TRANSPORT OF UREA AND AMINO ACIDS THROUGH THE ISOLATED HUMAN PLACENTA

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By means of a method of two-way perfusion of the isolated human placenta the transport of urea from the fetal to the maternal placental circulation and the transport of amino acids in the opposite direction were studied. Experiments showed that the method provides for sufficiently complete perfusion of the intervillous space and creates suitable conditions for the study of placental transport. If the amino nitrogen concentrations in the two circulatory systems are equal, its concentration in the fetal circulation rises in the course of the experiment. On the addition of an amino acid to the maternal circulation, this increase develops to a greater degree. The results of these experiments confirm the view that amino acids are "secreted" by trophoblast cells into the fetal circulation.

KEY WORDS: isolated human placenta; urea; amino acids; placental transport of materials.

Techniques for the study of the human placenta in vivo are limited and for that reason methods of studying the isolated, surviving placenta are of great importance as a second best solution. These methods, of course, reproduce to a greater or lesser degree the natural conditions of the placental circulation.

A technique developed previously, by means of which two-way perfusion of the surviving human placenta can be undertaken, was used. First experience with the method showed that it ensures perfusion of the intervillous space [1]. However, it was not known how complete this perfusion was or whether the conditions created for the study of placental transport were close to physiological.

It was therefore decided to make a more detailed study of the transport of materials to the placenta and, in particular, to study the transport of urea from the fetal placental circulation into the maternal circulation and the transport of amino acids in the opposite direction.

EXPERIMENTAL METHOD

Two-way perfusion of the placenta immediately after birth was carried out with a mixture of Hanks' solution and gelatinol (1 : 1) with the addition of placental blood and glucose. A pulsating flow of fluid (85 beats/min) with a velocity of 500-600 ml/min was maintained in the maternal circulation while the pressure in the cavity of the uterus was 40-60 mm Hg. Fluid circulated in the fetal circulation as a pulsating flow (140 beats/min) with a velocity of 27 ml/min and a mean pressure of 117 mm Hg. Constancy of temperature (37 ± 0.5°C) and pH (mean 7.28) was maintained during the experiments. The sodium and potassium concentration in the medium was determined with the FPL-1 flame photometer. The values were 174 meq/liter (165-181 meq/liter) for sodium and 5.8 meq/liter (3.6-8.1 meq/liter) for potassium. The glucose concentration in the medium also was determined by the method of Somogyi and Nelson. The initial glucose concentration (mean 98 mg %) fell gradually in the course of the experiment, reflecting the utilization of glucose by the placental cells. Urea was determined by Conway's microdiffusion urease method, and the amino nitrogen concentration was determined by Szentirmai's method [2] in Zhakhova's modification. Samples of

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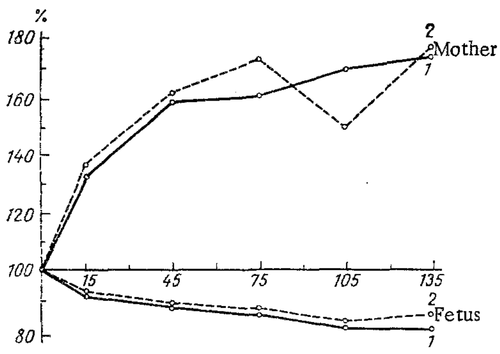

Fig. 1

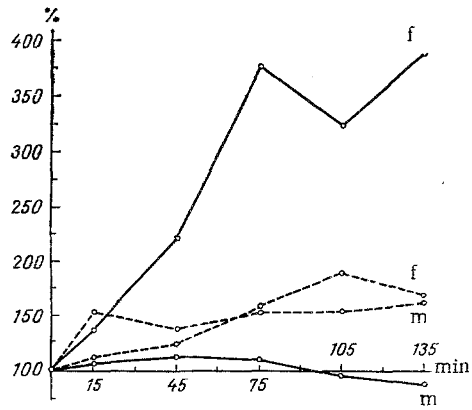

Fig. 2

Fig. 1. Changes in mean percentage concentration of urea in fetal and maternal circulations during perfusion of placenta: 1) normal, 2) toxemia. Abscissa, time (in min); ordinate, urea concentration (in % of initial value).

Fig. 2. Changes in mean percentage concentration of amino nitrogen during perfusion of placenta: f) fetal, m) maternal circulation. Continuous lines) experiments with addition of glycine, broken lines) experiments without glycine. Abscissa, time (in min); ordinate, concentration of amino nitrogen (in % of initial value).

fluid for testing were taken before the circulation started, 15 min later, and thereafter every 30 min for 135 min of the experiment.

EXPERIMENTAL RESULTS

In experiments on 34 placentas the transport of urea was studied from the fetal circulation into the maternal circulation. In the control experiment the urea concentration in the fetal circulation ranged between 5 and 12 mg % and in the maternal circulation between 6 and 14 mg %; its level remained virtually unchanged during perfusion.

