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FACILITATED TRANSFER OF GLUCOSE FROM BLOOD INTO BRAIN TISSUE

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It has frequently been shown that a number of small molecules and ions pass only slowly into the brain from the blood (Manery & Bale, 1941; Katzmann & Leidermann, 1953; Davson & Spaziani, 1959). This raises the question of how glucose can pass into the brain in quantities which are sufficient to supply the energy requirements of the tissue. It was shown in earlier studies (Crone, 1965)—in which the unidirectional flux of non-electrolytes from blood to brain tissue was measured—that only 3–5% of fructose and glycerol leave the blood during one passage through the brain. In contrast, glucose, which is chemically similar, has a net extraction of about 10%.

The fact that the extraction of glucose is much higher than that of similar substances indicates that glucose might pass the brain capillaries by means of a facilitating mechanism. Earlier experiments by Klein, Hurwitz & Olsen (1946) showed that the brain contained more glucose than fructose when those two substances were injected intravenously in cats. They do not give, however, quantitative data which permit a closer characterization of the phenomenon. Geiger, Magnes, Taylor & Veralli (1954) advance the view that the uptake of glucose in the brain requires the presence in the blood of co-factors, a finding which suggests that the mechanism of the transfer of glucose might be different from simple diffusion.

The idea behind the present work was to try to estimate the unidirectional transfer of glucose from blood to brain tissue by the measurement of the net extraction of labelled glucose during the first passage through the brain. If it could be shown that there is no proportionality between the concentration of glucose in the blood and the amount of labelled glucose leaving the blood, then this would indicate that there is a mediated transfer of glucose. In other words, if the extraction of labelled glucose varied with the concentration of glucose in the blood this would signify that Fick's law of diffusion was not obeyed. This was in fact found to be the case in the experiments which are described in this paper.

THEORY

A necessary requirement for these experiments is that the method should permit the measurement of the loss of labelled glucose during the first passage of the test molecules through the brain, as once the brain has taken up radioactive glucose the labelled material may return to the blood and one can no longer measure the unidirectional movement. This means that blood leaving the brain must be sampled within seconds of the intracarotid injection of the test solution. As dilution in the blood, and loss from the blood, occur at the same time, it is necessary to allow for the dilution in order to be able to determine the loss. This means that the solution injected must contain a non-diffusible reference substance in addition to the labelled material. A single-injection technique which makes it possible to measure the initial net extraction was devised by Chinard, Vosburgh & Enns (1955) and

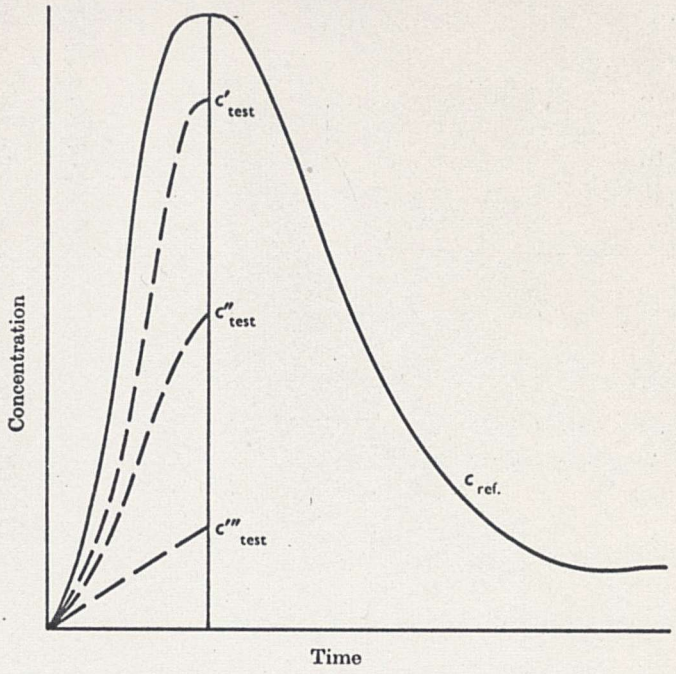

Fig. 1. Time–concentration curves in the effluent blood from an organ obtained after 'square wave' injection into the afferent vessel. $c_{ref.}$: reference substance which does not leave the capillaries. c_{test}: various diffusible test substances.

