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Experiments on Infarct Genesis Caused by Blockage of Carbohydrate Metabolism in Guinea Pig Placentae*

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Summary. The poisoning of pregnant guinea pigs with monoiodineacetate, an inhibitor of carbohydrate metabolism in the glyceraldehyde-3-phosphate dehydrogenase phase, leads within a few minutes to the formation of syncytial plasma protrusions in the maternal blood lacunae of the placenta. These protrusions cause a primary white infarct where the degenerative process takes place—in the interlobium. An accumulation of erythrocytes originates in its arterial inflow area owing to the obstruction of the blood flow. Both primary infarct and accumulation of erythrocytes alter to form a single homogenous white infarct within a few days, together with the degenerative products of all cellular and syncytial elements. Timely substitution of glycolysis for pyruvate, i.e. within 20 min, during continued blockage of the glyceraldehyde-3-phosphate dehydrogenase, prevents the formation of an infarct.

Introduction

Studies of the genesis of placental infarcts have led to the discovery of several possible causes. In particular the occlusion of spiral arteries (Wallenburg *et al.*, 1973) and the impedement of venous drainage (Wigglesworth, 1969) were generally accepted as pathogenetic factors. We recently showed that plasmal protrusions play an essential part in the formation and spreading of placental infarcts (Stark and Kaufmann, 1974). These plasmal protrusions are polypus-like growths of the syncytiotrophoblast in the placental villi, poor in organelles and growing into the intervillous space (Kaufmann, 1969). From this point, they may drift with the blood-stream into the maternal circulation (Stark and Kaufmann, 1971).

The formation of plasmal protrusions in the placenta seems to be due to block of the Embden-Meyerhof decomposition of carbohydrates. This was demonstrated histochemically (Kaufmann and Stark, 1972), biochemically (Thorn *et al.*, 1974) and in experiments on animals (Kaufmann *et al.*, 1974). In the latter study we had already referred to the formation of infarcts in a guinea-pig placenta after interruption of carbohydrate metabolism. In this process plasmal protrusions occupy a key-position.

The formation of placental infarcts through plasmal protrusions after experimental disturbance of carbohydrate metabolism is to be investigated more closely in present study. The guinea-pig placenta proved to be particularily suitable to this research, since the maternal blood here, unlike to that of human placenta, flows in defined streams through the blood lacunae.

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Material and Methods

We had 45 pregnant guinea-pigs at our disposal, they were 50 to 60 days pregnant—the average duration of pregnancy being 63 days—and weighed between 850 and 1200 g. The animals were given per kg body-weight 10–30 mg of monoiodineacetate (MIA) intraperitoneally. MIA primarily blocks glyceraldehyde-3-phosphate-dehydrogenase. This effect in the placenta should be considered as the main cause for the findings dealt with here, as was already demonstrated in a preceding study (Kaufmann *et al.*, 1974). Poisoning with sodium fluoride blocks the enolase and leads to similar changes. The morphological alterations caused by an existing block by MIA or sodium fluoride may be avoided by means of substitutions of glycolysis for pyruvate of lactate.

A certain proportion of the test animals received 500 mg of sodium pyruvate intraperitoneally, at first $^1/_2$ h, 2 hrs or 6 hrs after poisoning, subsequently in intervals of 2 hrs. The animals were killed by a blow on the head in intervals of 8, 16 and 32 hrs and 3, 5 and 10 days after the beginning of the experiment. Small parts of tissue were taken from the placentae of living foetuses only. These samples were fixed in 6% phosphate buffered glutaraldehyde, as well as in 1% phosphate buffered osmiumtetroxide. We must renounce perfusion fixation because of two reasons: A sufficed fixation from the fetal side is not possible, because the placenta of the guinea pig shows only a partially vascularisation. On the other hand, perfusion of the maternal part is not ingenious, because the examined experiments just aimed to the displacement of the maternal lacunes. That the ischaemic alterations by delayed fixation not yet are a decisive motive for our findings, show the experimentations. However, the plasma protrusions we see in a little number of the not-perfused control-animals may partly be ischaemically. After dehydration by alcohol, the material was embedded in Epon. 1 μ sections were stained with toluidin-blue/pyronin, as well as darrowred/toluidin-blue. Ultra-thin sections were contrasted with lead citrate and uranyl acetate. The ultrastructural proteoglycane proof was carried out with ruthenium red (Schwarz, 1973).

