimental data on this process in liver cells.

From work with liver slices *in vitro* (Berthet, Jacques, Hers & DeDuve, 1956) and with perfused rat livers (Hetenyi & Arbus, 1962; Hetenyi, Kopstick & Retelstorf, 1963), it has been concluded that D-glucose equilibrates rapidly between the cell water and the extracellular medium. Some non-metabolized analogues of D-glucose, 3-*O*-methyl glucose and L-glucose, also distribute in a similar intracellular volume (Hetenyi, Norwich, Studney & Hall, 1969). Mediation of monosaccharide transfer by a membrane component is suggested by the finding that L-glucose enters the cells of the isolated perfused liver of the rat very slowly, and that this transfer is inhibited by D-glucose (Williams, Exton, Park & Regen, 1968). There is ample evidence of the very high permeability of the walls of hepatic sinusoids (Goresky, 1965; Yudilevich, Renkin, Alvarez & Bravo, 1968; Bravo & Yudilevich, 1971), such that the sinusoidal vascular and interstitial spaces can for the present purposes be treated as one.

In the presence of a reference indicator which diffuses like the substrate in the extracellular spaces, but does not penetrate the cell, the cellular uptake of the substrate can be inferred from the change in the substrate/reference ratio during passage through the liver. In this way the rapid penetration of a cellular compartment of the livers of anaesthetized dogs was studied using glucose (Goresky, 1967) and galactose (Goresky, Bach & Nadeau, 1973).

The purpose of the present study was to examine the transfer of monosaccharides across the hepatocyte plasma membrane *in vivo*. Methods similar to those of Goresky & Bach (1970) have been adapted for use in sheep which were conscious and feeding, rather than starved, anaesthetized and supine, since the latter circumstances seemed likely to alter the hepatic uptake process.

## METHODS

*Abbreviations used*

The following is an alphabetical list of symbols and abbreviations used below. They are also defined in the usual way on their first use in the text.

| | |
|---|---|
| $A_p$ | activity of the indicator in plasma, |
| $A$ | activity (dpm.$l^{-1}$) of the reference indicator in blood, |
| $A_t$ | activity of the test indicator in blood, |
| $A_w$ | activity of the indicator in whole blood, |
| $E$ | the percentage or proportion of the test indicator removed from the blood in a single transit through the liver, |
| $E_o$ | the extraction estimated as early as possible after presentation of the indicator so that efflux is minimal, |
| $F_a$ | fraction of the activity present in plasma, |
| $H$ | haematocrit percentage, |
| HBF | hepatic blood flow, |
| HV | hepatic vein, |
| IVC | inferior vena cava, |
| Me-α-DG | methyl-α-D-glucoside, |
| PV | portal vein, |
| $R_F$ | chromatographic mobility as a fraction of that of the solvent, |
| $R_i$ | the ratio of reference and test indicator activities in inflowing blood, |
| $R_o$ | the ratio of reference and test indicator activities in outflowing blood. |

### *Catheter implantation*

Six month old cross-bred ewe lambs (25–35 kg body weight) were anaesthetized with pentobarbitone, oxygen, nitrous oxide and halothane. A long right subcostal incision was made and wound towels were secured to the skin edges with Michel clips.

The external oblique muscle was transected and a tunnel leading from the dorsal extremity of the wound was constructed. Three modified stainless steel Gordh needles were passed from the skin to the tunnel and secured by grub screws in a smooth perspex block (Fig. 1). The needle hubs stood about 5 mm clear of the skin surface to avoid impairing the blood supply of the skin close to the shank. A 0·3 m length of silicone elastomer tubing of internal diameter 1·3 m (Silescol, Esco Rubber Ltd) was secured with linen thread to the projecting shank of each needle below the block. A closely fitting 80 mm length of larger bore silicone elastomer tubing was threaded over each catheter and secured to the shank of each Gordh needle, so as to protect the inner catheter from bending and shearing at the needle tip.

The peritoneum was opened and the catheters separately brought through these layers to the peritoneal cavity. A retaining 'collar' of silicone elastomer tubing 5 mm long was then threaded on to the peritoneal extremity of each catheter.

The laterally perforated catheter was advanced into a pancreaticoduodenal vein until it could be palpated in the common portal vein (PV) about 30 mm below the liver. The collar was then moved up to the ligature, tied in place and oversewn with peritoneum using a 3/0 braided silk continuous suture. An end-hole catheter was similarly advanced from a caecal vein so that it lay in the PV about 10 mm below the liver and secured in the same way.

