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PAPER

COMPENSATION MECHANISMS FOR EXPERIMENTAL REDUCTION OF THE CAPACITY IN THE GUINEA PIG PLACENTA

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ABSTRACT
After experimental reduction of its functional capacity the labyrinthine part of the placenta of the guinea pig has been studied microscopically for changes in the trophoblast and vascularization pattern, which could be interpreted as compensation for this reduction. In the experimental groups the volume density of fetal capillaries was increased significantly compared with untreated controls. The surface density of these vessels remained the same. The volume density of the maternal lacunae did not change significantly. No decrease of the thickness of the maternofetal barrier could be measured, nor did enlargement by microvilli of the apical and basal surface of the syncytium change significantly. The conclusion is that after experimental reduction of its functional capacity no pronounced structural modification in the guinea pig placenta occurs, which can be interpreted as a compensation mechanism.

INTRODUCTION

During the course of the development of the human placenta several typical histological differentiations occur in the trophoblast resulting in an increase of the exchange area between maternal and fetal blood. In maturitas praecox placentas some of these phenomena, e.g. vasculosyncytial membranes and microvilli, develop much earlier (Becker, 1963) or in the case of strongly infarcted placentas, show a relative increase (Bender, 1974). Obviously the histological changes concerned are a partial compensation for the reduction of functional capacity occuring in these placentas. Next to the changes in the placenta the human fetus

has another, very important possibility for adaptation to a reduced functional capacity by decreasing its growth. These rather large adaptation capacities can possibly be connected with the relative low degree of development of the human neonates. The newly born guinea pig, however, is born after a long pregnancy of about 9 weeks, is very well developed and the weight of the newborn is already 10% of that of its mother.
In human placentas the chance of a sudden reduction in capacity is rather high as a consequence of the frequent occurrence of infarction. In guinea pig placentas, however, infarction is practically absent. The question can be raised whether a reduction of the functional capacity of the guinea pig placenta could also develop some histological changes which might be interpreted as compensatory changes.

MATERIALS and METHODS
Young primigravid albino guinea pigs were used throughout the study. The reduction of the functional capacity of the placenta was attempted in four different ways: (1) Pregnant animals were made anaemic by subcutaneous injections of phenylhydrazine chloride (PHZ). About 3 weeks before the expected birth the animals were given 0.5 ml of a 2.5% solution of PHZ on day 1 and 0.25 ml on the next day. They were then given 0.25 ml every 3 or 4 days, but after about 10 days the dosage had to be raised to keep the haemoglobin concentration below 7 mmol/l. Controls which were given the same dosage of Ringer's solution had a haemoglobin concentration of 8.6 mmol/l. (2) Pregnant animals were anaesthetized with halothane. One lateral abdominal incision was made, and the rim of the disk-shaped placenta was brought into view by a little incision in the uterine wall. With a fine soldering bolt a peripheral segment of the placenta was destroyed by heat coagulation. These lesions were made about 14 days before the expected birth. (3) Injections of monoiodic acetate (MIA) in pregnant guinea pigs cause an explosive formation of typical protrusions of the syncytiotrophoblast in the placenta (Kaufmann, 1974). These protrusions are cut off from the surface of the syncytium where they have been formed and can obstruct maternal blood flow. As a consequence infarcts are found. Pregnant guinea pigs were given a single dose of 25 mg MIA per kilogram body weight intraperitoneally about 3 weeks before the expected birth. (4) Colchicine also causes the formation of protrusions, but more intensely than MIA. Animals were given a single dose of 100 mg colchicine per kilogram body weight

