The Effect of Several Inhibitors on the Metabolism and Transfer of Glucose in the Human Term Placenta

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The author studied the uptake and metabolism of glucose in the human term placenta *in vitro* and obtained the following results.

(1) Glucose uptake by the placenta appeared to be approximately proportional to the glucose concentration in the medium, except for its range of from 100 mg to 130 mg/100 ml, while the placental fructose production had its maximum around the glucose concentration of 100 mg/100 ml, which is near the upper limit of normal human blood sugar.

(2) As to the influence of several chemicals on the glucose uptake, the largest increase was caused by phloridzin, and slight increase by monoiodoacetic acid, estradiol and dinitrophenol. A decrease of the glucose uptake was caused by alloxan. Ouabain had hardly any effect on the glucose uptake. As to fructose production, its increase was caused most remarkably by estradiol and slightly by monoiodoacetic acid, and a decrease was caused by ouabain, alloxan, dinitrophenol and also slightly by phloridzin.

(3) From these results the author suggested the lack of direct participation of ouabain-sensitive ATPase in the active transport of glucose in the placenta. On the contrary, the participation of phosphorylation or hexokinase activity was indefinite. These had rather distinct influence on fructose production.

Studies on the passage and metabolism of sugar in the placenta were carried out in animals and primates by many investigators^{1,4,6-13} who all acknowledged the existence of active transport of some kinds of sugar and the production of fructose from glucose. The mechanism of the active transport of sugar in the placenta, however, still remains obscure. Villee¹ was of the opinion that some glycolytic system participated in this process.

On the other hand, as the energy source to the active transport in biological membranes, the ATP-ATPase system is assumed to play an important role especially in that transport of sodium and potassium ions through the red cell membrane. Such a hypothesis has gradually been accepted for the last few years.

Although the mechanism underlying membrane permeability in living body is not the same in microorganisms, red blood cells, muscular membrane and others, the ATP-ATPase system is thought to be present on the surface of every kind of

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membranes. We have carried out experiments to know the effect of enzyme inhibitors on the uptake of glucose and production of fructose by the placenta.

EXPERIMENTAL MATERIALS AND METHODS

Human term placentas within 30 minutes after delivery were used as the material. Early placentas were not used because they had high activity to produce glycogen from glucose and were considered rather inadequate for the present study.

Immediately after delivery the placenta was put into ice-cold physiologic saline. Fetal membrane, chorion plate, chorion stalk, decidua, blood vessels, and blood were carefully removed. About 0.3g of the placental specimen was incubated for 20 minutes¹ at 37°C with 0.3 ml of Krebs-Ringer phosphate buffer (pH 7.2–7.3) containing known amounts of glucose. After the incubation, the homogenate of placental specimen and the incubated solution were deproteinized with $\text{Ba}(\text{OH})_2$ and ZnSO_4 , and the supernatant was used for the detection of glucose with Somogyi's method² and for the detection of fructose with Dische's cysteine-carbazol sulphuric acid method.³

EXPERIMENTAL RESULTS

Control Experiment

1) Leakage of sugars due to injuries of the villi

It is considered inevitable to produce some injuries to the villi during the procedure of obtaining 0.3 g as a specimen from the placenta. For this reason the following experiment was performed.

Ten-fold volume of a 80 mg/100 ml glucose solution was added to 50–10g of the

Fig. 1. Glucose and fructose contents in the external layer of the placental tissue were compared with those in the internal layer after incubation of the placental tissue in a medium containing 80 mg/100 ml of glucose.

placental tissue and the mixture was incubated for 20 minutes at 37°C. After the incubation, glucose and fructose contents were determined of the external layer of the tissue, probably with some injuries, and of the internal layer probably without injuries (Fig. 1). The result showed that there was a slight decrease in the averages of fructose and glucose contents in the external layer of the placenta than in the internal layer, but the difference was statistically insignificant.

2) *Optimum concentration of glucose for placental uptake of glucose*

Hayakawa⁴ studied glucose and fructose uptake by the placenta by using four media with concentrations of glucose ranging from 60 to 500 mg/100 ml, and found that at a concentration of 125 mg/100 ml of glucose the placental glucose value was the lowest, while the fructose value rose to its maximum. In the present study seven samples of placental tissue, weighing 0.3 g each, obtained from one

Fig. 2. Concentrations of glucose and fructose in the medium.

