J. Physiol. (1970), **208**, *pp.* 187–201
With 4 text-figures
Printed in Great Britain

OUABAIN-SENSITIVE CARRIER-MEDIATED TRANSPORT OF GLUCOSE FROM THE CEREBRAL VENTRICLES TO SURROUNDING TISSUES IN THE CAT

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(*Received 8 October 1969*)

SUMMARY

1. Artificial cerebrospinal fluid containing isotopically labelled sugars was perfused from the lateral cerebral ventricles to an effluent catheter inserted into the cerebral aqueduct of anaesthetized cats. This system was used for a quantitative study of the absorption of the sugars during steady state.

2. A saturable mechanism was involved in the absorption of [U-^{14}C]D-glucose and [^{14}C]D-galactose. Absorption of [U-^{14}C]D-glucose in the dead animal was similar to that of [^{3}H]D-mannitol.

3. 5×10^{-5} M ouabain in the inflow reduced cerebrospinal fluid formation and the unidirectional fluxes of glucose from the ventricles into brain tissue and plasma. Ouabain did not alter the absorption of [^{3}H]D-mannitol.

4. Three types of unidirectional fluxes of glucose from the cerebral ventricles were separated. One was ouabain-sensitive and followed Michaelis–Menten kinetics. The second was insensitive to ouabain and the third occurred by simple diffusion.

5. At normal ventricular glucose concentrations (3·5 mM) the three fluxes comprised (roughly): 25% (ouabain-sensitive), 35% (ouabain-insensitive) and 40% (simple diffusion) of total, unidirectional transport.

INTRODUCTION

The transport of glucose through the membranes separating the cerebrospinal fluid (c.s.f.) from blood and brain has recently been investigated in the rabbit by Bradbury & Davson (1964) and in the dog by Fishman (1964). A carrier mechanism was involved in the transport in both instances. The quantitative role of simple diffusion has not been determined; but in the dog the rate of carrier-mediated transport (facilitated diffusion) of glucose usually seemed to be greater than the rate of simple diffusion, both in the direction from blood to c.s.f. and from the sub-arachnoid space to venous blood (Fishman, 1964).

Only detailed studies of glucose transport between the c.s.f. and surrounding tissues can explain why the normal c.s.f./plasma glucose ratio is less than unity. It was therefore of interest to study the absorption of glucose from the ventricular c.s.f. and to see how important simple diffusion is at this site.

In a following section are presented the results from a quantitative study of the absorption of glucose and isotopically labelled sugars from the ventriculo–aqueductal perfusion system in anaesthetized cats. Concurrently, the possibility that glucose exchange might be in some way linked to active transport of cations was investigated.

In order to exclude the subarachnoid space from the investigations, the perfusion fluid was collected from the cerebral aqueduct (Bhattacharya & Feldberg, 1958). This technique offers an advantage over the ventriculo–cisternal perfusion technique in that the brain stem will not be directly exposed to toxic substances introduced into the cerebral ventricles, e.g. ouabain, which is toxic to the respiration centre (Vates, Bonting & Oppelt, 1964).

METHODS

Animal preparation. Adult cats weighing between 3·0 and 5·0 kg were anaesthetized with chloralose, 70 mg/kg I.P. When necessary, small amounts of sodium pentobarbitone (Nembutal) were later given I.P. Tracheotomy with intubation was carried out and a polyethylene catheter was introduced through a femoral artery into the abdominal aorta. The catheter served for blood sampling and for monitoring of mean arterial blood pressure by means of a mercury manometer. Rectal temperature was maintained at 37·5–38·5° C.

Ventriculo–aqueductal perfusion. Steady-state perfusions were carried out in thirty-four cats with an artificial c.s.f. (Merlis, 1940) containing D-glucose or other sugars as described in Results and in addition [U-^{14}C]D-glucose, [U-^{14}C]sucrose or [^{14}C]D-galactose. In some experiments [^{3}H]D-mannitol was added. The solution was equilibrated with 5% carbon dioxide to give a final pH of $7·3 \pm 0·05$. Two inflow cannulae (0·35 mm o.d.) were placed, one in each lateral ventricle (Davson, Kleeman & Levin, 1962; Oldendorf & Davson, 1967) and a polyethylene catheter was inserted into the cerebral aqueduct (Bhattacharya & Feldberg, 1958; Feldberg & Fleischhauer, 1960). The rate of perfusion was 110 μl./min (S.D. $\pm$ 0·8 μl./min; $n = 72$) and one half of this flow was diverted to each of the lateral ventricles.