The third catheter, also with an end-hole, was introduced into a main right hepatic vein (HV) by a procedure developed from that of Harrison (1969). The wall of the

IVC was pierced through a purse-string suture with a sharp stillette and a guide wire was introduced through a curved needle into a right HV, and advanced until it entered the peritoneal cavity 20–30 mm from the liver margin (Fig. 1). The collar was positioned about 80 mm from the catheter tip and the catheter fed over the end of the guide wire projecting from the liver surface. The catheter was then gently gripped on to the guide wire and the wire retracted, so pulling the catheter

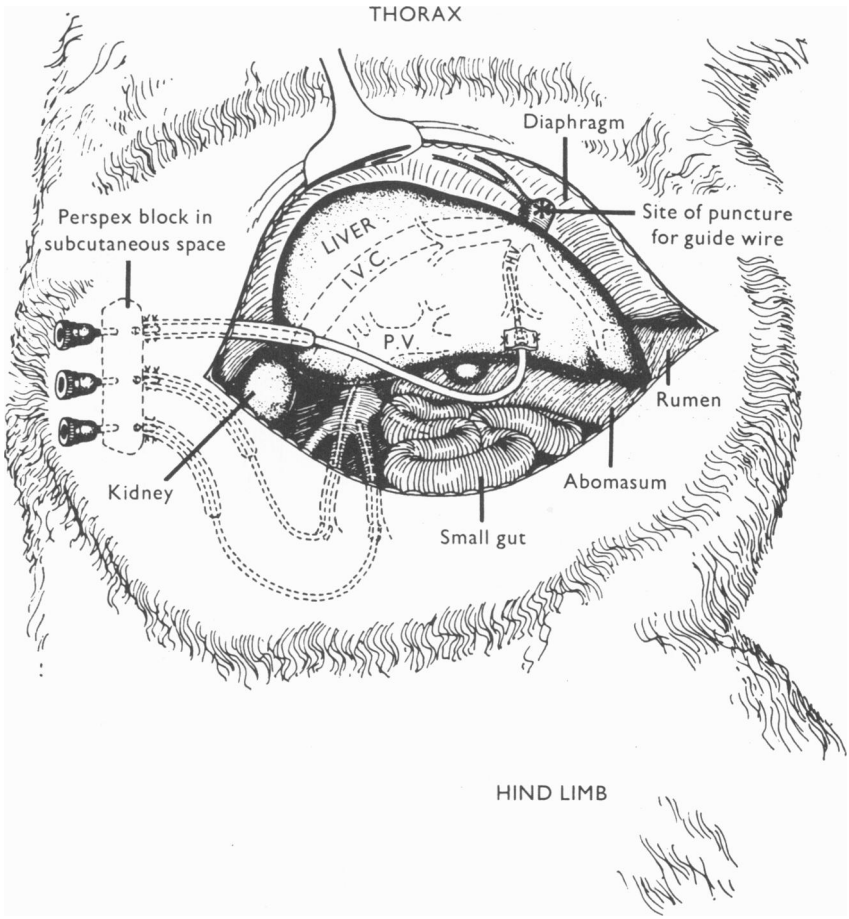


Fig. 1. Position of the catheters at operation. The right subcostal incision provided an unrestricted view of the liver as well as the means of implanting the Perspex block in a tunnel at the dorsal extremity of the wound.

into the HV. The collar was secured to an overlying 20 mm by 10 mm rectangle of polyvinyl alcohol sponge (Prosthex, A. R. Horwell Ltd.) secured by two loose transfixing sutures of 2/0 plain catgut to a similar sponge on the opposite surface of the liver.

The wound was closed in the usual way and the catheters filled with a 0·154 M-NaCl solution containing 1000 i.u. ml.$^{-1}$ heparin ('Pularin', Evans Medical). Penta-

zocine ('Fortral', Winthrop, 15 mg I.M.) was given post-operatively. It was not necessary to flush the catheters daily. After experiments they were refilled with the heparinized saline solution.

The animals were housed indoors in individual pens and given 1·5 kg of pelleted feed once a day with water *ad libitum*.

In the present study, operations were attempted upon twenty-five animals of whom fifteen bore a complete set of patent catheters for more than 30 days, twelve for more than 60 days and five for more than 100 days.

### *Effect of implanted catheters on liver morphology*

In three sheep who had borne catheters for 22 days, 73 days and 75 days respectively, percutaneous liver biopsies were performed under local anaesthesia using a 0·8 mm Menghini needle. These were compared with similar tissue samples removed from defined parts of the liver at terminal laparotomy under pentobarbitone general anaesthesia.

The tissue samples were fixed in glutaraldehyde (2%, v/v, in 0·1 M phosphate buffer) post-fixed in osmium tetroxide and embedded in Araldite (Ciba) and sectioned on a Huxley microtome. Sections 1 $\mu$m thick were inspected by visible light after staining with alkaline toluidine blue. Sections 60 nm thick stained with uranyl acetate and lead citrate were examined in an AEI EM 6B electron microscope at magnifications of 10,000× and 30,000×. We are indebted to Mr J. H. Kugler and Mrs M. Hodgkiss for the preparation of this material.