Electron microscope: Philipps EM 300. For better survey halved placentae were fixed according to Gendre and embedded in paraffin. The sections were stained with hematoxilin and eosin or with alcian blue.

Results

As the morphology of the guinea-pig placenta differs greatly from that of the human (Kaufmann, 1969), we shall describe it here briefly for this is of considerable importance to the comprehension of the experiments dealt with (Fig. 8).

The guinea-pig as well as the human placenta belong to the hemochorial type. The maternal and fetal blood is only separated by fetal capillary endothelium, fetal connective tissue and by the syncytotrophoblast, which is in direct contact with the maternal blood. Contrary to the villous human placenta, that of the guinea-pig is lacunal, i.e. an originally massive trophoblast is interspersed with a system of parallel channels, the lacunae. Through these the maternal blood flows in a constant direction. The greater part of this tissue contains in addition a network of fetal capillaries—the labyrinth. The capillaries lie parallel to the arterial and capillary part of the lacunae and their blood flows in the opposite direction to that in the lacunae. The area of the trophoblast devoid of capillaries is termed the interlobium. The structure of interlobium and labyrinth is as follows: Lobes of the labyrinth tissue, in which we find radially arranged capillaries and lacunae, are surrounded by thin layers of interlobium, through which the maternal arterial blood coming from the center of the lobes flows into the uterine veins.

Energy metabolism is particularly intense in the interlobium, judging by histochemical criteria (Vollrath, 1965). The interlobium is accordingly strongly affected by a block of energy metabolism, e.g. through monoiodine acetate. We

have already discussed the early ultrastructural alterations which occur 8 mins. to 7 hrs after intravenous injection of MIA (Kaufmann *et al.*, 1974).

8 hrs after intraperitoneal injection of 30 mg MIA, the rough endoplasmic reticulum, the mitochondria and the Golgi apparatus of the interlobial syncytium appear extremely dilated. Groups of vacuoles of 0.5 to 3 μ in diameter occur; they contain varying amounts of electron-dense fibrous material. The lacunae are totally obstructed by plasmal protrusions of 2–30 μ in diameter, poor in organelles, which were cut off from the free surface of the syncytium (Fig. 1). The syncytium of the labyrinthal lacunae only forms very few of these protrusions. There we find a considerable accumulation of maternal blood since the drainage of venous blood through the interlobium has been blocked (Fig. 2). The lacunae are thus widened and thereby diminish the diameter of the capillaries. One can demonstrate no qualitative or quantitative difference to the normal placenta in the distribution of proteoglycane, neither with the electron microscope and ruthenium red, nor with the light microscope and alcian blue.

16 hrs after intraperitoneal injection of 20 mg of MIA, the syncytium shows more vacuolar space due to increased dilatation of the mentioned cell organelles, whereas the nuclei have shrunk considerably. The plasmal protrusions start decomposing. The blood plasma between them shows a fine fibrillous structure. The erythrocytes accumulated in the labyrinth stick so closely together that one can no longer distinguish parts of the cell membranes. These disintegrate partly. The proteoglycane proof with alcian blue shows a diffuse increase of the reaction in the interlobium.

Microscopically, there is little change definable in the syncytium 32 hrs after injection of 10–20 mg of MIA. Nearly all the plasmal protrusions have disintegrated by now. One finds bundles of fibrils 10–20 Å thick, in the syncytoplasma and in the lacunae. The blood accumulation is more pronounced. In the labyrinth big confluing hematomes have been formed under compression of the syncytium.

Owing to the small dose of MIA—necessary to keep the animals alive long enough—these changes no longer occur diffusely in the entire placenta, but are only to be found in approximately half of the organ, while in the other half an alteration of the organelles also takes place, but the formation of protrusions remains slight and the blood accumulation accordingly unimportant. Proteoglycanes can now be shown with the help of alcian blue in the interlobial syncytium and, to a lesser extent, in the plasmal protrusions. Their distribution is clearer in the electron microscopical picture after the application of ruthenium red: the fibrillous content of the vacuoles, 0.5–3 μ in size, is strongly ruthenium red positive. This also applies to the bundles of fibrils in the maternal blood plasma of the lacunae and in the disintegrated protrusions, however not to such an extent. Similar bundles of fibrils can be seen faintly in the syncytoplasma with the use of this method. In addition, one finds rosette-shaped ruthenium red positive material in the matrix of the mitochondria (Fig. 3), as well as in the form of small and very dense druses in the nuclei of the syncytium. The specificity of the last mentioned findings is questionable. The only ruthenium red positive material in the syncytium of the labyrinth are the bundles of fibrils. Nuclei and mitochondria are inconspicuous. The same can be said of practically all the fetal vessel and connective tissue cells.