Anthonisen & Crone (1956). A detailed description of the development of a single-injection method into a method for quantitative evaluation of transcapillary passage processes has been published by Crone (1961, 1963). This 'Indicator Diffusion' method consists essentially of the intra-arterial 'square-wave injection' of two substances at the same time. One is a reference substance which cannot leave the capillaries and therefore indicates the degree of dilution in the blood. The other is the test substance, the transcapillary movement of which is to be measured. After intra-arterial injection and fractional collection from the vein draining the organ a number of blood samples are obtained which permit the construction of time–concentration curves as shown in Fig. 1. The curve for the

reference substance indicates the concentration to be expected had no loss of the test substance occurred. The greater the diffusion out of the capillaries the greater the displacement of the 'test' curves from the 'reference' curve.

The extraction (E) of a test substance is determined in each sample as $(c_{ref.}-c_{test})/c_{ref.}$. As the molecular concentrations of the reference and test substance in the injection solution are not equal, all concentrations are expressed relative to the concentrations in the injection solution. If one wishes to express the permeability of the capillaries in terms of permeability coefficients the following expression is used (Crone, 1963): $P = -(\dot{Q}/A)\ln(1-E)$, where P is the permeability coefficient ($cm.sec^{-1}$), $\dot{Q}$ is the rate of blood flow/g tissue/sec and A is the capillary surface area/g tissue. The unidirectional flux of labelled test substance is equal to $\dot{Q} \times E \times Ca$, where Ca is the arterial concentration of glucose. If it is assumed that the rate of blood flow per unit of tissue is relatively constant from animal to animal, then it is possible to determine whether or not the unidirectional flux is proportional to the glucose concentration. If, in particular, the extraction diminishes with rising blood-glucose concentration, then this implies that the flux does not rise proportionately to the intracapillary concentration, in other words Fick's law of diffusion does not apply. The conclusions in this paper, therefore, are essentially based on determinations of the initial extraction of radioactive glucose at varying levels of blood glucose concentration.

Methods

The experiments were performed on dogs anaesthetized with sodium pentobarbital (Nembutal), 25–30 mg/kg intravenously. A free air passage was ensured by means of an endotracheal tube. A small polythene cannula was introduced through the superior thyroid artery, almost into the lumen of the carotid artery, so that intracarotid injections could be given without interference with the blood flow in the carotid artery. A hole was drilled in the skull immediately above the torcula and a Perspex adapter (Rodnight & Tresize, 1957) was screwed firmly into the skull. A polythene cannula was attached to the adapter. Immediately before the experiment an incision was made in the superior longitudinal sinus so that the blood could flow freely through the cannula. Small test-tubes with dry heparin and sodium fluoride were mounted on a slowly moving chymograph (Asmussen & Nielsen, 1952) to collect the cerebral blood in small fractions—each collection lasting for 1-2 sec.

Approximately 1 ml. of a test solution was injected quickly and sampling was begun immediately afterwards. The solution contained Evans blue as a reference substance and both [^{14}C]glucose and inactive glucose. Isotonicity was ensured by addition of appropriate amounts of NaCl.

The concentration of *Evans blue* was determined spectrophotometrically on a Beckman spectrophotometer, model DU, at 620 mμ.

Fructose was determined by Bojesen's method (1952) after precipitation of the proteins with $ZnSO_4$ and NaOH.

[^{14}C]*glucose* was determined as the total radioactivity in the supernatant after precipitation of the protein. Wet combustion was carried out by the method described by van Slyke, Plazin & Weisiger (1951) and, in more detail, by Sakami (1955). No attempt was made to isolate glucose from the blood before measuring the radioactivity, as no other radioactive fractions could possibly have appeared in the plasma within the duration of an experiment. The CO_2 evolved by the combustion procedure was collected in NaOH and precipitated as $BaCO_3$ on filter paper. The dry disks were weighed and the activity expressed as the activity which would have been present in a disk weighing 16 mg. The loss of radioactivity due to self-absorption was determined on a number of samples weighing from 8 to 32 mg and appropriate corrections were made. All samples were plated and counted in duplicate. The coefficient of variation, as found by repeated analyses of the same sample containing radioactive glucose, was 2·3 %. The counting was made on Tracerlab Equipment using a

TGC-14 Carbon counter with a thin end-window. The background activity was 11 counts/min. In the majority of the samples the registered activity was more than ten times the background activity.