intraperitoneally.
In all experiments the survival of the dams was poor and about 50% died or aborted before the end of the experiment. At the end of the experiment the pregnant guinea pigs were anaesthetized with halothane and the placentas fixed by perfusion via an umbilical cord artery. A solution of 2.5% glutaraldehyde in 0.07 M Na-cacodylate was used at room temperature. For electron microscopical examination small tissue fragments were fixed by immersion for 1 h in 1% osmium in 0.07 M Na-cacodylate, pH 7.4. After dehydration the material was embedded in epon. For light microscopical examination 1 µm sections were cut from the epon embedded material and 6 µm sections from the paraffin embedded tissue. In a projection microscope (Olympus UM 350) the volume density of the interlobium and labyrinth in the 6 µm sections was measured using a point grid and an enlargement of 20. For the measurements of the volume density (V_v) and surface density (S_v) of the maternal lacunae, fetal vessels and syncytiotrophoblast in the labyrinth of the 1 µm sections a Zeiss integration ocular with a 25 point grid was used and an enlargement of 1000. In each placenta 2,000 points were counted. In the 1 µm sections also the diameter of capillaries and lacunae was measured using an ocular with a linear calibration scale. As the relative section thickness (i.e. the ratio between section thickness and diameter of the blood vessels) was very small, the applied corrections for the Holmes effect on the V_v and S_v of the fetal and maternal vascularization had a minor influence (Weibel, 1979). The epon embedded material from the colchicine treated group and the control group was examined under the electron microscope and photographed systematically, starting in the upper left corner of the section and taking a photograph after each tenth field. The outline of the syncytiotrophoblast, lining the maternal lacunae, was measured, with and without microvilli, on the electron microscopic pictures using a digital curvimeter to determine the surface enlargement by means of microvillous-like structures. The same was done on the basal side where the trophoblast forms microvilli-filled spaces between the basal lamina. The mean thickness of the maternofetal barrier was determined on electron microscopic pictures with the aid of an isotropic test grid and a precision measuring gauge. The thickness of the barrier was measured only in places where the semicirculartest lines crossed the haemal barrier, however, with the restriction that no trophoblastic nucleus was enclosed and that both sides of the barrier made

an angle of under 45°. To calculate the mean value, a logarithmic transformation of the measurements was employed to eliminate as much as possible the influence of tangential cuts. Of all measured parameters the mean value was calculated and these values have been corrected statistically (analysis of variance) for the influence of the litter size and the difference in the numbers of fetuses in both uterus horns.

RESULTS

It appears that only in the colchicine treated group the mean placental and fetal weights are significantly lower than in the controls (table I). In the placental lesion group only the fetal weight is significantly lower. But in both groups no significant alternation occurs in the ratio (P_i) between placental and fetal weight.

Table I. Mean placenta weight and fetus weight per dam in gram.

Group	Placenta weight			Fetus weight			P_i.1,000		
	n	mean	SD	n	mean	SD	n	mean	SD
Control	19	7.0	2.2	19	104	19	19	66	12
PHZ anaemia	12	7.6	2.0	12	111	23	12	68	9
MIA	2	7.1	1.2	7	104	10	2	66	13
Lesion	5	6.0	1.4	5	87[a]	18	5	68	7
Colchicine	14	5.5[b]	0.9	14	91[c]	20	14	62	6

n = Number of pregnant females.

P_i = Ratio placental:fetal weight.

[a] $p = 0.037$; [b] $p = 0.026$; [c] $p = 0.023$.

The V_v of the labyrinth (fig. 1) part of the placenta remains the same in all groups. The V_v of the interlobium did not change in any experimental group (table II).

After experimental reduction of the functional capacity of the placenta, the fetal capillaries in the labyrinth show an increase of the V_v, but no difference in the S_v (table III).

V_v and S_v of the maternal lacunae do not change significantly in any of the experimental groups. Although the mean diameter of capillaries and lacunae did not change significantly, there is an increased variance in dia-

Table II. V_v of the tissue compartments in the placenta in percent.