No morphological differences could be discerned between the biopsy samples obtained under local or general anaesthesia, nor between tissue samples from close to the catheter tracks and tissue removed from a remote lobe. No structural damage likely to distinguish the function of these livers from the normal appears to have been caused by the implanted silicone elastomer catheters.

### *Infusion procedures*

Each sheep was accustomed to stand in a crate during experiments and for the preceding 30 min. A companion sheep was placed in an adjacent crate. Free access to food and water was provided.

Approximately 15 ml. HV blood was collected into a previously heparinized syringe. To this a $^{14}$C-labelled substance and a $^{3}$H-labelled substance were added so that the motor driven syringe, set to deliver 21 ml.min$^{-1}$, produced the following rates of infusion into the PV:

| | $\mu$c.sec$^{-1}$ |
|---|---|
| [U-$^{14}$C]sucrose or D-[U-$^{14}$C]glucose | 0·5 |
| 6,6′-[$^{3}$H]sucrose or D-[1-$^{3}$H]glucose | 0·8 |
| D-[6-$^{3}$H]fructose | 0·3 |
| D-[1-$^{3}$H]galactose | 1·2 |

These radiochemicals were obtained as sterile solutions from the Radiochemical Centre, Amersham.

In each experiment a 40 sec constant infusion was delivered into the PV, and HV blood was collected at 30 ml. min$^{-1}$ using a Watson-Marlow roller pump. This blood was split into timed samples by a Hooke & Tucker A 40 sample changer modified to collect samples at 1/sec, both infusion and sampling being monitored on a two-channel oscillograph (Devices M 2, chart speed 10 mm sec$^{-1}$).

The procedure of infusion with sample collection was repeated within an experiment, 20–40 min elapsing between each 'run'.

*Measurement of isotopic activity*

0·2 ml. of each sample (duplicated for low activity samples) was washed into 0·5 ml. 8% (w/v) perchloric acid with 0·9 ml. 0·154 M-NaCl using a Hooke & Tucker autodiluter, mixed, centrifuged and 0·5 ml. of the supernatant pipetted into 9·5 ml. liquid scintillant (1 volume Triton X-100, Rotoan & Haas Ltd: 2 volumes xylene, Fisons SLR, containing 5·4 g.l.$^{-1}$ diphenyloxazide, Koch-Light Laboratories Ltd). The $^{14}C$ and $^{3}H$ activities were counted in a Packard Tri-Carb Model 3375 Liquid Scintillation Spectrometer with external standardization.

*Computation of uptake*

The $^{14}C$ and $^{3}H$ counts were converted to dpm.ml.$^{-1}$ by reference to the external standard ratios of the sample and of a series of variously quenched $^{14}C$ and $^{3}H$ standards of known dpm.

The reference substance, sucrose, does not enter liver cells but behaves in a closely similar manner to the monosaccharides within the extracellular space (Goresky & Bach, 1970). The sucrose dilution is used to estimate the hepatic blood flow (HBF) and also as a reference point for estimating the proportion of the monosaccharide which leaves the extracellular space. Having measured the net activity of the reference indicator ($A_r$) and of the test indicator ($A_t$) in the infusate and in each HV sample, the ratio $A_t/A_r$ in the infusate ($R_i$) and in the output HV blood ($R_o$) was calculated. The extraction $E$ of the test substance could then be calculated for each sample in turn:

$$E = 1-(R_o/R_i).$$

On the assumption of adequate mixing of the infusion in the total inflow,

$$\text{HBF, l.min}^{-1} = \frac{\text{sucrose infusion rate, dpm.min}^{-1}}{\text{mean plateau sucrose, dpm.l}^{-1}}.$$

In the case of D-glucose, the inward flux into liver cells could be estimated from the earliest measurable extraction ($E_o$), the HBF and the total blood glucose level. The total glucose concentration of HV and PV samples were estimated for some experiments by a glucose oxidase autoanalyser method modified from that of Trinder (1969). We are indebted to Dr G. Walker and the Pathology Laboratory of the Nottingham General Hospital for the performance of these measurements.

*Erythrocyte penetration by indicators*

Aliquots of [$^{3}H$]sucrose, [$^{3}H$]D-glucose or [$^{14}C$]D-glucose, [$^{3}H$]D-galactose, [$^{3}H$]D-fructose, [$^{14}C$]inulin or tritiated water were added to a series of samples of heparinized sheep blood and incubated for 40 min in a shaking water bath at 37° C. The proportion of added radioactivity present in plasma after incubation ($F_a$) was calculated from the ratio of plasma activity ($A_p$) after centrifugation, to the whole blood activity ($A_w$) before centrifugation and corrected for the haematocrit $H$, namely

$$F_a = \frac{A_p}{A_w} \times \frac{100-H}{100}.$$

If none of the label penetrated the erythrocyte, the value of $F_a$ would be unity. The final haematocrit was of the order of 25% in these experiments, and no correction for trapped plasma was attempted (Table 1).