RESULTS

Two types of experiments were performed: (1) Experiments in which the extraction of [^{14}C]glucose was determined at different blood-sugar concentrations. (2) Experiments in which the ratio between the relative concentrations of [^{14}C]glucose and fructose in the effluent cerebral blood were determined after simultaneous injection of the two substances in the carotid artery.

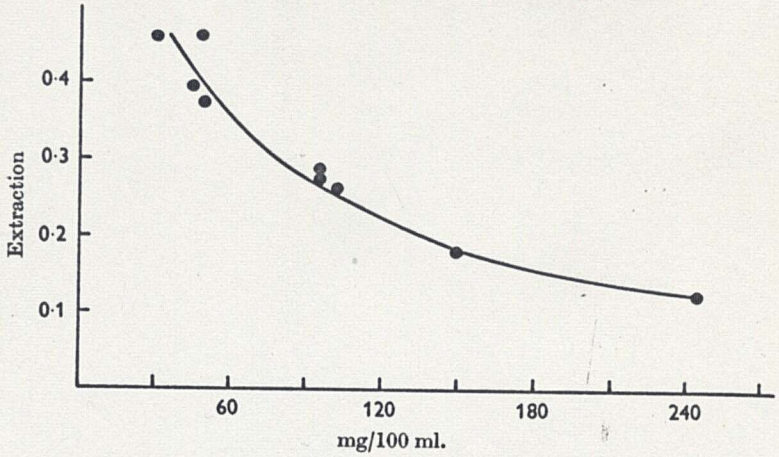

Fig. 2. *Ordinate.* Extraction of [^{14}C]glucose during the initial passage through the brain. *Abscissae.* Concentration of glucose in plasma. Every point on the figure represents the results from one experiment in which 5–9 samples were collected.

Extraction of [^{14}C]labelled glucose. A range of glucose concentrations varying from hypoglycaemic values to concentrations well above normal was established either by injection of insulin or by continuous infusion of glucose. Just before the experiment the blood sugar of the animal was determined and the concentration of glucose in the solution to be injected (containing glucose and [^{14}C]glucose) was adjusted so as to correspond to that of the blood. Ten experiments were performed. The results are presented in Fig. 2, and show that the fraction of glucose which passes in the direction from blood to brain increases with decreasing blood-glucose concentrations. Each point on the figure represents results from one experiment and is the average of 5–9 individual samples. The figure clearly shows that the fraction of glucose which travels in one direction (from the blood) during the passage through the brain is de-

pendent on the concentration. It is seen that at normal blood-sugar concentrations (about 90–100 mg/100 ml.) this extraction is more than twice the net extraction of glucose (which is about 10 %). The findings summarized in Fig. 2 place glucose in a different category from other non-electrolytes that pass from blood to brain, as these have never been shown to have a concentration-dependent extraction (Crone, 1965). The results of an experiment in which the blood-glucose concentration was reduced to 22 mg/100 ml. fell outside the general trend shown on the figure, as the extraction was found to be only 24 %.