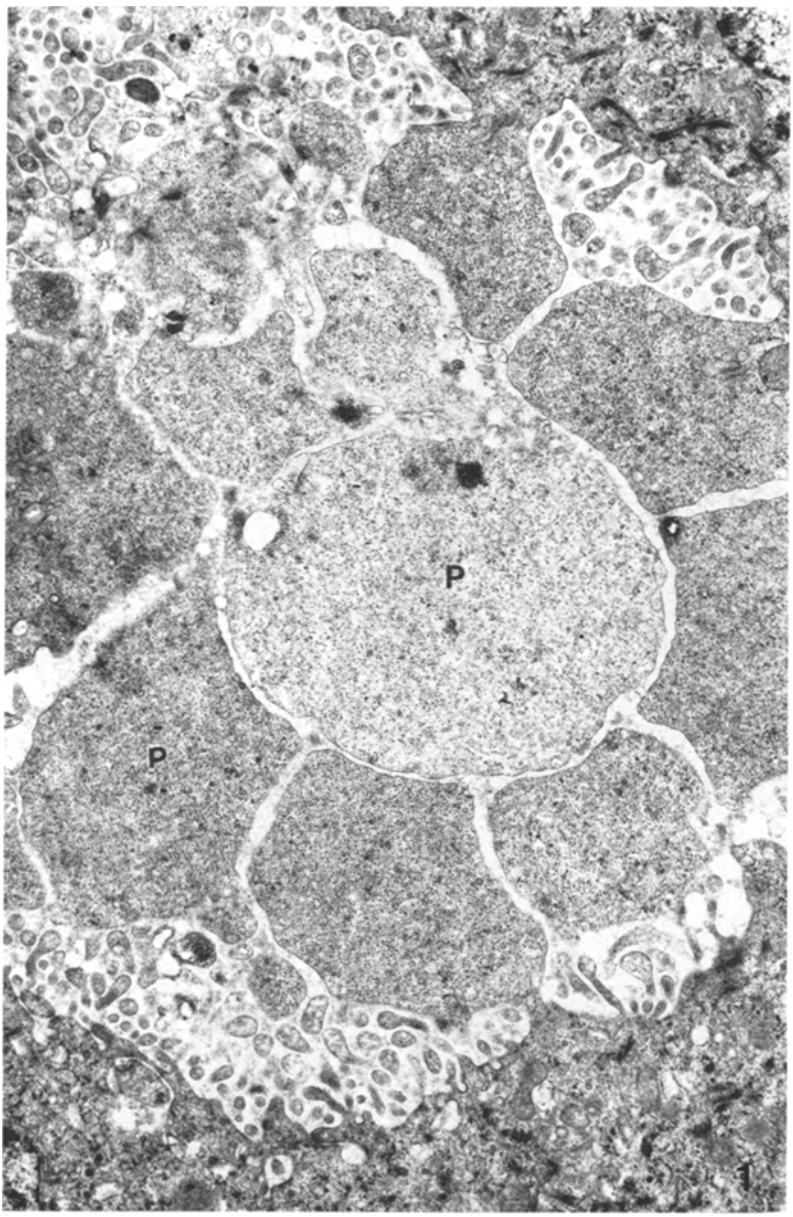
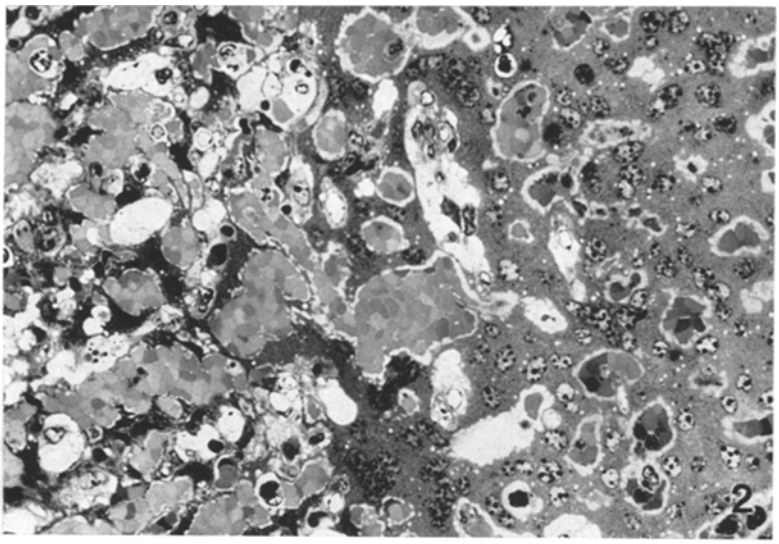