The effluent was collected during successive periods of 15 min and effluent volumes were determined by weighing to the nearest 0·1 mg. Only effluent samples which were clear and free from blood were used for analyses. Throughout the experiment the rate of respiration, pulse rate, reflexes, etc., were controlled. Materials and instruments were sterilized before coming into contact with tissues or fluids containing glucose.

Analytical. Samples of arterial blood were taken every half hour, and glucose was determined in plasma (in some cases also in whole blood) and in effluent samples using a glucose oxidase method (Hjelm & de Verdier, 1963). Mean recovery of glucose added to effluent samples was 99·2% (S.D. $\pm$ 1·0; $n = 6$), and of glucose added to plasma, 100·0% (S.D. $\pm$ 1·7; $n = 7$). Twenty determinations of plasma glucose from the same sample gave an average figure of 14·1 mM (S.D. $\pm$ 0·27 mM).

^{14}C activity in the effluent was determined using a Packard Tri-Carb liquid scintillation system. A minimum of 50,000 counts were registered. Simultaneous counting of [^{14}C]glucose and [^{3}H]mannitol was carried out in two channels together with standard solutions and with appropriate gain and window settings. A minimum of 35,000 counts was registered.

Less than 0·5 % of total ^{14}C activity in the effluent was due to [^{14}C]carbon dioxide (determined by the method of Clausen, 1966). [^{14}C]lactate was separated from [^{14}C]-glucose by paper chromatography (descending) using the solvent system described by Goodner (1964); lactate was localized using lactic acid dehydrogenase and diphosphopyridine nucleotide, and [^{14}C]glucose by liquid scintillation counting; no ^{14}C activity was found to indicate [^{14}C]lactate. Therefore, the measured ^{14}C activity in the effluent was taken as representative of [^{14}C]glucose. Sodium and potassium in the effluent was determined using an Eppendorf flame photometer.

Computations. Determination of the fluxes of glucose between the cerebral ventricles and surrounding tissues during steady state were based upon principles described elsewhere (Pappenheimer, Heisey & Jordan, 1961; Bradbury & Davson, 1964; Pappenheimer, Fencl, Heisey & Held, 1965). In the present experimental arrangement the rate of net formation of c.s.f. was equal to the rate of outflow from the aqueductal catheter (F_o ml./min) minus the rate of inflow (F_i ml./min) (see Results).

The unidirectional flux of [^{14}C]glucose or of other labelled substances from the ventricles into plasma and brain ($J^*_{v,pb}$ (counts/min) per minute) was computed as the rate of inflow of tracer minus the rate of outflow of tracer:

$$J^*_{v,pb} = C^*_i F_i - C^*_o F_o, \tag{1}$$

where C^*_i and C^*_o represent activity ((counts/min) per millilitre) in outflow and inflow respectively.

The unidirectional flux of glucose from the ventricles ($J_{v,pb}$ μ-mole/min) was derived from the following equation:

$$J_{v,pb}/\bar{C}_v = J^*_{v,pb}/\bar{C}^*_v, \tag{2}$$

where $\bar{C}_v$ (mM) is the mean ventricular concentration of glucose; the arithmetical mean of the concentrations in the inflow (C_i mM) and in the outflow (C_o mM) was used (Bradbury & Davson, 1964). $\bar{C}^*_v$ is the mean ventricular concentration of tracer ((counts/min) per millilitre); assuming an exponential decrease of concentration within the system, $\bar{C}^*_v = 0{\cdot}37C^*_i + 0{\cdot}63C^*_o$ (Pappenheimer *et al.* 1961).

The net flux of glucose between the ventricles and surrounding tissues (N μ-mole/min) was determined as the rate of outflow of glucose minus the rate of inflow of glucose:

$$N = C_o F_o - C_i F_i. \tag{3}$$

Net fluxes were called positive if they were directed from the tissues into the ventricles.

The unidirectional flux of glucose from blood and brain ($J_{pb,v}$ μ-mole/min) was determined as the sum of N and $J_{v,pb}$.