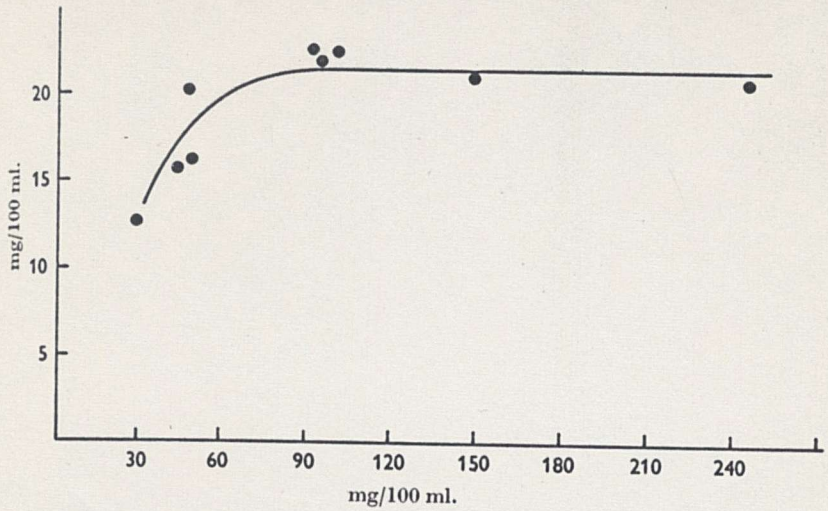

Fig. 3. *Ordinate.* The amount of glucose which leaves the cerebral capillaries per 100 ml. of blood by means of a facilitating mechanism. The results are calculated from the extractions shown in Fig. 2 after subtracting the amount of glucose which leaves the capillaries purely by diffusion *Abscissa*: Plasma glucose concentration (mg/100 ml.).

If it is assumed that the disappearance of glucose takes place by diffusion as well as by some other mechanism then it is reasonable to assume that the passive component is of the same order as that of fructose (about 4 %). If the amount of glucose leaving the blood by passive diffusion is subtracted from the total unidirectional flux then the amount of glucose passing by a facilitating mechanism can be calculated at different glucose concentrations. Figure 3 shows the amount of glucose leaving the brain capillaries by the 'transport' mechanism per 100 ml. blood. It is seen that the system has a limited capacity and is saturated at about 70 mg/100 ml.

Glucose-fructose ratios in effluent cerebral blood. In order to check the results described in the previous section, experiments were carried out to compare the relative concentrations of [^{14}C]glucose and fructose after

simultaneous intracarotid injection of the substances. The advantage of such a procedure is that it omits all indirect steps in the calculation. If, in such experiments, the extraction of fructose is less than that of glucose, as measured with labelled glucose, this would support the contention that glucose is carried by a transport system. Experiments in which glucose and fructose are injected together have the advantage that variations in extraction due to random variations in cerebral blood flow will affect the two substances equally, as will any other variable parameters.

This method of demonstrating a special transfer mechanism for glucose is, however, not very sensitive. If, for example, the extraction of fructose is 4 %, then 96 % remains in the blood. If the extraction of glucose is twice as high, 8 %, then 92 % will remain, the glucose-fructose ratio in this case being 92/96 = 0·96 and so forth.

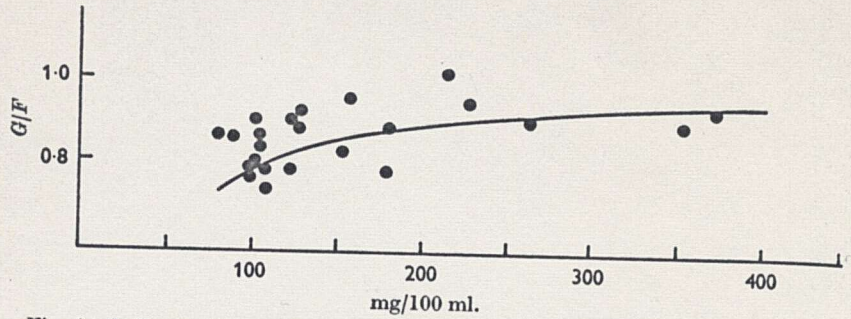

Fig. 4. *Ordinate.* Glucose-fructose ratios in effluent cerebral blood after injection of a mixture of [^{14}C]labelled glucose and fructose into the carotid artery. *Abscissa.* Concentration of glucose in that blood sample from which the G/F ratio was determined. The curve was drawn on the assumption that the extraction of fructose is 4% and that the extraction of glucose has a passive component of the same magnitude as the extraction of fructose, in addition to a 'facilitated' component.