Fig. 1. Electron microscopical presentation of an interlobial lacuna, 30 min after iv. injection of 50 mg of MIA. The lacuna is totally obstructed by plasmal protrusions (*P*). ×10500

In the course of the following days, up to the fifth day, the mass of the proteoglycanes increases histo- and cytochemically. The ultrastructural parallel is a drastic increase of the fibril bundles in the syncytoplasma and in the lacunae (Fig. 4), where the plasmal protrusions were previously to be found. These have shrunk to an extreme degree, or else are only recognisable as membrane remain-



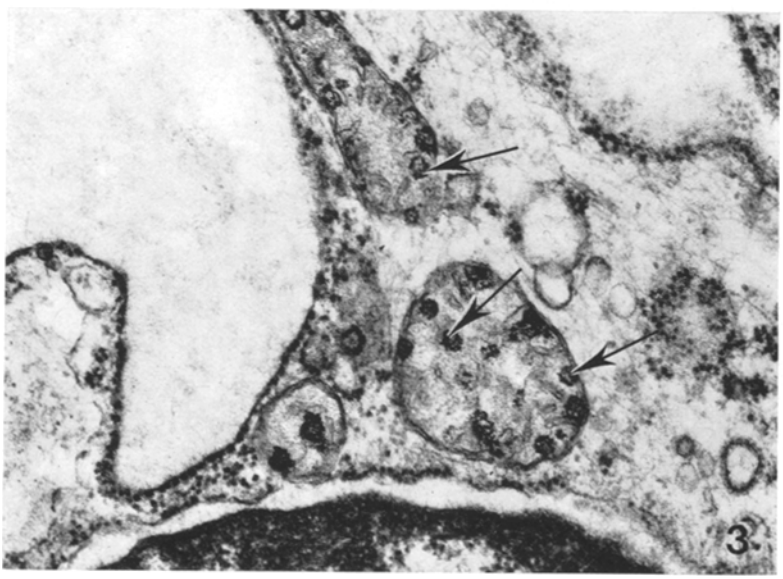


Fig. 2. 8 hrs after ip. injection of 20 mg of MIA. The lacunae in the interlobium (on the right) are blocked by plasmal protrusions. The lacunae of the labyrinth (on the left) are dilated by the accumulation of erythrocytes, syncytium and capillaries in between are displaced (1 μ-epon-section; toluidin-blue pyronin). ×450

Fig. 3. Electron microscopical proteoglycane proof with ruthenium red in the form of small rosettes (arrows) in the mitochondria of the syncytium. The specificity is questionable. 32 hrs after ip. injection of 15 mg of MIA. ×40000

ders (Fig. 5). The erythrocytes in the labyrinthal lacunae turn increasingly into a homogenous electron-dense mass. The labyrinthal syncytium between them degenerates to a fibrillous mass.