RESULTS

Rate of c.s.f. formation. The rate of net c.s.f. formation within the two lateral and the third cerebral ventricles was determined as the difference between the rate of outflow and the rate of inflow ($F_o - F_i$). This gave an average figure of 9·4 μl./min (S.E. $\pm$ 4·0 μl./min; n = 17). Another method

often used in ventriculo–cisternal perfusion experiments is the application of the inulin dilution technique. Instead of inulin, sucrose may be used, as this too is considered to be confined to extracellular spaces and like inulin diffuses from perfusate into surrounding tissues to a slight degree only (Davson *et al.* 1962; Welch & Sadler, 1966). So, in order to compare the dilution technique with the method used in the following experiments, [^{14}C]sucrose was perfused through the ventricular system together with carrier sucrose for 3–4 hr. In three experiments the recovery of [^{14}C]sucrose from the aqueductal catheter during steady state was 99·1, 96·7 and 98·4 %. The following expression for calculation of the rate of c.s.f. formation was applied, $F_i(C_i^* - C_o^*)/C_o^*$ (Heisey, Held & Pappenheimer, 1962). Here, a correction was made to C_o^* for the measured loss of activity to surrounding tissues. The results obtained by this method did not differ by more than 1 % from the simultaneously determined difference, $F_o - F_i$. This is in agreement with the reports by Pollay & Davson (1963), Bradbury & Davson (1964) and by Hochwald & Wallenstein (1967) and it further demonstrates that no appreciable loss of substances occurred along unwanted routes, e.g. needle tracks or around the aqueductal catheter.

Steady state. It was assumed that steady state was attained when the ^{14}C activity in successive 15-min effluent samples (C_o^*) remained constant. This was usually the case after 45–60 min, and the ^{14}C activity in the following samples varied in the majority of the experiments within 2·8 %.

Ouabain intraventricularly. After a steady-state control period of 45–120 min, ouabain was added in a small volume to the inflow perfusion fluid so as to give a final concentration of 5×10^{-5} M. The effects of ouabain were irreversible and they were similar, whether it was added early or late during the perfusions. No effects of ouabain were observed upon the rate of respiration, pulse rate, arterial blood pressure, reflexes, etc. Electrocardiograms recorded during some of these ouabain periods remained normal for up to 3·5 hr until the sacrifice of the animals. No signs of oedema were observed by post mortem examination of the opened brain.

Ouabain reduced the rate of c.s.f. formation to 1·5 μl./min (S.E. $\pm$ 3·1 μl./min; n = 17), corresponding to an average reduction of 84 %. During many sampling periods the c.s.f. formation was reduced to zero and in a few periods it became even slightly negative.

Ouabain caused an increase of [^{14}C]glucose in the effluent. At low concentrations of glucose within the system ($\bar{C}_v$ around 2 mM) the increase was 16·1 % as compared to the values during control periods and at high concentrations ($\bar{C}_v$ around 50 mM) the increase was only 6·4 %. The specific activity of glucose in the effluent during control periods varied between

25 % of the inflow values at low glucose concentrations and 95 % at high concentrations. Ouabain caused a small increase of these figures.

After 2–5 hr of perfusion both of the unidirectional fluxes of glucose often began to increase. This was also found in several preliminary experiments in which ouabain was not used, so this increase in permeability could not be attributed to an effect of ouabain (periods with increased permeability are not included in the calculations).

Absorption of [^{14}C]glucose. This was not proportional to the mean concentration of glucose within the cerebral ventricles ($\bar{C}_v$ mM), thus indicating that Fick's law of diffusion was not obeyed. This is seen in Table 1, where the absorption during steady state is given as % of the amount of activity entering the inflow cannulae ($100 \times (C_i^* F_i - C_o^* F_o)/C_i^* F_i$). The % absorption decreased with increasing concentrations of glucose, thus indicating a mediated transfer of glucose. Table 1 also shows the reduced absorption of [^{14}C]glucose when ouabain was added to the inflow later during the experiments.

TABLE 1. Simultaneous rate of absorption of [U-^{14}C]D-glucose and of [^{3}H]D-mannitol before ouabain perfusion (control period) and during 5×10^{-5} M ouabain in the inflow and the absorption of [^{14}C]glucose in the dead animal, presented as % of rate of inflow. $\bar{C}_v$ mM is the mean ventricular concentration of glucose. In experiments with [^{3}H]mannitol there was an inflow concentration of D-mannitol of 5·6 mM

Control period (45–180 min)			Ouabain period (45–180 min)			Dead animal (30–45 min)	
$\bar{C}_v$ mM	% ^{14}C	% ^{3}H	$\bar{C}_v$ mM	% ^{14}C	% ^{3}H	$\bar{C}_v$ mM	% ^{14}C
2·5	19·2	5·2	2·5	13·5	6·2	—	—
2·8	18·9	4·7	2·7	16·0	4·6	—	—
4·4	18·8	—	—	—	—	3·5	3·0
6·5	17·4	2·9	6·5	13·6	2·7	—	—
6·5	17·8	5·4	—	—	—	—	—
11·7	15·5	2·0	11·8	12·0	2·4	—	—
21·0	14·2	—	—	—	—	21·4	4·8
30·9	13·7	4·2	32·3	11·9	5·2	—	—
31·3	12·2	5·3	33·2	10·1	5·8	—	—
50·8	14·0	—	—	—	—	54·7	2·4
Mean		4·2			4·5		3·4