TABLE 1. Fractions of labelled substances remaining in sheep plasma after incubation with whole blood

| Labelled substance | $n$ | $F_a$ Mean | $F_a$ S.D. | Significance of difference from sucrose ($t$ test) |
|---|---|---|---|---|
| Sucrose | 4 | 0·98 | 0·02 | |
| Inulin | 8 | 1·00 | 0·02 | $P > 0·1$ |
| D-glucose | 6 | 0·96 | 0·01 | $P > 0·1$ |
| D-fructose | 4 | 0·97 | 0·03 | $P > 0·1$ |
| D-glactose | 4 | 0·95 | 0·03 | $P > 0·05$ |
| Water | 4 | 0·76 | 0·03 | $P < 0·001$ |

*Identity of the label found in HV samples*

Water from pooled deproteinized blood drawn from the HV in the period 15–28 and 29–42 sec after the onset of infusion of [$^3$H]D-glucose into the PV was trapped at low temperature and its $^3$H activity compared with that recoverable from the infusate. The early samples were significantly *depleted* of the [$^3$H]water activity present in the infused material. No activity attributable to metabolism of the glucose label was detected within 42 sec of the onset of the infusion. All the data of this report pertain to the period 10–30 sec after the onset of the infusion.

TABLE 2. Percentage of $^3$H activity recoverable as water

| Source | $n$ | Recovered (%) Mean | Recovered (%) S.D. | Significance of differences from infusate ($t$ test) |
|---|---|---|---|---|
| Infusate | 6 | 4·2 | 0·6 | |
| HV (15–28 sec) | 4 | 2·5 | 0·7 | $P < 0·05$ |
| HV (29–42 sec) | 6 | 3·5 | 0·8 | $P > 0·1$ |

A search was made by paper chromatography of similar blood extracts for evidence of the appearance of labelled lactate or pyruvate in HV blood 10 and 30 sec after the beginning of PV infusion of labelled glucose. Even 1 % of the residual activity would have been detectable at the $R_F$ of lactate or pyruvate. None was detected.

## RESULTS

### *Blood glucose levels*

The total concentrations of glucose in the PV and HV blood were determined on seventeen occasions in seven sheep before experiments were begun. The results are shown in Table 3.

TABLE 3. Blood glucose levels in the PV and HV of feeding sheep

| Site | $n$ | Mean (mM) | S.D. | Paired $t$ test |
|---|---|---|---|---|
| PV | 17 | 2·42 | 0·51 | |
| HV | 17 | 2·77 | 0·57 | |
| Difference | 17 | +0·35 | 0·28 | $P < 0·001$ |

Table 3 shows that even during feeding there was a consistent output of glucose by the sheep liver. During each of the experiments an estimate of HBF was made. For twenty-four runs in nine sheep the HBF estimate was $2{\cdot}4\,l.min^{-1} \pm 0{\cdot}8$ (S.D.). These values of glucose concentration and HBF mean that glucose was being delivered to the livers of these sheep at $56{\cdot}1$ m-mole.$min^{-1} \pm 14{\cdot}4$ (S.D.) and leaving the liver at $62{\cdot}2$ m-mole.$min^{-1}$, implying that gluconeogenesis was proceeding at $6{\cdot}5$ m-mole.$min^{-1}$. These are expressed per sheep more readily than 'standardized' to a body weight or liver weight basis. The latter weights were not available at the time of experiment, while the former contained an unmeasured but progressive contribution by fat deposition and fleece growth. The rate of gluconeogenesis calculated on the liver weights ruling at autopsy in four of the sheep would have been 12 $\mu$mole.$min^{-1}.g^{-1}$ (wet wt.).

*Entry of a cellular compartment by indicator monosaccharides*

*Glucose* extraction, $E$, plotted as percentage in one experiment is shown in Fig. 2. The uptake rises to a peak between 1 and 5 sec after the first appearance of the sucrose label, that is, about 11–16 sec after onset of the infusion. The ensuing decline in extraction of glucose is attributable to efflux of labelled material, and attention is here concentrated upon the initial extraction ($E_0$) estimated in the period 1–5 sec after sucrose appearance, before the efflux of label from the cell can have become notable. In twenty-four runs using nine sheep the $E_0$ for glucose was $81{\cdot}1 \pm 10{\cdot}0$ (S.D.). This implies a hepatocellular entry rate for glucose, on the basis described above, of some 83 $\mu$mole.$min^{-1}.g^{-1}$. It was immaterial whether the $^3$H-label was on glucose and the $^{14}$C-label on sucrose or vice versa. When runs of identical plan were repeated in the same animal on the same day, with about 40 min between runs, six or eight estimates of $E_0$ were made for each run in each of four animals; in no instance was there a significant difference between the paired mean $E_0$ values. If, however, runs were performed on the same animal but separated by long intervals of time, significant differences were sometimes found. Two of the six animals studied showed such differences at intervals of 42 and 99 days respectively. The practice was therefore adopted of basing comparisons, for such purposes as studying competitive effects, on runs performed within the same period of 2 hr in the same animal.