Fig. 3. Concentrations of glucose and fructose in the placenta.

placenta were incubated in media in which glucose concentrations ranged from 40 to 220 mg/100 ml stepwise by 30 mg/100 ml, and the concentration of glucose and fructose in each sample were determined and compared with each other (Figs. 2 and 3). Fig. 2 shows the changes of glucose and fructose concentrations in the media above quoted.

The decrease rate of glucose in the medium presumably due to the uptake by the placenta was approximately proportional to the concentration of glucose in the medium. The fructose concentration in the medium reached the maximum value when the medium contained glucose in a concentration of 70 mg/100 ml, and it was not increased even when the glucose concentration was increased over 70 mg/100 ml.

It was found that glucose contents in the placental specimens was increased in proportion to an increase in the concentration of glucose contained in the media so far as the latter was in the range of from 40 mg to 100 mg/100 ml and from 130 mg to 220 mg/100 ml. When the media containing glucose at concentrations ranging from 100 mg to 130 mg/100 ml were used, the contents of glucose in the placental specimens were found to be almost of the same levels. On the other hand, the intraplacental fructose reached the highest value at 100 mg/100 ml of glucose in the incubation medium. The data suggest that the placental tissue has a regulating function to the uptake of glucose within a certain range of

glucose concentration in the media, and that the fructose production is the most efficient at such a range of glucose concentration in the media which corresponds to the upper limit of blood sugar level in normal pregnant women.

An additional experiment was performed by employing graded glucose concentrations of 10 mg/100 ml intervals in the range of 70 to 130 mg/100 ml of glucose concentration in the media. Such small changes in the glucose concentration in the media, however, did not cause remarkable changes in the glucose uptake by the placenta, nor in the intraplacental fructose production. It is probable that the placenta hardly reacts to such minimal changes.

Effect of Inhibitors

For the experiment of inhibitors, the medium containing 100 mg/100 ml of glucose, which was considered as the optimal concentration from the results above-mentioned, was used. All experiments on the effect of inhibitors were performed simultaneously with control experiments (Fig. 4). The amount of fructose in the

Fig. 4. Control experiments for six inhibitors.
K.R.P.: Krebs-Ringer phosphate solution.

placenta did not show a remarkable difference before and after incubation with glucose solution of a concentration of 100 mg/100 ml. and after incubation with Krebs-Ringer phosphate used as a glucose-free control medium. The amount of glucose going into the medium from the placenta was rather small after incubation with glucose solution of 100 mg/100 ml in concentration, but extremely large after the incubation with Krebs-Ringer phosphate although the amount of fructose in the media was rather unchanged.

1) *Ouabain*: Ouabain, known as an inhibitor of ATPase, was used at con-

Fig. 5. Ouabain was added at a concentration of 10^{-5} M to the medium containing 100 mg/100 ml of glucose.

Fig. 6. Ouabain was added at a concentration of 10^{-4} M to the medium containing 100 mg/100 ml of glucose.

centrations of 10^{-6} M and 10^{-4} M. Fig. 5 shows the results of 10^{-6} M ouabain. The upper part of the figure shows intraplacental glucose and fructose contents. Any inhibitory action upon glucose uptake was not noted, while the fructose production was markedly inhibited. Fig. 6 shows the results of 10^{-4} M ouabain.

Even when the concentration of ouabain was increased, the glucose uptake was scarcely influenced as in the case of 10^{-6}M , while the intraplacental fructose production was more intensely inhibited than in the case of 10^{-6}M . From these two figures it might be concluded that ouabain apparently did not cause impairment to the uptake of glucose into the placenta, but inhibited the production of fructose in the placenta more distinctly as the concentration of ouabain increased.

2) *Estradiol 17 β* : This agent is considered to inhibit the permeability of the red cell membrane to uric acid and phosphate probably through its inhibitory action on ATPase. The results with $4 \times 10^{-6}\text{M}$ of estradiol are shown in Fig. 7. In

Fig. 7. Estradiol was added at a concentration of $4 \times 10^{-6}\text{M}$ to a medium containing 100 mg/100 ml of glucose.

this case, the production of fructose was intensely increased contrary to the case of ouabain, while the glucose uptake did not change so remarkably. No change was noted in the glucose and fructose concentrations in the medium.

