On the Transportation Channels of Substances in the Stroma of Placental Villi

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Works in the past upon the details of the mechanism of substance exchange in the placenta have been regrettably few, the mechanism being generally interpreted only in a schematic way in analogy with the process of gas exchange in the lung. There is, however, a wide anatomical difference between the placenta and the lung, the exchange of matters between the fetal blood and the maternal blood in the placenta being effected through the epithelium and the stroma of the villi and the walls of the capillaries. So, we can readily surmise the existence of some mechanism or channel of matter transportation in this part, but the past studies have been limited to the researches on the epithelium of the villi and little, if any, research has been conducted on the villous stroma and blood capillaries.

At this Department, we have been studying on the metabolism and the permeability of the placenta, availing ourselves of the histochemical technics rapidly advanced in recent years. Several reports\(^1\),\(^2\),\(^3\),\(^4\) have been already published on the results of these studies, and the present authors will report hereunder on the interesting findings suggestive of the transportation channels of matters in the stroma of the placental villi.

Experimental

Materials and Methods

The materials used were human placentae obtained at term, and with copper, iron, calcium and glucose, we made the three series of experiments described below, then sections were sampled choriodecidualy from the central and the marginal parts of the placenta and stained for microscopic examination, as given below.
1) Injection through the maternal cubital vein (Group M). Copper was used in the form of copper sulphate, 20 cc. of its 0.5% isotonic solution being mixed with 200 cc. of isotonic saline for slow intravenous injection. For iron administration a mixture of 15 cc. of 1% iron gluconate solution and 40 cc. of 20% glucose solution was used and calcium was injected in 40 cc. of 2% calcium chloride solution, while 100 cc. of 50% glucose solution was used in the glucose administration tests. The placental specimens were sampled upon parturition taking place immediately following or within 12 hours after the injection.

2) Injection through the umbilical artery (Group U). 200 cc. of the copper sulphate solution above, 200 cc. of a 0.03% iron gluconate solution in isotonic saline, 300 cc. of a calcium chloride solution in isotonic saline containing calcium in the concentration of 100 mg/dl or 200 cc. of a 5% glucose solution was injected into the placenta through the umbilical artery immediately after delivery of placenta.

3) Incubation (Group I). The decidual membrane was carefully peeled off the placenta immediately after expulsion and the placenta was incubated for 5–30 minutes at 37°C in isotonic copper sulphate, iron gluconate, calcium chloride or glucose solution.

Staining

For histochemical detection of copper, iron, calcium or glucose, Okamoto-Utamura’s paradimethyl-aminobenzylidenrhodanine staining and rubeanic acid staining, Prussian blue and Turnbull’s blue staining, Yamashita’s variation of Kossa’s method, or Okamoto-Kadoto-Aoyama’s method of using toluidine blue staining in combination were respectively applied to stain the sections concerned. The original Bielschowsky’s method, a variation of it by Foot or Gomori was used for impregnating argyrophilic fibrils.

Results

After copper administration, in the placentae from mothers subjected to the injection (Group M) no finding was observed different from the untreated placentae, but in the incubated specimens (Group I), a diffuse copper reaction was already observed in the syncytium cells of the villi and copper granules were found arranged linearly along the basement membrane beneath these cells. These copper granules stretched in lines further into the stroma and forming a net-work by mutual anastomosis, partially reached the walls of capillaries. In the placentae injected with copper sulfate solution through the umbilical cord (Group U) too, such reticules of copper granules stretching from the capillary walls into the stroma were observed and copper granules were detected also in a part of the basement membrane, but the result of searching for them in the syncytium cells proved negative. Such findings were observed only in a part of peripheral villi with normal epithelium, while in the villi of which the epithelial layer had disappeared and the stroma directly touched the fibrinoid tissue, a quantity of copper was observed in the fibrinoid mass alone, but no copper could be found in the stroma.

Iron administration was followed by results nearly identical with those after
copper injection as above.

In the experiments with calcium, the Group M showed little findings different from the control specimens, as in the preceding series, but in the Group I, we found a large quantity of calcium diffused moss-wise in the surface of the syncytium cells, and a small quantity of the element was found also in the cytoplasm of syncytium cells in nearly uniform distribution. Calcium was also found aggregated in lines in a somewhat larger quantity along the basement membrane beneath the syncytium cells, and in some part stretching into the stroma in the form of fibres and nets. However, it rather accumulated much in the fibrinoid tissue. In the Group U, a net-work of calcium was found running along the fibres stretched from the capillaries into the stroma and linear aggregation of calcium was observed beneath the epithelium of the villi, but the calcium granules in the cytoplasm of the syncytium cells were very small in number.

In the experiments of glucose administration, no appreciable difference was observed in the Group M, except in the placenta expelled 30 minutes after the administration, which contained glucose in its villous epithelium. In the Group I, glucose granules were found arranged in a line beneath the syncytium cells and forming a net-work between the same part and the capillary walls. The granules of glucose were absent in the stroma of the villi covered by fibrinoids and devoid of epithelium, as in the copper and iron. Similar pictures were seen also in the Group U and I.