Group	n	Interlobium		Labyrinth	
		mean	SD	mean	SD
Control	7	10.8	2.0	75.2	2.2
PHZ anaemia	7	9.6	1.5	74.8	3.7
MIA	5	12.6	1.7	71.9	2.0
Lesion	8	12.8	3.4	73.7	3.1
Colchicine	6	13.3	2.9	75.9	3.3

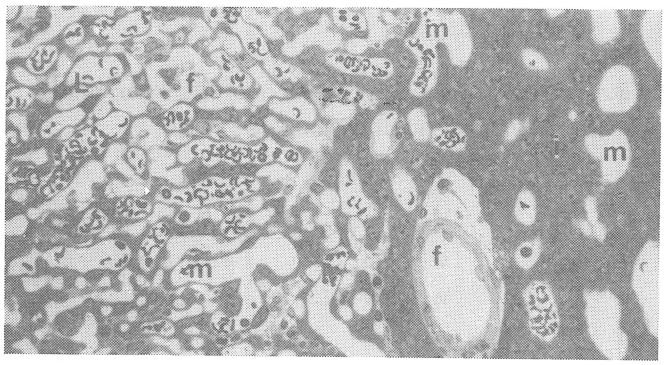

Fig 1. Interlobium and labyrinth in the guinea pig placenta. f=fetal vessel; L=labyrinth; m=maternal lacuna; i=interlobium. Light micrograph x 237.

Table III. V_v, S_v and diameter of the blood systems in the labyrinth.

Group	n	V_v in percent				S_v, mm^2/mm^3				Diameter in µm			
		fetal capillaries		maternal lacunae		fetal capillaries		maternal lacunae		fetal capillaries		maternal lacunae	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Control	6	15.3	3.2	40.8	12.6	146.8	17.9	175.3	19.7	18.5	0.6	34.6	12.6
PHZ anaemia	6	[illegible]	5.6	42.4	12.7	153.5	29.5	174.9	15.7	17.0	5.4	33.9	13.1
MIA	4	[illegible]	7.2	40.8	9.9	141.5	15.0	170.0	18.0	18.6	8.0	34.7	12.0
Lesion	5	[illegible]	2.6	40.2	4.1	149.5	19.5	160.6	20.5	18.8	1.5	35.6	12.0
Colchicine	6	17.6	4.1	34.7	11.5	140.0	24.8	141.3	32.0	20.0	7.2	39.3	10.8

[a]p = 0.020; [b]p = 0.012; [c]p = 0.036.

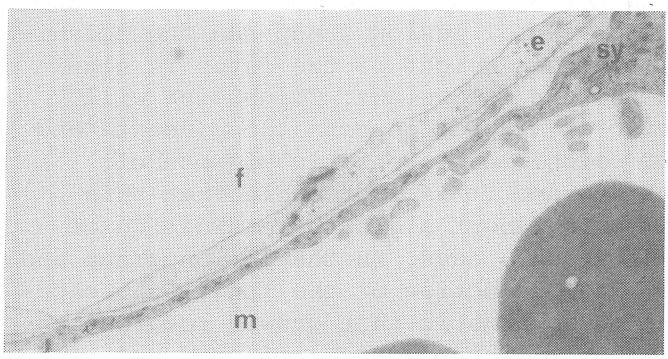

Fig 2. Very thin part of the maternofetal barrier in the labyrinth. f=fetal vessel; m=maternal lacuna; e=endothelium; sy=syncytiotrophoblast. Electron micrograph. x 64,600.

meter of the fetal capillaries in the experimental groups. In the guinea pig placenta, after reduction of the exchange area, no significant decrease of the maternofetal barrier thickness was observed in the colchicine-treated group (table IV, fig. 2). The exchange area of the placenta can be considerably increased by microvilli on the surface of the trophoblast. In the control placentas we found an enlargement of the surface of the syncytiotropho-

Table IV. Mean thickness of the maternofetal barrier.

Group	n	mean	SD
Control	3	1.58 µm	0.20
Colchicine	9	1.46	0.19

Table V. Enlargement factors of the syncytiotrophoblast surface lining the maternal lacunae and on the basal side.

