Studies on the Carbohydrate Metabolism in Chorionic Villi of the Human Placenta

By

Sumio Hayakawa

From the Department of Obstetrics and Gynecology, Tohoku University School of Medicine, Sendai; Director: Prof. K. Kushima

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Chemical studies on the metabolism of glucose, fructose and glycogen in human chorionic villi are reported with the following conclusions: 1) In chorionic villi the level of glucose was the mean value between that of the maternal blood and the fetal blood. The level of fructose was three times as high as that in the fetal blood. 2) Chorionic villi convert glucose into fructose. The best conditions for the conversion were as follows: to keep the glucose level at 125 mg/dl in the incubation solution, to keep the pH at 7.4, to have the temperature at 37°C, and to perform the experiment under saturated oxygen. The chorionic villi play an important role in the conversion of sugar in the placenta. 3) The rapid transportation of glucose in chorionic villi does not occur through simple diffusion mechanism, but through biological action in connection with the sugar metabolism in chorionic villi. 4) Human full term placentae have stronger activities in decomposing than in synthesizing glycogen. 5) Sugars in chorionic villi are convertible to each other, such as the schema, glycogen → glucose → fructose, following changes of environmental condition.

The author stresses the existence of a regulation mechanism for the passage of sugar through the placenta. This mechanism might be called the metabolic regulation system.

Making progress in research on the quantitative differentiation of the substances in the maternal and fetal blood, which were given as a load test, the feto-maternal exchanging mechanism of substances across the human placenta has been explained not only by the diffusion theory, but also by the biologically active function in the transport of substances through the placenta.

In the mammalia glucose concentration is high, while fructose is low in the maternal blood, compared with that in the fetal blood. When glucose or fructose is given to the maternal body, glucose passes more rapidly through the placenta than fructose does. This fact shows that the placenta has selective permeability for sugars. So far, for the investigation of the function and metabolism of the placenta, placenta slices and placental homogenates have been used as materials, but in this way function of cells will be inves-
tigated rather than that of tissues. Metabolism in the chorionic villi of the human placenta occurs at the passage of substances across the placental barrier. Therefore, the chorionic villi which were kept in near physiological conditions must be used for the experiment on metabolism in the placenta.

This paper is mainly concerned with the metabolism of sugar using chorionic villi separated attentively from the placenta.

METHODS

The chemical determination of sugar
A) Assay method of glycogen in tissue

There have been many reports of assay of glycogen in tissue. The author used Montgomery’s method\(^1\) that has the advantage of simplicity in technique and high sensitivity.

The tissue is extracted with 30% potassium hydroxide solution, and is glycogen precipitated with alcohol. Diluted solution of the precipitate is filtered through glass-wool-plugs to remove extraneous insoluble polysaccharides. The color produced by phenol-sulfuric acid was measured with a spectrophotometer at 490 m\(\mu\). This method proved to be superior in precision and reproducibility.

B) Assay method of glucose

Somogyi’s method\(^2\) was used for determination of glucose. This is a sensitive and stable method. Glucose was measured with a spectrophotometer at 660 m\(\mu\).

C) Assay method of fructose

Dische’s method\(^3\) was used. Comparing Roe’s resorcinol reaction\(^3\) and Dische’s cystein-carbazol-sulfuric acid reactions\(^4\), the latter showed higher sensitivity than the former. In the Dische’s reaction, the color production rate by glucose is approximately 1/400 of the rate by fructose. Therefore, this reaction is considerably less affected by simultaneous presence of glucose (or other aldohexose) in the solution. Fructose was measured at 560 m\(\mu\).

RESULTS

I. Glucose and fructose concentration in chorionic villi of the normal human placenta

There are considerable amounts of glucose and fructose in the human placenta and passage rate of these two sugars through the placenta is different. Although the levels of glucose and fructose content in the chorionic villi may be a matter of interest, we have as yet very little information as to the determination of these sugars by chemical analysis.

Term placentae were obtained immediately after delivery. Chorionic villi were separated attentively from amnion, chorionic plate, decidua, blood vessels
and blood, and homogenized and deproteinized with Ba(OH)₂ and ZnSO₄. Glucose and fructose in the homogenates were determined by Somogyi's method and Dische's cystein-carbazol-sulfuric acid method, respectively.