Figure 4 shows the results from twelve experiments. It is seen that the ratio is always below unity at glucose concentrations between 80 and 175 mg/100 ml. The curve shown in the figure has been drawn as the ratio to be expected if the transfer of glucose has a passive component of the same magnitude as that of fructose, which means that 4 % of the glucose passes the capillaries by diffusion—in addition to the special transfer mechanism. It is also assumed that the transfer mechanism is saturated at a concentration of 70–80 mg/100 ml., whilst above this level a constant amount of glucose, about 20 mg/100 ml., is transferred 'actively'. The following expression should then apply:

$$G/F \text{ ratio} = \{1-(20/c_G+0{\cdot}04)\}/0{\cdot}96,$$

where the G/F ratio is the ratio between the relative glucose and fructose concentrations in the effluent blood, c_G is the concentration of glucose in

the plasma, 0·04 is the 'passive' extraction, 0·96 is the relative amount of fructose remaining in the effluent blood.

Is insulin necessary for the passage of glucose into the brain? As a first step towards the elucidation of the nature of the mediated transfer of glucose into the brain, investigations were carried out to establish whether insulin affects the transfer rate. Pancreatectomized dogs were used. The experiments were carried out 10–12 days after removal of the pancreas. The dogs were investigated twice: once without injection of insulin and later the same animal was used again after receiving an injection of insulin (3 i.u. crystalline insulin intravenously). There was no difference between the results obtained in the two experimental situations. It is concluded that insulin does not affect the transcapillary passage of glucose in the brain. The results are shown graphically in Fig. 5. It can be seen that the G/F ratios on the graph representing the values obtained from dogs that had, and had not received insulin are all scattered around the theoretical curve (drawn on the same principles as that in Fig. 4).

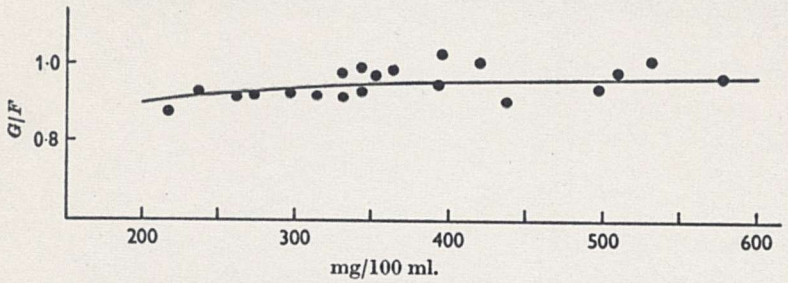

Fig. 5. *Ordinate.* Glucose-fructose ratios in effluent blood from the brain after injection of a mixture of [^{14}C]labelled glucose and fructose into the carotid artery in pancreatectomized dogs. *Abscissa.* Concentration of glucose in that blood sample from which the G/F ratio was determined. Curve drawn according to the same principles as Fig. 4.

DISCUSSION

The results presented in the previous section show that the flux of glucose varies with its concentration in the blood. If glucose were only transferred passively, then one would expect the flux to be proportional to the intravascular concentration; the extraction would therefore be constant and independent of the concentration in the blood. Without any sophisticated interpretations the present results show that this is not the case, and the conclusion is that glucose is *transported* from blood to brain tissue. Assuming that fructose passes by diffusion only, and that under similar experimental conditions the extraction is 4%, it seems most probable that a similar amount of glucose is also transferred purely by

diffusion whilst the remainder of the transfer is probably carrier-dependent, as is the case in other instances of facilitated transfer of glucose (erythrocytes and muscle cells).

The carrier-mediated transfer of glucose may either be of an 'up-hill' type or of an equilibrating type. The present experiments do not permit the differentiation of these possibilities, but the transport is most likely of the equilibrating type as no demonstrable quantities of free glucose have been found in the brain (Gey, 1956).

The passage of glucose across the blood–brain barrier is possibly analogous to the passage of glucose into erythrocytes of primates where there is evidence supporting the presence of a carrier-mediated transfer process of the equilibrating type (Wilbrandt & Rosenberg, 1961).