Absorption of [^{3}H]mannitol. The relative rates of passive diffusion of [^{14}C]glucose from the perfusate out through the ventricular walls were determined by adding [^{3}H]D-mannitol and D-mannitol as a carrier (final concentration in inflow solution, 5·6 mM). The mannitol did not influence the fluxes of glucose or the rate of c.s.f. formation. Mannitol resembles glucose closely in molecular weight and in configuration and both of them are highly polar substances. Furthermore, as mannitol has never been seen

attached to specialized transport systems, it could be used as a measure of glucose diffusion. From Table 1 it can be seen that the absorption of [^{3}H]mannitol was independent of the concentration of glucose within the cerebral ventricles as well as of the addition of ouabain. This last finding indicates that the membranes under investigation remained intact during ouabain perfusion, at least as far as the diffusional pathways for mannitol were concerned. From Table 1 it can further be deduced that part of the glucose transfer was neither due to simple diffusion nor sensitive to ouabain.

Dead animal experiments. Another approach to finding the rate of passive diffusion was the determination of [^{14}C]glucose absorption in the dead animal. The animals were killed by an overdose of sodium pentobarbitone given I.P. and the perfusion was continued. Within the first 30–45 min the flux of glucose from blood and brain and the c.s.f. formation approached zero, while the absorption of [^{14}C]glucose continued at a reduced rate. The average absorption values for [^{14}C]glucose are seen in Table 1. The values are from 30–45 min after the animals were killed and onwards, i.e. when a new steady state was achieved. It can be seen that the absorption of [^{14}C]glucose proceeded independently of the concentrations of glucose within the cerebral ventricles and at rates similar to mannitol diffusion in the intact system.

TABLE 2. Absorption of [^{14}C]D-galactose (similar calculations as for Table 1). Three different inflow concentrations of D-galactose were used. Each absorption value is the mean of three experiments. Mean ventricular concentrations of glucose were within 3·5–4·8 mM

Inflow concn. (mM)	% absorbed [^{14}C]galactose
0	10·7
5·6	9·4
11·1	6·1

Absorption of [^{14}C]galactose. The absorption of another monosaccharide with the same molecular weight as glucose was studied at a constant concentration of glucose within the cerebral ventricles. D-galactose was chosen because it is very poorly metabolized in cerebral tissues (Maddock, Hawkins & Holmes, 1939). It was perfused through the system in different concentrations together with [^{14}C]D-galactose. The results from nine experiments are shown in Table 2. Two to eight 15-min sampling periods during steady state were used. The % absorption decreased with an increase in the inflow concentration of galactose. The transfer mechanism thereby exhibited a similar pattern to that of glucose (Table 1). But with galactose the rate of absorption was only about half the rate of absorption of glucose.

Ouabain and unidirectional fluxes of glucose. An example of the effects of ouabain upon the fluxes of glucose is seen in Fig. 1. Both of the unidirectional fluxes were reduced. The effects were frequently seen during the first 15-min sampling period and they increased during the following one or two periods. The unidirectional flux from the cerebral ventricles to blood plasma and brain ($J_{v,\,pb}$) was thereby reduced to 60–85% of the steady-state values during control periods. Similarly, reduction of the unidirectional flux in the opposite direction ($J_{pb,v}$) varied between 46 and