The values of glucose and fructose in the chorionic villi were shown in Fig. 1.

![Diagram showing normal values of glucose and fructose in chorionic villi.](image)

Fig. 1. Normal value of glucose and fructose in chorionic villi.

In twenty-four cases, the average of glucose content in chorionic villi was 79.4±40 mg/100 g wet weight and the average of fructose content was 12.1±4.7 mg/100 g wet weight, whereas that in fetal blood was 4.8±2.1 mg/100 ml.

Hagerman et al.⁵) reported that the value of glucose in the maternal blood was 88±6 mg/100 ml and that in the fetal blood was 74±7 mg/100 ml.

Accordingly, the value of glucose in chorionic villi, 79 mg/100 g, in our experiments is the mean values between the maternal and fetal blood. But there is no highly significant quantitative differentiation among them; chorionic villi, maternal blood and fetal blood. On the other hand, the value of fructose in chorionic villi was the highest of the three.

According to Hagerman's report⁶), fructose in fetal blood is approximately 4 mg/100 ml, which is about 45% higher than the fructose in maternal blood. As mentioned above, the fructose content in chorionic villi was about 12 mg /100 g, which is almost three times the level in fetal blood. In explaining the fact that the fructose value in chorionic villi is extremely high, the problem of passage of sugar through the placenta and also the problem as to whether fructose is produced in chorionic villi or not must be discussed.

Since Cohnstein’s⁶) and Scholssmann’s⁷) experiments on the comparison of the sugar content in maternal blood with that in fetal blood, interest has developed in the placental passage of sugars. Chinard et al.⁸) observed that the content of
glucose in maternal blood was nearly parallel to that in fetal blood and after giving glucose to the maternal body, the glucose concentration in fetal blood was elevated in a short time.

Bickenbach's theory9) that sugars pass through the placenta by simple diffusion is based on the observations described above. But if his theory is right, it will be difficult to explain the following facts; fructose which has the same molecular weight with glucose takes more time to pass through the placenta than glucose does9). Also, as experimental results showed clearly, there is remarkable difference in the concentration of fructose among maternal blood, fetal blood, and the placenta. Even though the placenta is separated from the fetus, the level of fructose in chorionic villi is increased through the placental perfusion method11). These facts suggest that fructose will be produced in the placenta by some biological action and it passes slowly, accumulating in the chorionic villi. In order to clarify these above-mentioned problems, the next experiment was made on the relationship between fructose production and carbohydrate metabolism.

II. The vicissitude of glucose at the incubation of chorionic villi in the glucose solution

To study the mechanism of the transplacental passage of sugars, a considerable amount of research has been made on the comparison of glucose concentrations for fetal blood and for maternal blood. Since the demonstration by Passmore12), Aron13) and Needham14), that much fructose is contained in the sheep fetal blood, whereas only a little in the maternal blood, the placenta has been considered to be the place of fructose production (Hitchcock15)). Controversy continued as to whether monkey or human placenta can produce fructose5) or not5). Hagerman and Villee suggested that glucose turns to fructose in the placenta and alkaline phosphatase participates in the conversion. Although it is well documented in histochemical researches that the alkaline phosphatase is contained much in chorionic villi of human placenta16). Little work has been done to study the conversion of glucose to fructose using chorionic villi.

In our experiments placentae obtained immediately after delivery were put in cold saline. Chorionic villi were quickly removed from amnion, chorionic plate, chorionic stem, decidua, vessels and blood, and were suspended in the saline. Even 0.3 g of the chorionic villi was incubated in 3 ml of the Krebs-Ringer solution containing glucose at 37°C for 20, 40 and 60 minutes, respectively. After incubation, chorionic villi were filtered, homogenized, deproteinized with Ba(OH)₂, ZnSO₄, and then centrifuged. The supernatant fluid was used for the determination of glucose and fructose. The solution separated from chorionic villi at the first step after incubation was also deproteinized and centrifuged. The supernatant was used for the determination of these two sugars also. Fig. 2 shows these procedures.
As the control, the chorionic villi soaked in the saline which contains 0.03% corrosive sublimate for twenty minutes were used.