Staining of argyrophilic fibrils

No argyrophilic fibrils were found in the chorionic plate and the connective tissue of the stem of villi, light brown-red collagenous fibres alone being detected under Bielschowsky’s original staining. Rarely, these were some parts under the epithelium in the stems of villi and branches where argyrophilic fibrils were detected, blood capillaries being found in such parts also. No collagenous fibres could be found in the villi. In thick specimens, the capillaries were represented by argyrophilic nets forming tubes (Gitterröhr). These argyrophilic fibrils extend into the stroma, forming reticules, and were found connected with the basement membrane consisting of coarse argyrophilic fibrils under the syntygium cells. In the villi lacking in epithelium and covered by fibrinoids, no argyrophilic fibrils but only collagenous fibres were observed.

SUMMARY AND DISCUSSION

When copper, iron, calcium or glucose was injected into the maternal veins, no noteworthy finding was obtained, but in the incubation experiments, the administered substance was always found aggregated into very perceptible lines along the basement membrane of the villous epithelium and in varying degrees also in the stroma in fibrous or reticular arrangements, reaching up to the capillary walls. The fibres in the connective tissue of iron and glucose granules were nearly as fine as the argyrophilic fibrils stained out by silver impregnation, but those of copper and calcium were somewhat coarser.
In the experiments of administering these substances through the umbilical artery too, similar results were obtained, except that calcium and iron were found nearly absent in the villous epithelium.

As cited above, the authors have obtained experimental results indicating the presence of the respective substance used in incubating the placental specimens, arranged in lines and reticules. From the fact that the running courses of these lines and nets are similar to those of the argyrophilic fibrils detected by silver impregnation, that no such reticular formation has ever been proved to exist outside the stroma of the villi and that the said substances were not to be found in the parts lacking in argyrophilic fibrils, such as the part of villi under fibrinoids, we feel nearly assured in assuming the location of the administered substances within the argyrophilic fibrils in the stroma of the villi. If we accept the contention of Nageotte\textsuperscript{7} and Doljanski-Roulet\textsuperscript{8,9} that the argyrophilic fibrils are essentially flows of tissue fluids mainly consisting in protein in vital stage, they should not be such solidly fixed entities as we see in stained preparations, but should be more free formations, and the findings that the fibres formed by different substances are not always of the same size does not constitute any ground for disputing the above our assumption. Thence, we are led to the hypothesis that the argyrophilic nets in the stroma serve as transportation routes of substances between the epithelium and the capillaries of the villi.

The argyrophilic fibrils in the placenta were found independently by Costero\textsuperscript{10} and Zaltan\textsuperscript{11} in the connective tissue of the villi by Bielschowsky\textsuperscript{'}s silver impregnation, and these authors discribed these as collagenous fibres in a preliminary stage of development. Later, Wienbach\textsuperscript{12} found argyrophilic fibrils in existence around the capillaries of the villi and at the same time observed that the cells of the epithelium over the parts rich in argyrophilic fibrils are denucleated and flattened, while those in the positions poor in argyrophilic fibrils contain nuclei and remain unflattened.

On the other hand, Hofbauer\textsuperscript{13} found that the iron arranged in reticularly in the stroma of the villi, but he did not touch upon the relation between the argyrophilic fibrils and such reticules.

In Japan, Kihara\textsuperscript{14} has made an interesting study on many organs and tissues and published the opinion that the net-work of argyrophilic fibrils serve as extravascular pathways for fluids, supplying channels for absorption and efflux to lymph capillaries and for absorption to venules. His disciple Tatsumoto\textsuperscript{15} claims that the argyrophilic fibrils in the placental villi also have the meaning as extravascular fluid pathways.

The results obtained by the present authors as described above may be said to have given positive grounds endorsing the opinion of Tatsumoto.
CONCLUSION

Placentae obtained at term were incubated in solutions of copper, iron or calcium compounds or of glucose, or injected with similar solutions through the umbilical artery, and we could demonstrate the existence of reticular arrangements of the administered substances in the villous stroma in all the cases. This finding, coupled with our finding in silver-impregnated specimens, led us to conclude that the argyrophilic fibrils in the stroma of the villi can serve as transportation routes between the epithelium of the villi and the villous capillaries.

References

1) Imamura, Nippon Sanka-Fujinka Gakkai Zasshi (Jap.), in press.
2) Sugawara, ibid., in press.
3) Yamashita, ibid., in press.
6) Okamoto et al., Nippon Taishitsugaku Zasshi (Jap.), 1948, 14, 35.
15) Tatsumoto, San-Fujinka No Shinpo (Jap.), 1955, 7, 83.
Fig. 1. Argyrophilic fibrils in the stroma of the villi. High magnification.

Fig. 2. Glucose in the stroma of the villi observed in the Group I. High magnification.