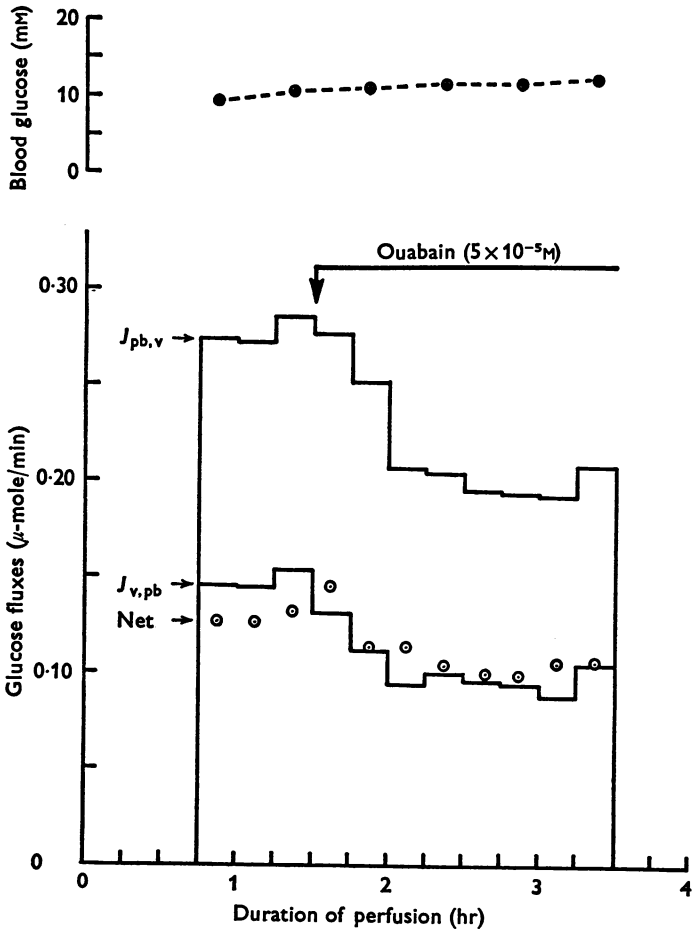

Fig. 1. Reduction of unidirectional flux of glucose from plasma and brain into the two lateral and the third cerebral ventricles ($J_{pb,v}$) and of the unidirectional flux during steady state in the opposite direction ($J_{v,pb}$) by addition of ouabain to the inflow perfusion fluid. Net = net flux (⊙). Concentration of glucose within the cerebral ventricles, 4·3 mM. Above: glucose concentrations in arterial whole blood.

91 %. Also, in Fig. 1, is seen an example of a net flux of glucose between the ventricles and surrounding tissues. Evidence for transport of glucose against a concentration gradient was never found.

The blood glucose concentrations are often very high in cats during anaesthesia and surgical operations (Fig. 1). The concentrations in blood plasma varied around a mean value of 14·6 mM (S.D. ± 3·0 mM; n = 17).

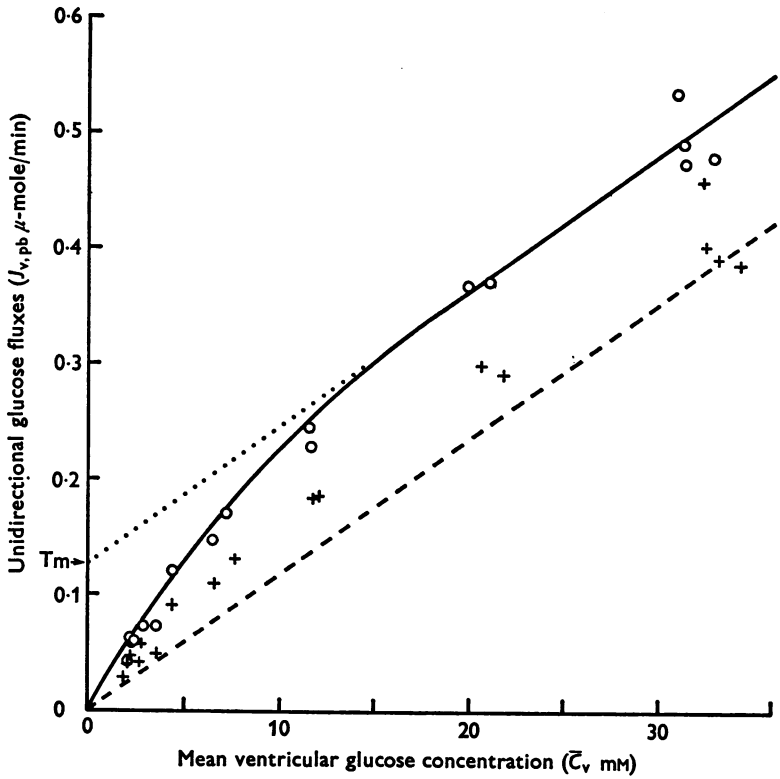

Fig. 2. Ordinate: unidirectional fluxes of glucose from the cerebral ventricles to plasma and brain ($J_{v,pb}$) during control periods (○) (upper curve), and the reduced fluxes during ouabain perfusion (+). Final concentration of ouabain in inflow perfusion fluid, 5×10^{-5} M. Abscissa: mean ventricular concentration of glucose ($\bar{C}_v$ mM). Interrupted line through zero represents simple diffusion; slope, 0·011 ml./min. Tm defines transport maximum for total unidirectional transport minus simple diffusion.