A) Experiment for fructose production

Under the conditions concerned, the following two factors were important; one is the concentration of glucose in the solution and another is the use of the saturated oxygen. The fructose production in the chorionic villi and the solution after incubating was greatest at 125 mg/dl of the concentration of glucose. Adding the saturated oxygen, at this level of glucose much more fructose was produced than adding the saturated nitrogen at the same level of glucose. That is, the fructose content rose to about eight times the normal in chorionic villi. When the level of glucose was lower or higher than 125 mg/dl, the fructose production rate decreased slightly under the saturated oxygen, compared with that under the saturated nitrogen, as shown in Fig. 3. Under the same condition the production rate of fructose in the control was inhibited by approximately 50% (Fig. 4).

B) Experiment of glucose

The change of glucose content at the incubation of chorionic villi in the glucose solution was investigated (Fig. 5). The test showed slight difference in the level glucose between the two conditions — under the saturated oxygen and the saturated nitrogen. Under the saturated oxygen the glucose level in chorionic villi became lower by 62.3%, 77.3%, 38.2% and 170.0%, when the glucose levels in the incubation solution were 500 mg/dl, 250 mg/dl, 125 mg/dl
Fig. 3. Fructose production rate following changes in the concentration of glucose.

Fig. 4. Fructose production rate after damage with 0.03% corrosive sublimate.
and 60 mg/dl, respectively. With the same incubation solution using saturated nitrogen, the percentages in the chorionic villi were 48.9%, 61.2%, 40.0% and 107.5%. Accordingly, excepting the level of 125 mg/dl the glucose level in chorionic villi under the saturated oxygen was higher than that under the saturated nitrogen. These facts suggest that the glucose level in chorionic villi is not parallel with the glucose level in the incubation solution and there is difference between the glucose levels under the saturated oxygen and the saturated nitrogen. The production of fructose in human placentae is still a matter of speculation; Chinard et al.8) made a denial of the fructose production in vivo and Huggett11) observed a small amount of fructose produced in the placentae. However, according to Hagerman and Villee's experiments9), incubation of placental slices in vitro resulted in a considerable production of fructose. In our experiments using chorionic villi, much more fructose production was observed, and the production rate was supposed to be influenced by experimental conditions. The discrepancy between Hagerman and Villee's and our results in the production rate of fructose seems to be due to the different conditions of experiment. For example, we used chorionic villi, while they used placental slices as a material, also, we applied different pH from that which Hagerman et al. did. Consequently, main factors to have influence on the fructose production will be the concentration of glucose in the incubation solution, use of saturated oxygen, pH

![Fig. 5. Comparison of values for glucose in chorionic villi and that in the incubation solution.](image-url)
and placental tissue, especially chorionic villi. Chorionic villi seem to be the most important part in the placenta in the production of fructose, because after damage with 0.03% corrosive sublimate the production rate of fructose decreases markedly. In our experiment, there is no production of fructose in fetal blood, so the hypothesis that fructose may be produced in a small amount of fetal blood contained in chorionic villi is unreasonable. If the glucose level in chorionic villi is always the same with that in the solution, in which chorionic villi are incubated, it is logical to believe that glucose diffuses simply to chorionic villi. However, in our experiments the glucose levels in chorionic villi became lower by 62% and 77% when the glucose levels in the incubation solution were 500 mg/dl and 250 mg/dl respectively. In the case of 125 mg/dl of the glucose level in the solution, the glucose level in chorionic villi decreased remarkably by approximately 38%. This result raised a question as to whether the low level of glucose was caused by low up-take rate of glucose into chorionic villi or by rapid conversion to the other substances. In the case of 60 mg/dl of the glucose level in the solution, the glucose level in chorionic villi increased by 170%. It is evident from the experiments described below that most of the glucose increase was produced as a result of glycogen analysis. Accordingly, the content of glucose in chorionic villi was influenced by the level of glucose in the incubation solution.