3) *Phloridzin*: This is an inhibitor of phosphorylation and also an inhibitor to the passage of galactose across the cell membrane of skeletal muscle in the insulin-facilitated state.⁵ Fig. 8 shows the results with $5 \times 10^{-4}\text{M}$ phloridzin. Increase in the glucose uptake into the placenta and increase of the fructose concentration in the medium were observed.

4) *2,4-Dinitrophenol (DNP)*: DNP, an uncoupler of oxidative phosphorylation, was used in a concentration of 10^{-4}M . As shown in Fig. 9, the fructose production was apparently inhibited, although no change was seen in the glucose uptake. It was interesting that the result was very similar to that with ouabain.

Fig. 8. Phloridzin was added at a concentration of $5 \times 10^{-4}M$ to the medium containing 100 mg/100 ml of glucose.

Fig. 9. DNP was added at a concentration of $10^{-4}M$ to the medium containing 100 mg/100 ml of glucose.

5) *Mono-iodo-acetic acid (MIA)*: MIA, known as an inhibitor of SH-enzyme group including hexokinase, was used in a concentration of $10^{-4}M$ (Fig. 10). Some increase in the glucose uptake and the fructose production was seen, but distinct differences were not observed as compared with other inhibitors.

Fig. 10. M.I.A. was added at a concentration of 10^{-4} M to the medium containing 100 mg/100 ml of glucose.

Fig. 11. Alloxan was added at a concentration of 100 mg/100 ml to the medium containing 100 mg/100 ml of glucose.

6) *Alloxan*: Alloxan, known as a causative agent of experimental diabetes mellitus through a continuous and specific injury to the beta cell of the Langerhans islet of the pancreas was used in a concentration of 100 mg/100 ml in this experiment simply as a cell toxin for comparison with others. As shown in Fig. 11,

Fig. 12. The rate of glucose uptake and fructose production in the placenta in reference to six inhibitors.

Fig. 13. The rate of glucose and fructose transfer into the medium in reference to six inhibitors.

both glucose uptake and fructose production were distinctly inhibited.

Figs. 12 and 13 show the rate of glucose uptake, fructose production, and the rate of transfer of glucose and fructose into the medium in percentage in reference to six inhibitors. The range of standard deviation shown in this graph represents the standard deviation of the control group when the placenta was incubated in a glucose-buffer solution of 100 mg/100 ml concentration. The uptake of glucose into the placenta was increased by phloridzin but not by others. The intraplacental production of fructose was remarkably increased in the case of estradiol, slightly increased in the case of MIA, and decreased in the cases of ouabain, DNP and alloxan. Ouabain especially showed a marked inhibition to the fructose production at a concentration of 10^{-4} M. The transfer rate of glucose into the medium from the placenta was slightly increased in the cases of ouabain (10^{-6} M) and MIA, and slightly decreased with DNP. Changes in corresponding values for fructose were practically not found in each group probably on account of the large scatter of individual measurements.

DISCUSSION

All living membranes are considered to have an active transport mechanism besides diffusion, and the placenta is not an exception. The most well known example is the transport of glucose. Davies⁶ studied the transport of glucose and many other sugars and made distinction between those transported by active transport mechanisms and those by non-active ones. According to Widdas,⁷ glucose is transported rapidly by an unknown carrier but fructose is not.

On the other hand, Alexander *et al.*^{8,9} demonstrated by means of ^{14}C glucose

Fig. 14. Concentrations of glucose and fructose in blood of pregnant and non-pregnant women.

loading that glucose was converted to fructose in the course of its transport through the placenta. Higher concentrations of fructose in the fetal blood than in the maternal blood and higher concentrations of fructose in the blood of pregnant women than in non-pregnant women, as shown in Fig. 14, are regarded as the result of fructose production in the placenta. The site of conversion of glucose to fructose is the syncytium cell (Yamaguchi *et al.*,¹⁰ Imamura,¹¹ Murakami¹²), and the pathway of the conversion apparently shares a part of the glycolytic pathway of Embden-Meyerhof (Vilée,¹ Ikuta¹³). Vilée¹ assumed a close relation between active transport of glucose and its conversion to fructose through the pathway of $\text{glucose} \rightarrow \text{G-6-P} \rightarrow \text{F-6-P}$.