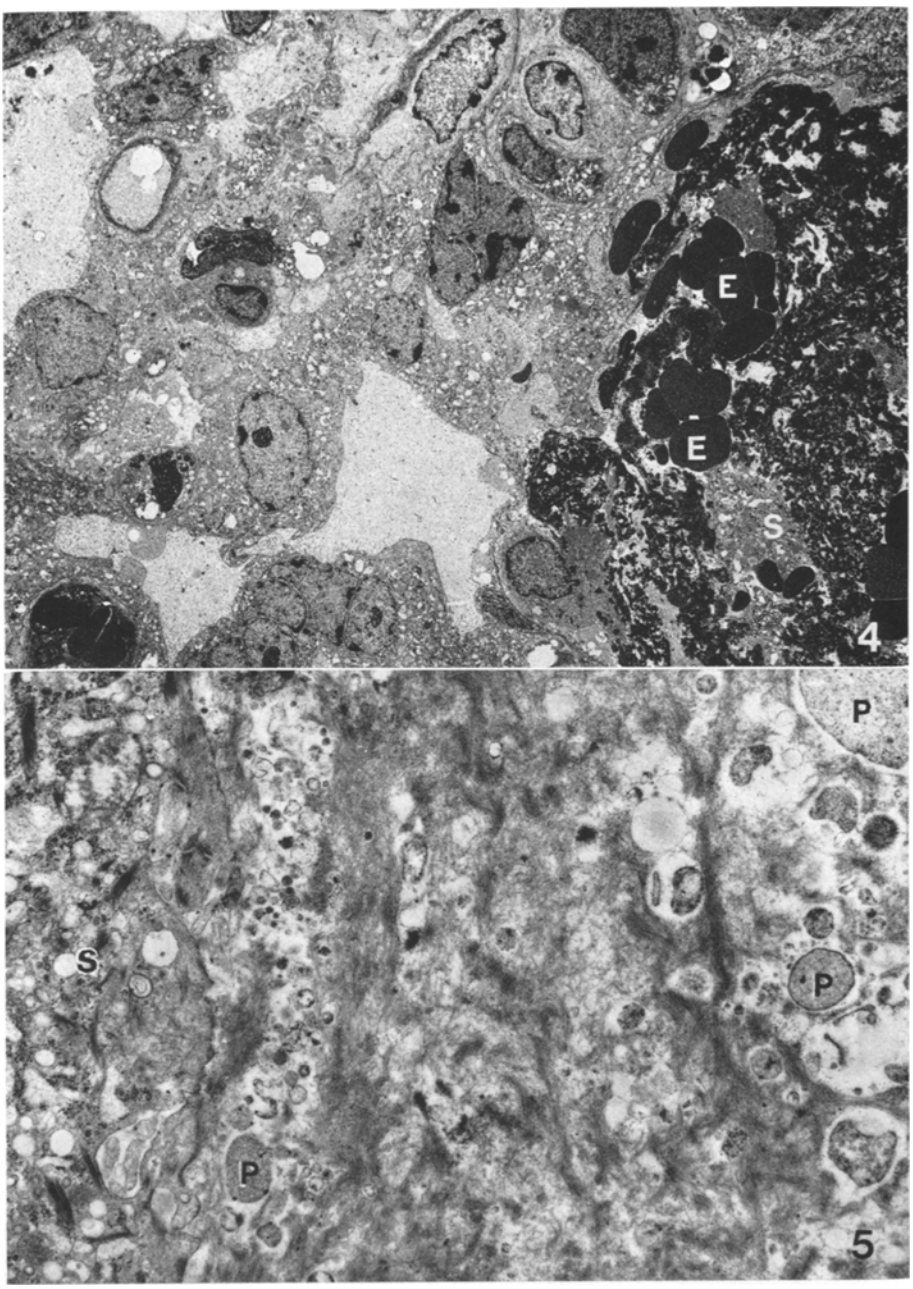
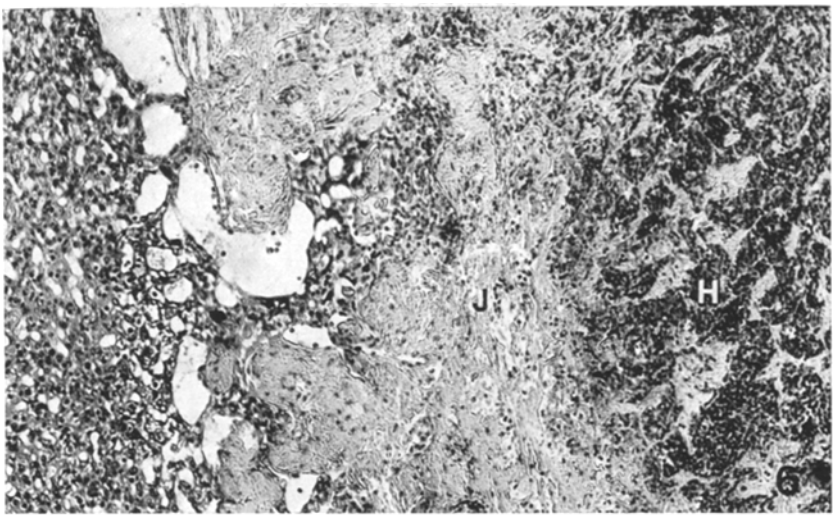