III. The vicissitude of fructose at the incubation of chorionic villi in the fructose solution

Fructose has the same molecular weight with glucose, and chemical differentiation between these two sugars is that fructose corresponds with aldohexose while glucose corresponds with ketohexose. These two sugars are supposed to be different in the passage through chorionic villi in the placenta which will be the barrier. As shown in Fig. 6, there were negligible differences in the
fructose level in chorionic villi between the saturated oxygen and the saturated nitrogen. The level was almost parallel to the fructose content in the incubation solution and the passage rate ranged from approximately 32 to 53%. The total concentration of fructose decreased whereas that of glucose increased by incubation. The decrease rate of fructose ranged from 15 to 54% (Fig. 7). Under the saturated oxygen the glucose levels in chorionic villi were approximately 180 mg,

Fig. 7. Decrease rate of total fructose at the incubation of chorionic villi in the fructose solution.

Fig. 8. Production rate of glucose at the incubation of chorionic villi in the fructose solution.
240 mg and 210 mg when the fructose levels in the incubation solution were 500 mg/dl, 250 mg/dl and 125 mg/dl respectively. Under the saturated nitrogen, 170 mg, 248 mg and 190 mg in chorionic villi at 500 mg/dl, 250 mg/dl and 125 mg/dl in the incubation solution. The fructose level in chorionic villi was almost parallel with that in the incubation solution. The fact suggests that the placental passage of fructose occurs by diffusion. Because the passage rate was low, the diffusion will be very slow in the placenta.

Our findings — that fructose diffuses slowly and the passage rate of fructose is inferior to that of glucose in the placenta — are essentially similar to those in the Davies' experiments on rabbits.

In our experiment, the more fructose decreased, the more glucose was produced. This result suggests the conversion of fructose into glucose in chorionic villi. But in the level of fructose, 250 mg/dl and 125 mg/dl, much more glucose was produced compared with the decreasing fructose. Inferences are drawn from the fact mentioned above that glucose may be produced not only by conversion of fructose, but also by conversion of other substances.

IV. The vicissitude of glycogen at the incubation of chorionic villi

A considerable amount of research has been reported on the state of glycogen in placentae, which is closely related to the metabolism of sugar. Most of the research was based on histochemical method and we have very little information as to the chemical studies of sugar in chorionic villi.

An attempt was made in the following experiment to investigate the vicissitude of glycogen in chorionic villi incubated in the glucose or fructose solution. Chorionic villi separated from human full term placentae were incubated in the glucose or fructose solution under saturated oxygen for 60 minutes. First, changes of glycogen in chorionic villi incubated in the high and low concentration of glucose solution were observed (Fig. 9). When the glucose levels in the incubation solution were 500 mg/dl, the glycogen levels in chorionic villi increased by approximately 10%, while when the glucose levels were 60 mg/dl, the glycogen levels decreased about 30%. Secondly, as the incubation solution, various concentration of fructose were used (Fig. 10). When the fructose levels in the incubation solution were 500 mg/dl, 250 mg/dl and 125 mg/dl, the glycogen levels in chorionic villi decreased by 68%, 57% and also 57% respectively.

According to the results of Villee's experiment using placenta slices, placentae in the early stage of pregnancy showed high ability in synthesizing glycogen, but in the later stage they have only a little ability in synthesizing glycogen.

Our experimental results suggest that chorionic villi have both abilities of composition and decomposition of glycogen; if glucose high in concentration is,
glycogen may be synthesized in chorionic villi and if it is low in concentration glycogen may be decomposed. But in chorionic villi of placentae in the later stage of pregnancy, the ability of decomposition is stronger than that of synthesis. This is related to the fact that the content of glycogen in the placenta decreases after the middle stage of pregnancy.

When chorionic villi was incubated in the glucose solution containing 60 mg/dl in the concentration, glucose was produced in the chorionic villi simultaneously with a decrease of glycogen. Therefore it may be that glycogen is decomposed into glucose. On the contrary, when chorionic villi were incubated in the solutions, of various fructose concentrations glycogen in chorionic villi decreased in all
cases, making glucose increase in the fructose solution. So it is quite possible that under the condition by adding oxygen glycogen is decomposed into glucose, but is not synthesized from fructose.

The transfer system of sugar in chorionic villi was summarized in Fig. 11. It is reasonable to presume that there is an intimate mutual relationship between glucose, fructose, and glycogen and they can be converted each other according to the environmental situation. Glycogen obtained from the maternal blood and stored in the placenta, and as demanded it can be released to the fetal blood in the form of glucose or fructose.

References

4) Dische, Z. & Borenfreund, E., ibid., 1951, 192, 583.
    1963, 15, 1221.