Ouabain perfusion had no effect upon these concentrations and reduction of glucose fluxes were seen at all levels of blood glucose concentrations. Neither were the blood glucose concentrations influenced by the concentrations of glucose within the cerebral ventricles. This finding confirms the observations from dogs and rabbits (Sloviter & Sakata, 1963)

and from dogs (Vuylsteke, 1949; Leusen & Demeester, 1949; Fishman, 1964).

In Fig. 2 are shown the unidirectional fluxes of glucose from the cerebral ventricles ($J_{v,\,pb}$) at different concentrations of glucose within the cerebral ventricles ($\bar{C}_v$ mM) before ouabain administration and during that part of the ouabain period where the effect was maximal. Each point represents the average value from three to twelve 15-min sampling periods during steady state in one experiment. Because of increases in blood glucose concentrations there was an average increase in $\bar{C}_v$ of 0·45 mM relative to control periods, despite the fall of the unidirectional flux from blood during

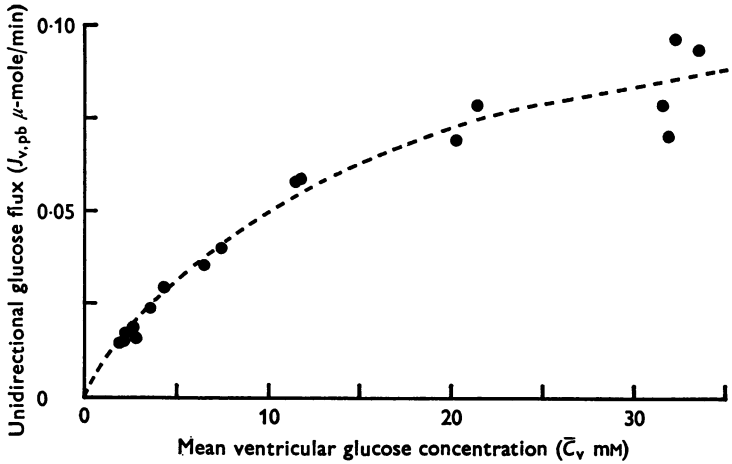

Fig. 3. Saturation of the unidirectional, ouabain-sensitive transport of glucose from the cerebral ventricles to plasma and brain ($J_{v,\,pb}$) by increasing concentrations of glucose within the cerebral ventricles ($\bar{C}_v$ mM). The points represent the values during ouabain periods subtracted from the values during control periods (Fig. 2). The interrupted line was calculated from the Michaelis–Menten equation, using the values for K_m and V as calculated from the Lineweaver–Burk equation (see Fig. 4).

ouabain perfusion. The intercept between the ordinate and linear portion of upper curve for total transport defines transport maximum (Tm) for total unidirectional transport before ouabain administration minus simple diffusion. It was approached at glucose concentrations above 10 mM. The interrupted line through zero was drawn parallel to the linear portion of the upper curve and it represents unidirectional transport of glucose by simple diffusion. The slope of the line for diffusion gives a transfer constant of 0·011 ml./min for simple, unidirectional diffusion of glucose. From this, a figure of about 5 % can be derived for simple diffusion in relation to the amount of glucose entering the inflow cannulae per minute. This figure is in good agreement with those obtained for absorption of [^{3}H]mannitol

and [^{14}C]glucose in the dead animal (Table 1). The points representing ouabain periods (Fig. 2) do not quite follow the line for diffusion, indicating that not all of the mechanism for glucose transport was inhibited by ouabain.

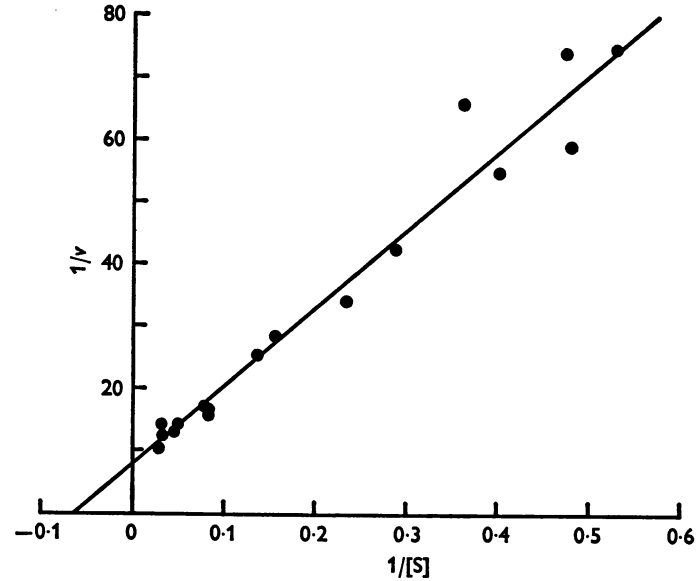

Fig. 4. Lineweaver–Burk plot, giving the reciprocals of the values depicted with the saturation curve (Fig. 3). x-axis: reciprocal of mean ventricular glucose concentration (1/[S]mM). y-axis: reciprocal of unidirectional, ouabain-sensitive transport of glucose from the cerebral ventricles ($1/v$ μ-mole/min). The regression line was computed by the method of least squares. The reciprocals of the intercept with the y-axis gives V = 0·13 μ-mole/min and of the intercept with the x-axis gives K_m = 16 mM.