Figure 2 shows that the extraction as measured with labelled glucose is about 25 % at normal blood glucose concentrations (90–100 mg/100 ml.). The steady state (or net) extraction at this level of blood sugar is about 10–14 % (Himwich & Fazekas, 1937). The finding that the extraction determined from the unidirectional passage of glucose is much greater than the net extraction shows that there must be a passage of glucose in both directions across the blood–brain barrier. This may be a reflexion of the occurrence of 'exchange-diffusion'. The unidirectional flux of glucose at normal concentrations is 6–7 times as high as that of fructose so that the difference between the behaviour of glucose and that of similar substances is much more pronounced than would appear from the steady-state extraction of glucose. Krogh's (1946) statement, that the blood-brain barrier shows many of the features encountered in the passage of non-electrolytes and ions through cell membranes is certainly corroborated by the present findings as carrier-mediated transfer of glucose is very typical for mammalian cells.

The findings in the present article have been interpreted as evidence for the existence of a special transport system which allows glucose to pass into the brain. *Where* it goes to is unsettled, and the answer given depends partly on one's views about the existence or non-existence of an interstitial space in the brain. Dempsey (1958) advanced the concept that the astrocyte-foot processes engulfed material from the blood and nourished the neurones with this material. However, in fact, the experimental evidence for such a mechanism is strikingly meagre. Nobody has ever demonstrated the presence of, for example, glucose in the glial cells before it can be found in the neurones.

What is the morphological structure responsible for the blood–brain barrier? Davson (1964), in a very thorough discussion of the various hypotheses, concludes that the question of whether the barrier is at the endothelium, the basement membrane, or the glial covering, must remain

open until several conflicting morphological and physiological observations can be resolved. It is, however, tempting (once again) to propose that it is the permeability and transport characteristics of the endothelial cells that are reflected in the blood–brain barrier. The recent electron microscope observations support this concept. The overlapping of endothelial cells in the brain capillaries (Bennett, Luft & Hampton, 1959) may signify that dissolved substances must cross the endothelial cells in order to leave the blood. Passage through the endothelial cells proper, rather than through pores *between* them, would mean passage through two plasma membranes, the one facing the blood and the one facing the interstitial fluid. Having crossed to the *trans* side of the capillaries the molecules and ions could reach the neurones and glial cells by diffusion. This interpretation seems to the author to be consistent with, and a consequence of, the special construction of the endothelial lining of the brain capillaries. The model leaves to the endothelial calls the task of regulating the composition of the brain's interstitial fluid. In this connexion the finding of Torack, Besen & Becker (1961) that the cerebral capillaries contain ATPase in the basement membrane and in the endothelial cell may be relevant. As the erythrocyte membrane can effectively regulate the composition of its intracellular fluid it is not more surprising that the endothelial cells can regulate the composition of the fluid which they allow to pass.

SUMMARY

1. The mechanism of transfer of glucose from blood into brain tissue was studied in anaesthetized dogs by means of the 'Indicator Diffusion' technique. The initial unidirectional transfer of glucose was measured after intracarotid injection of glucose + [^{14}C]glucose with subsequent sampling from the superior sagittal sinus.

2. The fraction of glucose which passed into the cerebral tissue fell with increasing concentration of glucose in the concentration range 25–240 mg/100 ml. The extraction at low concentrations was almost 50 % and that at high concentrations about 10 %.

3. The rise in extraction at low concentration is taken as evidence of a carrier-mediate transport mechanism which facilitates the passage of glucose across the blood–brain barrier. The transfer probably takes place both by diffusion and by the special transport mechanism, which is saturated at concentrations of about 70 mg/100 ml. The capacity of this process is 20–22 mg/100 ml. blood.

4. Further evidence for a special transport mechanism for glucose was found in experiments in which the unidirectional passage of [^{14}C]glucose was compared with the passage of fructose by relating the concentrations

of the two substances in the effluent cerebral blood after one passage through the brain. The glucose/fructose ratio was less than one in all instances.

5. Insulin does not influence the passage of glucose from blood to brain tissue as judged from experiments in which the glucose/fructose ratio was determined in pancreatectomized animals before and after injection of insulin.

6. It is suggested that the endothelial cells in the cerebral capillaries are responsible for the transport of glucose across the blood–brain barrier.

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