On the other hand, studies on active transport through biological membranes of various tissues and organs have made a great progress. The present experiment was designed to see if active transport for glucose exists in the placenta, whether such process requires ATPase activity as seen in the red cell membrane for the exchange of sodium and potassium ions (Yoshikawa¹⁴) and in the intestinal mucosa for the absorption of sugars (Kaneko¹⁵) and whether the process is related to glycolytic pathway as suggested by Vilée.¹

The data obtained offered some interesting suggestions: Ouabain, an ATPase inhibitor, exerted no influence on glucose uptake. On the contrary, the rate of fructose production decreased in proportion to its concentration. Since glucose is readily metabolized in the cell and forms G-6-P by hexokinase accompanied with ATP-ADP reaction, ouabain apparently inhibits G-6-P formation as a result of suppressed ATPase activity in the syncytium cell. The assumption seems to be justifiable because ATPase activity in the microvilli of the syncytium cells is demonstrated by electron microscopy (Lister¹⁶). In our own electron microscopic observation of the placenta on the effect of some inhibitors (Sekii¹⁷), ouabain had a pronounced effect on the form of the syncytium cell which has been thought to have a biological transport activity despite the absence of demonstrable changes in the organelles and microvilli of the syncytium cells. From these facts it may be concluded that ouabain selectively inhibits ATPase activity in the membrane, and such inhibition causes a decrease of fructose production and the morphological changes of the syncytium cells.

According to Kasama¹⁸ the increased production of fructose by using estradiol probably suggests the facilitating action of the latter on Warburg-Dickens and Embden-Meyerhof pathways, rather than its inhibitory action on ATPase activity as mentioned by Christensen.¹⁹

The inhibitory action of DNP on fructose production, which is an uncoupler of oxidative phosphorylation, is thought to be due to decreased G-6-P formation from glucose on account of lowered hexokinase activity induced by inhibition of ATP formation in the villi. This is brought about by blockade of oxidative phosphorylation with DNP. The presumption is consistent with the result of electron microscopy by Sekii¹⁷ that DNP causes destructive changes of the mitochondria, deformation or swelling of the endoplasmic reticulum and decrease

of RNP particles. According to the Siekevitz's hypothesis,²⁰ intracellular sugar metabolism occurs on the surface of the endoplasmic reticulum. From these points of view, the effect of DNP on the sugar metabolism of the placenta suggests a close relation between morphological changes of intracellular organelles of the syncytium cells and the changes in sugar metabolism. Although the results obtained with DNP were similar to those with ouabain, it is better to assume an essential difference between the mechanisms of DNP and ouabain activities.

The results obtained with MIA and phloridzin were quite difficult to interpret. With both MIA and phloridzin, increase of glucose uptake into the villi occurred, and it was especially remarkable in the case of phloridzin.

If the placenta had a mechanism for glucose absorption similar to that in the tubular epithelial cells of the kidney, and if hexokinase activity could be directly related to the active transport of glucose in the placenta too, MIA and phloridzin would decrease glucose uptake.

On the other hand, the fact that the production of fructose in the villi was almost unchanged or only slightly increased compared with that in the control experiment suggests a deficiency of G-6-P and F-6-P induced by insufficient phosphorylation of glucose. From these points of view, the increase of glucose in the villi should be considered to occur without phosphorylation. The question still remains, if the active transport is accelerated by MIA and phloridzin, but it is rather improbable. According to the electron microscopic observation of Sekii,¹⁷ MIA causes more extensive changes in the cell membrane, mitochondria and other organelles than those in cases of DNP and ouabain.

In the case of alloxan, a definite inhibition of glucose uptake and fructose production was observed. It may be assumed, alloxan as a cytotoxin intensively destroys the cell membrane and intracellular structure so that a complete inhibition of active transport and sugar metabolism takes place.

When placental tissue was incubated with Krebs-Ringer phosphate alone as the control experiment, decrease of fructose in the placenta was not conspicuous. Glucose probably diffuses out to some extent and leads to a decrease in the glucose concentration within the villi. Although similar diffusion of fructose would also take place, fructose deficiency is rapidly compensated by other sugars, most probably by those supplied by decomposition of glycogen, and a constancy in the amount of fructose is maintained in the placenta. This interesting problem deserves further experimental study on sugar metabolism in relation to the pentose cycle (Onoue²¹).

In the experiment mentioned above, the passage of glucose through the placenta does not appear as simple as the passage of sodium and potassium ions or the sugar transport through the red cell membrane or other cell membranes. Even if a carrier exists within the placental membrane as suggested by Widdas⁷, and even if the active transport of glucose in the placenta belongs to the facilitating transport, the direct role played by ouabain-sensitive ATPase coupled with Na-K exchange as in sugar absorption of the intestinal mucosa is not very likely in our experiment.