Group	Apical side			Basal side				
				basal spaces without microvilli			inclusive microvilli	
	n	mean	SD	n	mean	SD	mean	SD
Control	3	2.62	0.5	5	1.14	0.07	2.37	0.35
Colchicine	8	3.16	0.6	8	1.18	0.08	2.28	0.44

blast lining the maternal lacunae with a factor 2.62 (table V). In the placentas of the colchicine-treated group there is no extra enlargement of the apical syncytial surface. On the basal side of the syncytiotrophoblast also no change in the microvillus area could be observed.

DISCUSSION

Any reduction of the functional capacity of the placenta means a reduction of transport of nutrients which could entail a retardation in the growth of the fetus. In two experimental groups, the placental lesion group and the colchicine-treated group, the mean weight of the fetus is significantly lower than the control weight. Colchicine will cause a short blocking of mitoses in both the placenta and fetus. The effect of colchicine on mitotic microtubuli lasts only for about 6 h and it is improbable that this short blockade could bring about a growth retardation of placenta and fetus of about 5 days. In the lesion group and the colchicine group, the reduction of the functional capacity of the placenta is so drastic that growth retardation of the fetus is inevitable. This decrease of growth is in proportion to the lower weight of the placenta because the ratio placenta : fetal weight (P_i) does not change. The placental lesion and the colchicine treatment cause a slowdown or a temporary stop of the placental growth which also means, in view of the lower weight of the fetus, that these smaller placentas have a reduced functional capacity without development of sufficient compensation mechanisms. In the placenta of the guinea pig no direct exchange can take

place in the interlobium between maternal and fetal blood as fetal capillaries are lacking here. Consequently, in the case of a reduction of the capacity, one would expect a decrease of the interlobium in favour of an increase of the labyrinth. In contrast to this expectation there is a somewhat higher V_v of the interlobium and no significant expansion of the labyrinth in most experimental groups. The function of the interlobium as maintainer of pregnancy by production of progesteron may play a possible role (Burgess and Tam, 1978).

Although the V_v of the labyrinth itself does not change in the experimental groups, the V_v of the fetal capillaries shows an increase. The S_v of the capillaries remains the same as in the control group, as does the V_v of the maternal lacunae. There is an increase of the variance in diameter of the fetal capillaries but the mean diameter did not change. It is difficult to interpret these changes in diameter in a way of a compensation mechanism. An increase of the diameter of the fetal vessels allows a better perfusion, and this is an adaptation which occurs in the human placenta with infarcts (Bender, 1974).

After an infarction the transport through the maternofetal barrier in the human placenta can be facilitated by an increase in the number of vasculosyncytial membranes leading to a decrease in the overall thickness of the barrier. Although during the normal maturation of the guinea pig placenta the intervascular distance falls significantly (Firth and Farr, 1977), no decrease of the barrier thickness was observed in our material after a reduction of the placental exchange area as occurred in the colchicine-treated animals. Comparing the mean thickness of the haemal barrier in the guinea pig placenta with that in the human placenta, the guinea pig placenta has a much smaller mean intervascular distance. In the guinea pig placenta we measured a mean thickness of 1.5 µm, whereas in the human placenta this distance is between 3 and 6 µm (Aherne and Dunnill, 1966). It might thus be possible that the histological structure of the labyrinth does not leave room for a further decrease in the barrier thickness.

With regard to the increase of microvilli in the guinea pig placenta during its normal development (Firth and Farr, 1977), it might be expected that after a reduction of the exchange area in the colchicine-treated group there would be a tendency to enhance this area by increasing the number of microvilli in the remaining functional labyrinth. However, the enlargement of the syncytial surface by development of

microvilli appears not significantly different from the control value.

In conclusion it might be deduced from the results of these investigations that in the guinea pig placenta after reduction of its functional capacity no explicit structural modifications are provoked which can be interpreted as compensation mechanisms. So, these observations seem to indicate that the guinea pig placenta has a very small reserve of functional capacity and that compensation of reduction of this capacity appears possible only on a very limited scale.

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