Fig. 4. 5th day after ip. injection of 10 mg of MIA. Large parts of the labyrinth (on the left and in the middle) are once again well supplied with blood, the syncytium only shows a few vacuoles. On the right, the remainders of a hematome, which was locally confined owing to the low dose of MIA. Its fibrinoid change has begun. A few erythrocytes (*E*) are still to be seen. Extensive bundles of fibrils and degenerating rests of syncytium (*S*) lie in between. (Electron microscopical picture.) ×1400

Fig. 5. On the 5th day after ip. injection of 15 mg of MIA, the plasmal protrusions (*P*) shrink or desintegrate, the mass of bundles of fibrils (fibrinoid) increasing enormously. On the left one sees rests of syncytium (*S*). (Electron microscopical picture.) ×10000



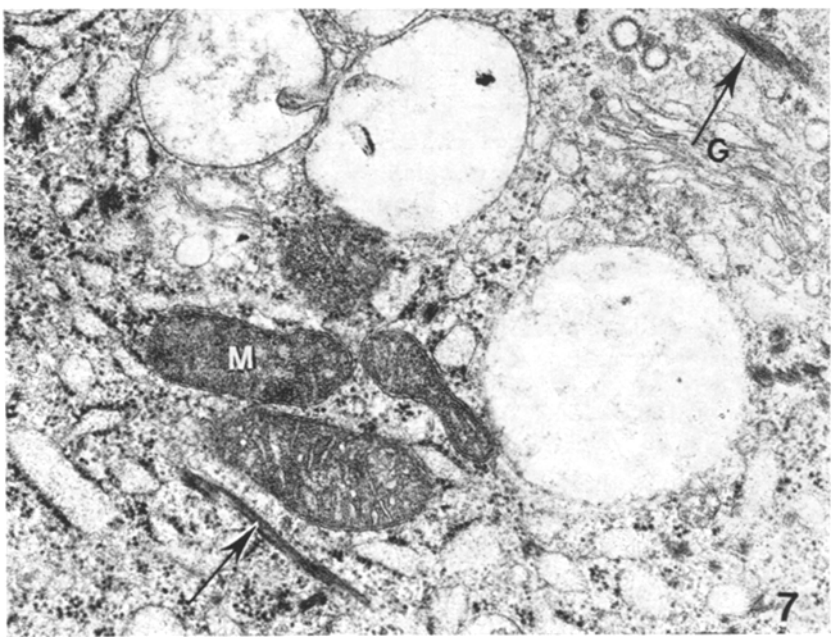


Fig. 6. A primary white infarct (*I*) in the interlobium, next to it on the right, in the labyrinth, an accumulation of erythrocytes (*H*) in the process of fibrinoid change. On the 5th day after ip. injection of 15 mg of MIA. (Paraffin section, hematoxilin-eosin stain.) ×80

Fig. 7. Placenta, poisoned with MIA, substituted with pyruvate. Thanks to the substitution with pyruvate, mitochondria (*M*) and Golgi apparatus (*G*) are well preserved. As a result of MIA poisoning, only vacuoles and single intrasyncytial fibrils (arrow) occur. (Electron microscopical picture.) ×30000