*Galactose* mean $E_0$ values were determined in ten runs on five sheep which yielded an over-all mean of $73{\cdot}6 \pm 5{\cdot}1\,\%$ (S.D.). This was consistently lower than that of glucose in the same animal in eight experiments on four animals (Fig. 2; Table 4).

*Fructose* mean $E_0$ values were determined in seven runs on four sheep. The mean of $32{\cdot}6 \pm 3{\cdot}4\,\%$ (S.D.) was significantly below (Fig. 2) the means found for glucose and galactose ($P < 0{\cdot}001$). Paired runs in the same animal showed the degree of repeatability reported for the D-glucose experiments.

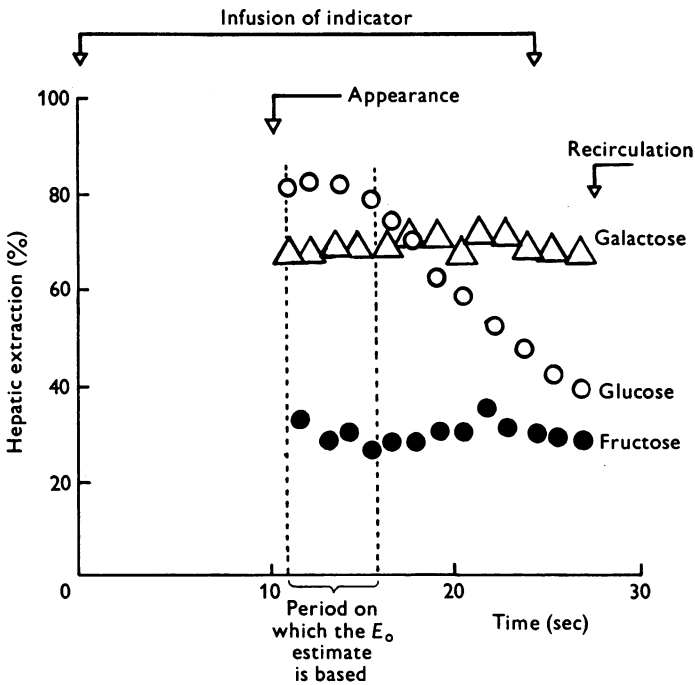


Fig. 2. The hepatic extraction measured from 11 to 28 sec after the start of the infusion in three runs for three different hexoses in one experiment in a single animal. Glucose extraction (○) was maximal in the first 5 sec of this period ($E_0$), and subsequently declined progressively. The trace inputs of galactose and fructose gave approximately constant extractions throughout this period.

L-*glucose* was used as a test substance in four runs. The mean $E_0$ was $-0{\cdot}9\,\% \pm 3{\cdot}4$ (S.D.). This was significantly below that of any other monosaccharide measured, and was effectively zero.

The relationship between the $E_0$ values for D-glucose, D-galactose and D-fructose is indicated by the experiment shown in Fig. 2, in which one run with each monosaccharide in the same sheep is shown. The absence of apparent efflux of galactose and fructose is presumed to reflect the fact that these are presented at trace levels which are capable of being almost completely sequestered within the cell, whereas glucose is already present at 2·8 mM and has the large fluxes mentioned above.

TABLE 4. Galactose and glucose $E_0$ values in paired runs

| Sheep no. | Glucose $E_0$ (%) | Galactose $E_0$ (%) | Difference | |
|---|---|---|---|---|
| 1391 | 86·1 | 73·4 | −12·1 | |
| | 83·8 | 73·7 | −10·1 | |
| Mean | 84·9 | 73·6 | | −11·3 |
| 1307 | 78·9 | 74·3 | −4·6 | |
| | 76·3 | 67·1 | −9·2 | |
| Mean | 77·6 | 70·7 | | −6·9 |
| 690 | 75·8 | 74·9 | −1·8 | |
| | 77·6 | 72·2 | −5·4 | |
| Mean | 76·6 | 73·1 | | −3·6 |
| 1767 | 77·1 | 70·8 | −6·3 | |
| | 77·8 | 75·5 | −2·3 | |
| Mean | 77·5 | 73·2 | | −4·3 |

Treated as eight separate pairs: $t = 4{\cdot}82$, $0{\cdot}01 > P > 0{\cdot}001$
D.F. = 7
Treated as four paired means: $t = 3{\cdot}74$, $0{\cdot}05 > P > 0{\cdot}02$
D.F. = 3

*Evidence of competition for entry amongst monosaccharides*

A series of experiments was performed to examine the saturability of the hepatocellular uptake process. An unlabelled monosaccharide of the same species as the labelled monosaccharide was infused into the PV at 6·5 m-mole.min$^{-1}$ for 1 min before and for the 40 sec duration of the indicator infusion given in the second of an otherwise matched pair of runs.