Subtraction of the unidirectional flux of glucose during ouabain perfusion from the unidirectional flux in the same experiment before ouabain administration gave the ouabain-sensitive transport which is shown in Fig. 3. Here, the concentration of glucose within the cerebral ventricles was calculated as the intermediate value between the concentration before and the concentration during ouabain perfusion. The interrupted curve was calculated from the Michaelis–Menten equation, using values for V and K_m which in turn were found from the Lineweaver–Burk equation. It can be seen that the points follow this saturation curve which is characteristic of carrier-mediated transport. The Lineweaver–Burk plot (Fig. 4) was made by plotting the reciprocals of the values represented in Fig. 3. From it the values of V and K_m can be derived. K_m resembles the Michaelis–Menten constant and defines that concentration of glucose within the cerebral ventricles where the velocity of the unidirectional, ouabain-

sensitive transport of glucose from the cerebral ventricles to plasma and brain is half the maximum ($\frac{1}{2}V$).

Estimation of the relative rates of simple diffusion, ouabain-sensitive and ouabain-insensitive transport under 'normal' conditions. If the normal concentration of glucose in ventricular c.s.f. in the awake animal is taken to be around 3·5 mM and if this figure can be related to the rates of transport found in the anaesthetized animal, it can be found from the values depicted in Fig. 2 that unidirectional transport of glucose from the cerebral ventricles by simple diffusion comprises around 40% of total, unidirectional transport. From Fig. 3 it can likewise be found that unidirectional, ouabain-sensitive transport comprises around 25% of total transport. That leaves around 35% for unidirectional, ouabain-insensitive transport.

Ouabain, sodium and potassium. The concentrations of sodium and potassium in the effluent were close to the concentrations in the inflow during control periods. After ouabain administration the effluent concentrations of sodium became 1–2 μ-equiv/ml. lower and effluent concentrations of potassium became 1–2 μ-equiv/ml. higher, i.e. ouabain caused a net absorption of sodium and a net loss of potassium from the tissues. These effects were always seen during the first 15-min sampling period and suggested a more prompt action of ouabain upon the sodium–potassium pumping mechanism than upon the glucose-exchange mechanism, where the effect of ouabain was often delayed.

DISCUSSION

The main result of this study is that glucose was absorbed from fluid perfusing the first three cerebral ventricles by three different mechanisms. One showed Michaelis–Menten kinetics and proved to be inhibited by 5×10^{-5} M ouabain. The second was insensitive to ouabain and the third occurred by simple diffusion.

Certain assumptions have been involved. The physiological conditions of the tissues around a perfusion system are usually considered not to be seriously affected. Criteria such as certain brain stem reflexes are probably not sufficient to establish whether all cellular membranes under study are intact, and it is not possible to state whether they were acting normally in the present experiments. A slow increase in permeability after some hours of perfusion was also found in perfusion experiments on dogs (Cserr, 1965). However, after death of the animal, the rate of absorption of [^{14}C]glucose decreased to the point of simple diffusion. The membranes absorbed closely related sugars with a high degree of selectivity, and ouabain caused profound alterations in the fluxes of cations. Such findings could not be expected if the membranes were seriously deteriorated. The possibility

that ouabain caused a reduction in brain extracellular space (Zadunaisky, Wald & De Robertis, 1965; Woodbury, 1968) was unlikely, as diffusion of [^{3}H]mannitol was unaltered and no signs of oedema were observed. Arteriolar contractions due to ouabain can hardly explain the results. The effect upon glucose fluxes and upon arterioles does not occur simultaneously and arteriolar contractions last only for 10–15 min (Lendle & Mercker, 1961; Crone, 1966). Possible interferences by the anaesthetics were not studied.

The observation that diffusion of mannitol from the ventricles into brain was similar to that of glucose after death was unexpected. It has been suggested that cellular elements imbibe intercellular water shortly after death, as indicated by a rise in electrical impedance of the tissues (Van Harreveld & Ochs, 1956) or by the lower volume of distribution of ventricular inulin (Rall, Oppelt & Patlak, 1962) or as deduced from electron micrographs (Van Harreveld, Crowell & Malhotra, 1965). It is possible that some anaerobic glycolysis was involved.