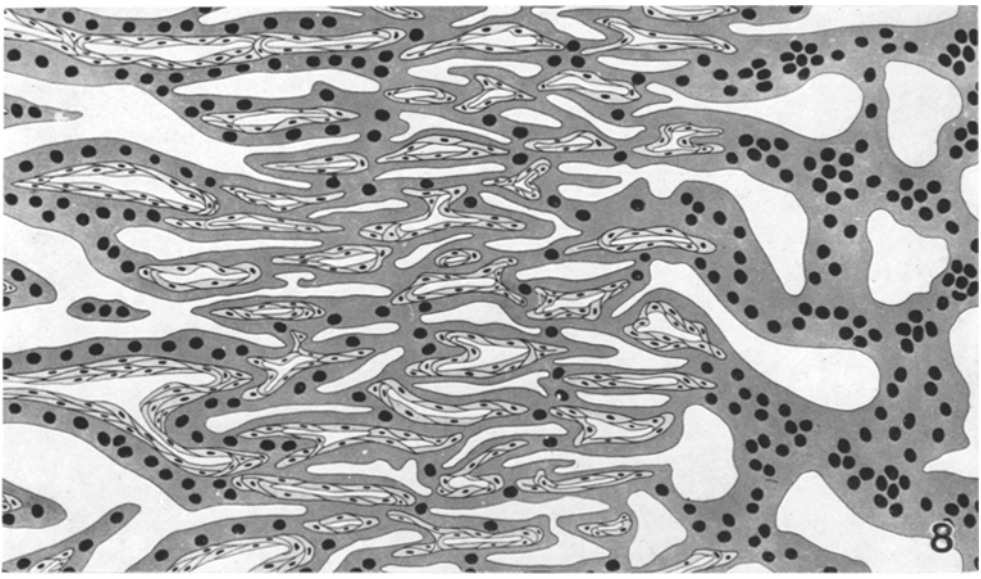
After the fifth day the histo- and cytochemical proof of the proteoglycanes diminishes rapidly. Structurally, however, the bundles of fibrils in the lacunae and syncytium increase further beyond this date. Organelles and nuclei in the cells now disintegrate completely, until finally a uniform ruthenium red negative network of fibrils is left; these were formed from the content of the lacunae and the syncytium. At the same time, around the tenth day, the plasmalemm decomposes. The former syncytium is no longer to be differed ultrastructurally and light microscopically from fibrinoid.

In the interlobium, where the primary degenerative process takes place, a white infarct developed after the formation of plasmal protrusions, deposition of protoglycanes, and emergeance of fibrinoid (Fig. 6). In this area, masses of plasmal protrusions—having partly degenerated by turning fibrillous—may be carried into the subsequent bigger venous lacuane and cause a new white infarct there. Within ten days, the accumulated erythrocytes have disintegrated to the extent that only precipitated fibrillous material remains, usually not to be distinguished from the fibrinoid formed in the interlobium. Thus the accumulation of erythrocytes in this area has changed secondarily to a white infarct. The fetal capillaries, compressed by the maternal blood accumulation, decompose and form star-shaped cells. Their volume increases owing to the formation of vacuoles. They appear subsequently to be the origin of the proliferation of connective tissue in the fibrinoid.

Provided the animals poisoned with MIA were given a sufficient amount of pyruvate from the 30th minute onwards, no particular pathological alterations were to be found in any of the following stages. Just the described vacuoles containing ruthenium red positive substance remained (Fig. 7). However, neither plasmal protrusions nor blood accumulation came into being. If one only starts substitution two hours after poisoning, single small infarcts occur and go through the changes indicated above. However, compared to the non-substituted animals, the alterations are much less explicit. In order to prevent secondary deterioration of the results, the substitution is to be maintained over a period of at least 48 hours by giving approximately 500 mg of pyruvate per kg body-weight in intervals of two hours. A substitution started later than two hours after poisoning leaves the course of pathological alterations, as described above, unchanged.

Discussion

The formation of plasmal protrusions is caused by a block of carbohydrate metabolism in the Embden-Meyerhof pathway, as we demonstrated in earlier histochemical (Kaufmann and Stark, 1972) experimental (Kaufmann *et al.*, 1974) and biochemical studies (Thorn *et al.*, 1974). Maintenance of the citrate cycle through sufficient quantities of substituted pyruvate or lactate prevents the formation of plasmal protrusions in spite of continuing block of the Embden-Meyerhof pathway. The attempt to provoke formation of plasmal protrusions through hypoxia or through uncoupling of oxidative phosphorylation with 2,4-dinitrophenol failed (Thorn and Kaufmann, in press). The reduced feeding of the citrate cycle due to the block of the Embden-Meyerhof pathway is held to be



maternal blood flow →

	labyrinth:	interlobium:
stage I:	block of maternal circulation	plasmal protrusions
	↓	↓
stage II:	accumulation of erythrocytes	primary white infarct
	↘	↙
stage III:	secondary white infarct	