In the case of D-glucose, the HV concentration was approximately doubled to $6{\cdot}8 \pm 0{\cdot}59$ mM (S.D., $n = 5$) measured in samples collected 40 sec after the onset of the infusion of unlabelled glucose. This increase did not significantly alter the D-glucose $E_0$ in paired runs compared by a $t$ test, there being six estimates of $E_0$ on each run ($P < 0{\cdot}1$). Larger competitor concentrations have not been studied because of the difficulties of interpreting results of this type of experiment in the presence of osmotic transients.

When unlabelled galactose was infused at 6·5 m-mole.min$^{-1}$ before and during a labelled galactose infusion, the $E_0$ was significantly ($P < 0{\cdot}001$) depressed from $77{\cdot}5 \pm 2{\cdot}4\%$ (S.D., $n = 3$) to $64{\cdot}5 \pm 2{\cdot}6\%$. Unlabelled fructose infusion in the same way depressed significantly ($P < 0{\cdot}01$) the fructose $E_0$ from 32·1 to 27·1 %, the comparison in this case being between six paired estimates of $E_0$ in 2 runs in a single animal.

When unlabelled glucose was infused as described above in the second of two runs in which D-galactose was the labelled substrate, the galactose

$E_0$ was significantly depressed ($P < 0{\cdot}01$) from $75{\cdot}5 \pm 3{\cdot}4\%$ (S.D., $n = 8$) to $70{\cdot}8 \pm 2{\cdot}8$. This depression obtained throughout the 18 second period in which the galactose extraction could be estimated before sucrose recirculation occurred. When data from two such pairs of runs over 18 sec is compared, the D-galactose $E_0$ was also significantly depressed ($P < 0{\cdot}001$) by a similar infusion of methyl-D-glucoside (Me-α-DG), from $77{\cdot}5 \pm 2{\cdot}4\%$ (S.D., $n = 16$) to $73{\cdot}0 \pm 2{\cdot}0$. This difference was not large enough to reach significant levels when only the data from the period 1–5 sec after sucrose appearance was considered.

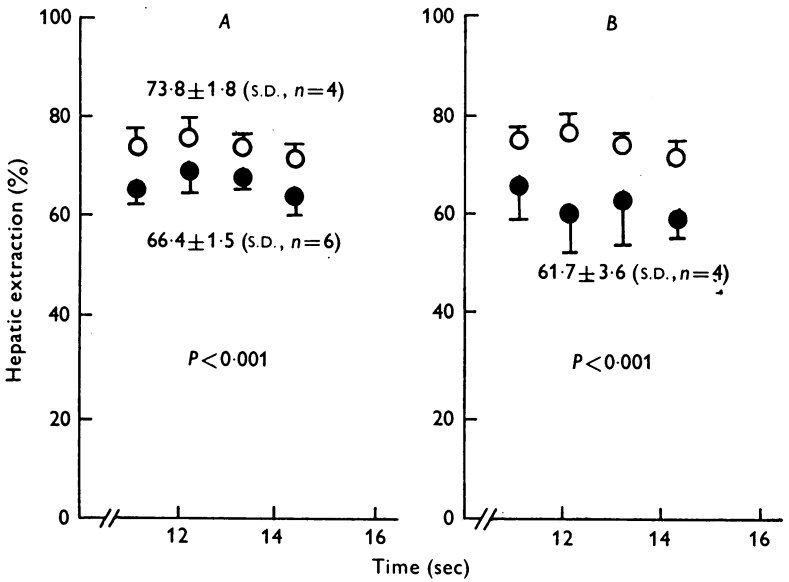


Fig. 3. Glucose extraction measured in the period 1–5 sec after sucrose appearance in three runs of the same experiment. *A*, Me-α-D-glucoside infusion at 6·5 m-mole.min$^{-1}$ significantly reduced glucose extraction (●) compared with the saline infused control (○). *B*, sucrose infusion at a similar rate produced an even more marked reduction. Sheep 1800.

A similar infusion of Me-α-DG at 6·5 m-mole.min$^{-1}$ significantly lowered the $E_0$ of D-glucose (Fig. 3*A*) from $73{\cdot}8 \pm 1{\cdot}8\%$ measured in the 1–5 sec period to $66{\cdot}4 \pm 1{\cdot}5$ ($P < 0{\cdot}001$, $n = 4$). The D-glucose $E_0$ was also lowered by an infusion of unlabelled sucrose (Fig. 3*B*) given in a third run of the same experiments to a value of $61{\cdot}7 \pm 3{\cdot}6\%$ ($P < 0{\cdot}001$, $n = 4$). On the other hand the $E_0$ of the labelled glucose was not significantly affected in any of three pairs of runs by the infusion of 2-deoxy-D-glucose at 6·6 m-mole.min$^{-1}$ before and during the labelled D-glucose infusion.