The remaining experiments were carried out with the addition of urea to the fetal circulation (0.5 g liter perfusion fluid) on 21 placentas from women with uncomplicated pregnancies. The original urea concentration in these experiments averaged 53.1 ± 1.7 mg % in the fetal and 7.0 ± 0.5 mg % in the maternal circulation. After perfusion for 15 min the urea concentration in the fetal circulation was significantly lowered (46.2 ± 1.3 mg %; $P < 0.01$); the decrease continued later (by 7.1-9.8 mg % compared with the initial value). The increase in the mean urea concentration in the maternal circulation after perfusion for 15 min (9.1 ± 0.9 mg %; $P = 0.05$) also was significant, and later the concentration continued to rise gradually (by 3.9-4.4 mg % compared with the initial value). However, the decrease in the urea concentration in the fetal circulation in absolute terms was 1.5-2.3 times smaller than the increase in its concentration in the maternal circulation.

During two-way perfusion of normal placentas, the concentration gradient of urea created on both sides of the placental membrane thus determines both its transport from the fetal to the maternal circulation and also, evidently, some retention of urine in the placental tissues in the case of a gradient of the magnitude studied. Urea transport did not follow an identical course in all the experiments, and in Fig. 1, which shows the dynamics of transport, it was expressed as the mean percentage change in the urea concentration, calculated from the percentage of changes in each experiment.

Twelve placentas from women with pregnancy complicated by late toxemia, running a mild course, also were studied. No significant differences from normal were found either in the absolute values of the urea concentration or in the dynamics of the mean percentage of the changes in its concentration (Fig. 1). Similar experiments on 20 placentas from women with an uncomplicated pregnancy were carried out in order to study the transport of amino acids from the maternal circulation to the fetal. In these experiments the initial concentration of amino acids in both circulations was the same: mean 12.9 ± 1.7 mg %. In 12 control experiments, by contrast with those to study urea transport, if the concentrations of the amino acids on both sides of the placental membrane were the same, their concentration in the fetal circulation rose in the course of the experiment. The mean concentration of amino nitrogen in the maternal circulation rose by 4.5% 15 min after the beginning of perfusion, and later it exceeded the initial value by 4.9-6.2 mg %. However, this increase was not statistically significant at any period during the 135 min of perfusion ($P > 0.05$). The concentration rose in only nine of 12 experiments and the increase was different in different experiments. The results are therefore shown in Fig. 2 as curves reflecting the dynamics of the mean percentage change in the amino nitrogen concentration, taking all the experiments into account. During perfusion the mean percentage of changes was 140-160 relative to the initial amino nitrogen level in the perfusion fluid. An increase in the amino nitrogen concentration in the fetal circulation was observed in all 12 experiments. During the first 75 min of perfusion its mean value was 0.3-4.5 mg % and it was not statistically significant ($P > 0.05$), although in individual experiments the increase during this period was considerable namely by 2.2-4.2 times. Meanwhile, at the end of perfusion the concentration of amino nitrogen in the fetal circulation in absolute terms rose significantly, to 21.6 ± 2.9 mg % ($P = 0.001$). It will be clear from Fig. 2 that the concentration of amino nitrogen in the fetal circulation rose in the course of the first 15 min of perfusion to 113% of its initial level, and by the end of the experiment to 170-190%.

These experiments showed that if the amino nitrogen concentration was the same on both sides of the placental membrane, the perfusion fluid became enriched with amino acids; the process took place more rapidly and to a significant degree on the fetal side.

In the remaining eight experiments an amino acid was added to the maternal circulation (1.5 g glycine to 1500 ml perfusion fluid). The original amino nitrogen concentration in the fluid was 38.3 ± 3.7 mg %. Later an increase in the concentration was observed in only five of eight experiments; however, the changes were slight, on the average by 1-3.5 mg %, and they were not significant ($P = 0.05$). In all experiments the concentration rose on average by only 11-14%. The initial amino nitrogen concentration in the fetal circulation was 8.9 ± 2.3 mg %. A subsequent increase in concentration was observed in all experiments, and in absolute terms the increase averaged 1.9 mg % after 15 min and 13.8 mg % after 135 min of perfusion. The increase compared with the initial concentration was highly significant ($P = 0.01$) after perfusion for 75 min. The dynamics of transport is illustrated in Fig. 2, which clearly shows the rapid increase in concentration of amino nitrogen in the fetal circulation of the perfused placentas. In individual experiments the concentration rose very considerably, by 5.8-7.9 times.

By creating concentration gradients of the substances used for testing, in a physiological direction, the processes of transplacental transport of these substances can be quantified. Differences between urea and α-amino acids were observed already in the control experiments with perfusion of the placentas. If the urea concentration was the same in both placental circulations, no transport from the fetal to the maternal circulation took place. Under the same initial conditions the concentration of amino acids in the fetal circulation invariably increased, by a greater or lesser degree. This confirms the view that the "secretion" of amino acids by the cells of the trophoblast into the fetal circulation is one factor in the mechanism of transport of amino acids from mother to fetus [3].

The use of the method of two-way perfusion of the human placenta showed that it ensures sufficiently complete perfusion of the intervillous space of the placenta and creates the conditions required in order to study placental transport.

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