Fig. 8. Scheme of the arrangement of lacunae and capillaries in the guinea-pig placenta: dark grey = syncytiotrophoblast, pale grey = fetal capillaries. Underneath, the development of experimental infarcts in relation to the maternal blood flow (Drawing by H. Hess)

responsible for the formation of plasmal protrusions. The experiments on guinea-pig placenta showed that this disturbance of metabolism alone may lead to the formation of infarcts (Fig. 8): white infarcts, poor in erythrocytes and primary fibrinoid occur where the degenerative process takes place, in the interlobium. From this point, parts of the protrusions—intact or already decomposed—get into the subsequent venous stream and cause a white "displaced infarct". In the vessels leading to both these primary infarcts, maternal blood accumulates within a few hours after the beginning of the experiment. This is especially pronounced in the guinea-pig placenta, as the direction of the blood flow is very defined owing to the parallel arrangement of the maternal blood lacunae. The degenerative centre blocking the venous stream cannot be evaded, or if so, only partly. Therefore, an extensive accumulation of erythrocytes is unavoidable in the lacunae in front of the degenerative centre. This expands under compression of the trophoblast.

In the days which follow, the erythrocytes perish, as in similar hematomes in the human placenta (Becker, 1970). The hemoglobin is washed out. Finally, after 10 days, a homogenous white, i.e. fibrinoid infarct remains; in this, one

can no longer distinguish between the original accumulation of erythrocytes in the labyrinth and the primary white infarction in the interlobium (Fig. 8).

The model experiments on the guinea-pig placenta show that not only primary obstruction of the maternal circulation in the placenta, but also metabolic defects in the syncytium itself may lead to the block of the maternal circulation and to typical infarcts. The very clear circulatory conditions in the guinea-pig placenta moreover allow one to recognize that the genesis of white infarcts and of accumulations of erythrocytes do not necessarily differ, but are to be exclusively attributed to the location of the degenerative process relative to the blood flow. A white infarct is formed at the emplacement of the primary degenerative damage. A similar white infarction may be expected in the venous drainage area. Accumulations of erythrocytes however, only occur in the arterial inflow area of a white infarct.

Up to now it is uncertain to which extent these findings are applicable to the human placenta in which the conditions are more difficult to survey owing to the lack of a strictly defined blood stream. However, it is not expected that fundamentally different rules should be valid here.

Even assuming that the majority of the human placental infarcts are primarily due to obstruction of the circulation (Becker, 1970; Wallenburg *et al.*, 1973), at least a secondary importance should be attributed to the polypous degeneration of the syncytium: As we showed in a preceding publication (Stark and Kaufmann, 1974), one can demonstrate a progression of the degeneration together with the formation of plasmal protrusions and subsequent block of intervillous space in the marginal area of a great many infarcts, the genesis of which was no longer to be traced. These "progredient infarcts" have zones of different grades of degeneration, structured in layers like an onion. They remain progredient until they have extended to an area in which the plasmal protrusions produced on the margin no longer influence the haemodynamics of the surrounding tissue negatively. The process only then comes to a standstill. The phenomenon also could be demonstrated in the guinea-pig placenta, but plays a much lesser part here as the haemodynamics are totally different.

The disturbance of intrasyncytial energy metabolism as described above may have—like on the margin of the progredient infarct—a nutritional origin, i.e. it may be caused by insufficient nutrition of the syncytium, the maternal haemodynamics having been affected. Therefore, they are certainly frequently connected etiologically with primary maternal circulatory trouble. But they may also be determined by a second etiological complex which, so far, has seldom been related to infarct genesis (Bartholomev *et al.*, 1961) and was usually rejected (Wallenburg *et al.*, 1973). Panigel and Myers (1972) demonstrated on the placentae of rhesus monkeys that fetal circulatory trouble is followed by damage of the syncytium; here too, plasmal protrusions were formed. We showed in earlier studies, that this process is probably caused by the reaction of the cytotrophoblast (Kaufmann, 1972; Kaufmann and Stark, 1972). The grade of the blood-flow in the fetal vessels regulates the proliferation tendency and the extent of the syncytial fusion of the Langhans cells. The Langhans cells in their turn are obviously necessary for the enzymatic regeneration of the syncytium. If, despite the presence of a large number of Langhans cells, too few Langhans cells change

into syncytium because of severe hypoxia, or if there are originally too few Langhans cells, e.g. in the case of hyperoxia, glycolysis of the syncytium comes to a standstill within a few days. At the same time, the syncytium degenerates in forming plasmal protrusions. Therefore, obstruction of the maternal circulation, as well as causes in fetus and placenta, may be related to the genesis of an infarct.

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