There were sometimes notable changes in the HBF estimate between pairs of runs (Fig. 4). In these experiments there appeared to be no relation

between the flow estimate and the monosaccharide extraction estimate. In seven of ten D-glucose pairs the flow estimate changed markedly without a change of the $E_0$; in two glucose pairs neither estimate changed; and in 1 instance the $E_0$ changed markedly in the absence of a change in the flow estimate. Of five galactose pairs, one showed change in neither estimate and two changed in the flow estimate only. The remaining two galactose pairs showed a change of $E_0$ in the same direction as the flow estimate, that is, in the opposite direction to that predicted from the hypothesis that the flow is determining the extraction. The comparison of flow estimates between runs in which one of each pair received an infusion of unlabelled competitor yields a similarly diverse set of changes in which no evidence that the competitor acts by blood flow change can be discerned.

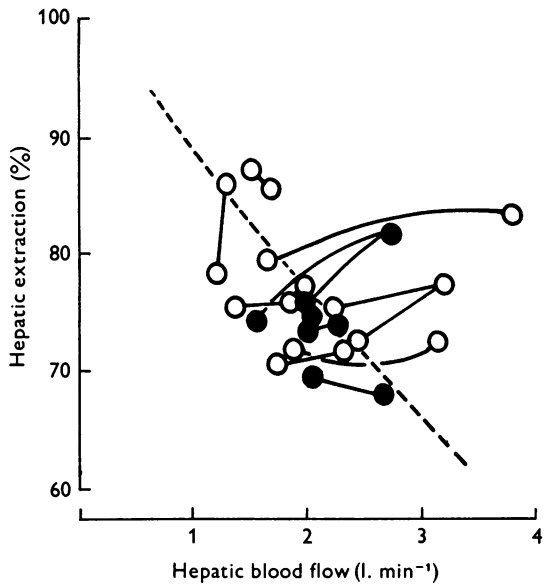


Fig. 4. The lack of dependence of hepatic extraction on the estimated hepatic blood flow. ○—○, glucose runs of the same experiment; ●—●, and galactose runs. The dotted line shows the relationship predicted by Yudilevich *et al.* (1968), see Discussion.

## DISCUSSION

The livers of these catheterized but otherwise intact sheep were in a normal condition as judged by the lack of cytological abnormality and by the fact that the estimated rate of gluconeogenies was sixfold greater than that of isolated perfused sheep livers in the hands of Linzell, Setchell & Lindsay (1971). The absence of an anaesthetic agent at the time of the

measurements may be significant since Green (1965) found measurable effects of a variety of anaesthetic agents in clinically effective concentrations on the permeability of the human erythrocyte membrane to various sugars.

The very low permeability of adult sheep erythrocytes to monosaccharides is well known (Kozawa, 1914; Widdas, 1955), and has been confirmed in this study in conditions which allow us to conclude that all the labelled monosaccharide infused into the PV remains in the plasma and available to enter the cellular compartment of the liver.

The assumption that the effluent label is a measure of the concentration of the same substance as that with which the label was associated in the infusate has been examined in the case of glucose. It was found that the label associated with glucose in the infusate was associated with glucose on leaving the liver. The fact that there is no evidence of efflux after infusion of galactose or fructose suggests that conversion of these sugars to glucose is not substantial.

The relationship between the extractions

$$\text{D-glucose} > \text{D-galactose} \gg \text{D-fructose} \ggg \text{L-glucose}$$

in a single passage through the liver was consistent and could not have been due to chance variations. The consistent effects of competitor substances could not be accounted for by chance.

The possibility that blood flow changes might have mediated the differences of extraction between substances or the reduction of $E_0$ on addition of a competitor is rejected on the basis of the contrast between the reliability of the $E_0$ estimates and the more capricious fluctuations of the blood flow estimate. The latter does, however, create a problem.

The blood flow estimates based on sucrose dilution were quantitatively similar to those reported for conscious sheep by Harrison, Linzell & Paterson (1972) and by Bergman, Brockman & Kaufman (1974) who used PAH dilution, and also by Webster & White (1973) who used thermal dilution. All these methods require adequate mixing of the indicator with the blood stream. Movement of the infusion catheter might produce variable flow estimates, or a stable but inappropriately positioned catheter might give consistently high or low estimates of flow. The present estimates show stability in the very short term (as evidenced by stable sucrose plateaux over the latter part of the 18 sec observation period) but sometimes change substantially over periods of an hour or two. It seems likely that the conscious sheep has a large but variable splanchnic blood flow.