After administration of ouabain intraventricularly, the tissues began to lose potassium and gain sodium and shortly afterwards both of the unidirectional fluxes of glucose decreased. The present investigations provide no evidence as to whether the effects of ouabain are localized to the blood–brain barrier, the blood–c.s.f. barrier, parenchymal cells or all three. Previous investigations suggest that ouabain inhibits the active uptake of potassium in the tissues, but it is not clear which sites ouabain acts upon (Bradbury & Davson, 1965; Cserr, 1965; Katzman, Graziani, Kaplan & Escriva, 1965; Davson, 1967). Eidelberg, Fishman & Hams (1967) found carrier-mediated and ouabain-sensitive transport of arabinose from blood to brain in cats. The carrier was probably shared with glucose and it was suggested that some degree of coupling between sugar and ionic transport processes might exist. Csáky & Rigor (1964) suggested a similar explanation for sugar uptake by choroid plexus *in vitro*. The effects of ouabain upon respiration of brain cortex slices are also inconclusive as fas as the perfusion system is concerned (Swanson & Ullis, 1966; Ruščák & Whittam, 1967).

The findings are in good agreement with those of Bradbury & Davson (1964) who found that the absorption of [^{14}C]glucose from the ventriculo–cisternal perfusion system in rabbits showed self-inhibition. As they pointed out, this phenomenon could be due to altered metabolism rather than altered transport. But with xylose (as with galactose, Table 2) the same phenomenon must be due to altered transport. They did not find evidence for transport of glucose against a concentration gradient. The findings of the present experiments agree with this. They furthermore found no inhibition of glucose absorption when dinitrophenol was per-

fused through the system. Absorption of glucose from the ventricles is thus in part governed by an equilibrating type of transport system.

The half-saturation constant (K_m) for ouabain-sensitive glucose absorption was 16 mM. It is within the same order of magnitude as for facilitated diffusion systems elsewhere, including erythrocytes and muscle cells (Stein, 1967).

The flux of glucose from the cerebral ventricles during ouabain perfusion was larger than could be accounted for by simple diffusion alone. The ouabain-insensitive flux comprised roughly 35 % of total flux at low concentrations of glucose within the cerebral ventricles while it almost disappeared at high concentrations. Similarly, Csáky & Rigor (1964) found that ouabain did not totally suppress the accumulation of sugars while they were taken up by choroid plexus *in vitro*. No explanation for this transport phenomenon is at hand and it is not possible to say whether it is confined to the choroid plexus alone.

The effect of ouabain upon the unidirectional flux of glucose from blood and brain varied considerably and the variation of the concentrations of blood sugar prevented an analysis of these fluxes. There was no correlation between reduction of this flux and reduction of the rate of c.s.f. formation during ouabain perfusion, and the flux proceeded even in those cases where c.s.f. formation was reduced to zero. This is in agreement with Bradbury & Davson (1964), who concluded that glucose enters from sources additional to those concerned in the secretion of c.s.f. Accordingly, acetazolamide (Diamox) intraventricularly reduces the rate of c.s.f. formation without affecting glucose fluxes (Brøndsted, 1970).

The rate of c.s.f. formation from the first three ventricles was 9·4 µl./min. It is slightly higher than that found by others who used different techniques. The following figures have been reported: 8·5 µl./min (found by conversion of the figures from Flexner & Winters, 1932); 8·89 µl./min (Vates *et al.* 1964); 6 µl./min (Graziani, Kaplan, Escriva & Katzman, 1967). The last two groups of investigators have also found reduction of c.s.f. formation when ouabain was administered intraventricularly.

The present studies have shown that unidirectional, simple diffusion of glucose from ventricular c.s.f. to surrounding tissues (probably into brain tissue) is important relative to more specific transport. Although quantitative studies of glucose transport from blood to c.s.f. are missing, it seems likely that simple diffusion out from ventricular c.s.f. is usually more important than from the subarachnoid space to surrounding tissues (Bradbury & Davson, 1964; Fishman, 1964).

The role of the fourth ventricle was not studied, but the absorption capacity at this site may be relatively low as compared with the lateral ventricles (Davson *et al.* 1962).

This investigation was supported by grants from F. L. Schmidt and Co. A/S's Jubilæumsfond and Aarhus Universitets Forskningsfond.

I am indebted to Professor J. C. Skou for support during the investigations and to Professor C. Crone for inspiring advice during the experimental work and help in preparing the manuscript.

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