The role of the glycolytic system in the glucose passage through the placenta was rather inconsistent with the concept of Villee.¹

References

- 1) Villee, C.A. The metabolism of human placenta *in vitro*. *J. biol. Chem.*, 1953, **205**, 113-123.
- 2) Somogyi, M. Notes on sugar determination. *J. biol. Chem.*, 1952, **195**, 19-23.
- 3) Dische, Z. & Borenfreund, E. A new spectrophotometric method for the detection and determination of keto sugars and trioses. *J. biol. Chem.*, 1951, **192**, 583-587.
- 4) Hayakawa, S. Studies on the carbohydrate metabolism in chorionic villi of the human placenta. *Tohoku J. exp. Med.*, 1964, **83**, 81-93.
- 5) Keller, D.M. & Lotspeich, W. Phloridzin inhibition of the insulin expansion of the galactose space in the eviscerated rat. *J. biol. Chem.*, 1959, **234**, 995-997.
- 6) Davies, J. Differential permeability of the rabbit placenta to various sugars. *Amer. J. Physiol.*, 1957, **188**, 21-24.
- 7) Widdas, W.F. Inability of diffusion to account for placental glucose transfer in the sheep and consideration of the kinetics of a possible carrier transfer. *J. Physiol.*, 1952, **118**, 23-39.
- 8) Alexander, D.P., Huggett, A. St. G. & Widdas, W.F. Rate of formation of foetal fructose investigated with ¹⁴C glucose. *J. Physiol.*, 1951, **115**, 36-39.
- 9) Alexander, D.P., Huggett, A. St. G., Nixon, D.A. & Widdas, W.F. The placental transfer of sugars in the sheep: The influence of concentration gradient upon the rate of hexose formation as shown in umbilical perfusion of the placenta. *J. Physiol.*, 1955, **129**, 367-372.
- 10) Yamaguchi, R., Okuda, Y., Hayakawa, S. & Kinugawa, H. Carbohydrate metabolism in the human placenta. *Sanfujinka no Sekai* (Jap.), 1963, **15**, 1221-1226.
- 11) Imamura, H. Studies on sugar metabolism of the placenta. *Nippon Sanka-Fujinka Gakkai Zasshi* (Jap.), 1958, **10**, 1387-1396.
- 12) Murakami, H. Studies on carbohydrate metabolism in the placenta. *Nippon Sanka-Fujinka Gakkai Zasshi* (Jap.), 1959, **11**, 723-732.
- 13) Ikuta, M. Studies on enzymes relating to intermediate carbohydrate metabolism in human placenta and fetal liver. *Nippon Sanka-Fujinka Gakkai Zasshi* (Jap.), 1959, **11**, 37-44.
- 14) Yoshikawa, H. Active transport of ions in erythrocytes. *Tanpakushitsu Kakusan Koso* (Jap.), 1963, **8**, 417-425.
- 15) Kaneko, T. Digestion and absorption of the carbohydrate. *Saishin Igaku* (Jap.), 1956, **20**, 3087-3092.
- 16) Lister, U.M. Ultrastructure of the placenta in hypertensive states. *J. Obstet. Gynaec. Brit. Cwlth.*, 1966, **73**, 439-451.
- 17) Sekii, M. Electron microscopic studies on the morphological changes of the chorionic epithelial cells under several conditions and its influence on the transport of fat. *Nippon Sanka-Fujinka Gakkai Zasshi* (Jap.), 1967, **19**, 441-448.
- 18) Kasama, Y. Studies on the influence of carbohydrate metabolism in the presence of several hormone in the human placenta. *Nippon Sanka-Fujinka Gakkai Zasshi* (Jap.), 1964, **16**, 359-368.
- 19) Christensen, H.N. Transport by membrane-bound sites or by free shuttling carriers? *Membrane Transport and Metabolism*, edited by A. Kleinzeller & A. Kotyk, Academic Press, 1961, pp. 470-478.
- 20) Siekevitz, P. & Palade, G.E. Functional variation in enzymatic activity of microsomes. *J. biophys. biochem. Cytol.*, 1958, **4**, 309-313.
- 21) Onoue, K. Studies on the metabolism of the fructose in several tissues. *Igaku no Ayumi* (Jap.), 1966, **59**, 68-74.