When the flow estimates are related to the extractions of either glucose or galactose in the same animal and experimental period (Fig. 4) no consistent effect of flow on extraction is visible. This is not consistent with

the flow–extraction relationship predictable for an organ whose blood-tissue interface has constant permeability and constant surface area (Crone, 1963; Yudilevich *et al.* 1968):

$$\text{extraction } \% = 100\left[1-\exp\left(-\frac{\text{permeability}.\text{area}}{\text{blood flow}}\right)\right].$$

The glucose $E_0$ average for this group of experiments was 78 % and the HBF averaged 2·1 l.min$^{-1}$, so an appropriate figure for the permeability area product was 3·2 l.min$^{-1}$. This value, used to predict the decline of $E_0$ as HBF increases, yields the interrupted line on Fig. 4. The data does not conform to this prediction. It is concluded that either the changes in the flow estimate are artifact, or that the permeability.area product is not constant but increases with increasing HBF.

The competing substances might not have acted primarily upon the entry process but upon the efflux of monosaccharide to produce the net effects observed. However, efflux of galactose label was not observed and yet Me-α-DG reduced its extraction equally from first appearance until sucrose recirculation 18 sec later. Competitive effects are, moreover, exerted by sucrose although it does not enter the cell.

We conclude therefore that the consistent differences in extraction of the hexoses studied, and the effects of competing substances seen, are due to the existence in the plasma membrane of the hepatocytes of a mechanism mediating monosaccharide transport.

The results reveal several of the characteristics of the transfer of glucose across liver cell membranes. The first is its stereospecificity. Williams *et al.* (1968) found the transport of L-glucose in the perfused rat liver was inferior by orders of magnitude to that of D-glucose. In our experiments L-glucose did not enter hepatocytes.

The second characteristic is the saturability of the transport process. The quantities of substrate needed to demonstrate this have high osmotic activity, a problem also noted by Goresky *et al.* (1973). We elected to restrict the osmotic increments to about 5 m-osmole (kg $H_2O^{-1}$). For D-glucose itself, an increment of the blood level from 2·6 to 6·8 mM produced no significant change in the $E_0$. If the process were saturable these levels must be far short of its $K_m$.

Saturability of the transport of galactose and fructose was demonstrated. A 6·5 m-mole.min$^{-1}$ infusion of galactose was enough to reduce the $E_0$ by 13 % from 77·5 %, while a similar infusion of fructose brought the $E_0$ down from 32·1 to 27·1 %. Both these results are highly significant though based on few animals.

Competition by other substrates is evidence of mediation which is conceptually similar to that of saturability. There is a constrast between the

failure of glucose to change the glucose $E_0$ and the definite inhibition of glucose transport by similar levels of Me-α-DG and of sucrose. The lack of effect of glucose is evidence that unspecific osmotic effects cannot explain the inhibition by Me-α-DG and sucrose. Glucose concentration differences between plasma and cells take 30–40 sec to approach zero (Hooper & Short, 1975) and therefore during the first few seconds would exert osmotic effects nearly as great as those of Me-α-DG and sucrose.

Me-α-DG penetrates the cell and could conceivably provoke an additional outward glucose flux by some intracellular competition. This is unlikely in view of the lack of effect of 2-deoxy-D-glucose on glucose $E_0$, even though 2-deoxy-D-glucose is a most effective intracellular competitor for glucose.

How then, is it possible to reconcile the difficulty in saturating a glucose-transporting mechanism by glucose or 2-deoxyglucose with the evidence that Me-α-DG and sucrose can occupy the same mechanism to a significant degree? The hypothesis that presents itself to us is that occupied or unoccupied glucose-binding sites diffuse between the membrane and the extracellular space. The successful competitors sucrose and Me-α-DG not only compete for the binding site but retard it or trap it in the membrane. Glucose, being released to the cell interior, frees the site to facilitate further transport.

Glucose increments of a size which did not change glucose $E_0$ nevertheless significantly diminished the $E_0$ for galactose. No outward flux of galactose label was provoked, so entry competition took place. Though the galactose $E_0$ is almost as high as that of glucose, it would not fit the facts that galactose is transported on the whole population of sites accessible to glucose. The latter were very hard to saturate for glucose but much affected by Me-α-DG, while galactose transport is distinctly diminished by raised glucose levels but only moderately by Me-α-DG. Our hypothesis is that in addition to a relatively large population of sites which transport glucose but not galactose, there is a smaller population of lower '$K_m$' which transports both, and on which Me-α-DG is a less successful competitor.

Much experimental work remains to be done on fructose transport, but it is worthy of note that this sugar exists in aqueous solution at 36° C in pyranose and furanose forms, in the ratio of about 60:40 (Allerhand & Doddrell, 1971). It is tempting to conjecture that these forms may differ greatly in their affinity for a monosaccharide carrier, and that this is the reason for the markedly inferior extraction of fructose in a single transit of the liver.

We conclude that the sheep hepatocyte plasma membrane possesses apparatus which facilitates the diffusion of sugars in a fashion readily

explicable by the Widdas (1952) carrier hypothesis. The carrier population would appear to be particularly dense, and probably inhomogenous, but it would appear to be qualitatively similar to that of the human erythrocyte which has been so extensively studied.

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