

EXPLANTATION EXPERIMENTS FOR STUDYING THE GROWTH OF TROPHOBLAST

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When studying the behaviour of explanted placental tissue, interest is generally focussed upon the production of hormones, and on the kind of cells involved in their production. It has been shown that chorionic gonadotropin (in the following: HCG) is produced by the cells of Langhans [8, 3, 10]. More recently, however, CLERCK [1] demonstrated a correlation between the production of oestrogens and the growth of syncytial elements. Investigations concerned with the growth of the placenta have revealed that the connective tissue components grow rapidly, whereas epithelial elements develop at a slower rate [2, 4, 6, 7]. In general, media composed of hen's plasma, chicken embryo fluid, Tyrode's solution and hen's serum have been used in these experiments. There are, however, no data as to the factors influencing the growth of the single cell types in the trophoblast.

Our experiments with trophoblast from early pregnancy sought an answer to two questions. First, as to eventual differences between pregnant's serum and other media in regard to the characteristics or quality of growth. Second, the influence of different culture media on the behaviour of the single morphological components of the trophoblast.

Material for study was obtained from cases of interrupted pregnancy. The interval between operation and explantation was about 1 hour. Two kinds of culture medium were used. In one series the medium was composed of plasma and serum from pregnant women who had been operated on, then Tyrode's solution and chicken embryo fluid. In the other series plasma and serum from non-pregnant subjects, Tyrode's solution and chicken embryo fluid were employed. Thus, the difference between the two series was that in the one serum and plasma from early pregnancy, and in the other serum and plasma from non-pregnant subjects were contained in the culture medium. Each series consisted of 20 to 30 cultures, kept at 37° C in an incubator. The explants were controlled every 24 hours, fixed in some cases on the 4th, but usually on the 7th to 10th days, and were stained according to Giemsa, or with HE. The cultures were washed at 3-day intervals.

Results

We have studied 300 cultures in each of the two media. The material was obtained from about 20 cases of interruption. In agreement with the data in the literature, connective tissue elements began to grow first, whereas the growth of epithelial elements ensued only later. The most marked growth of connective tissue and Langhans' cells occurred in the period between 72 and 96 hours.

Connective tissue cells grow from the culture in one plane, radially, and may extend to several times the size of the original culture surface. In both

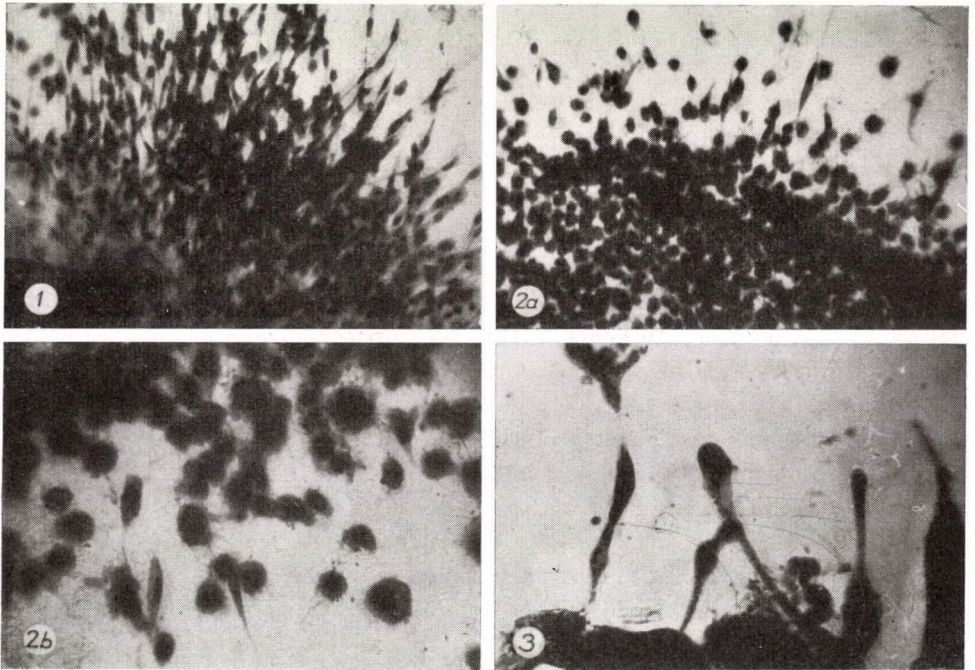


Fig. 1. 3-day placental culture in "non-pregnant" medium. Growth of fibroblastic type. Magnification, $\times 150$

Fig. 2a. 6-day placental culture in "pregnant" medium. Proliferation of Langhans' cells. Magnification, $\times 150$

Fig. 2b. 6-day placental culture in "pregnant" medium. Proliferation of Langhans' cells. Magnification, $\times 300$

Fig. 3. Cellular proliferation showing syncytial pattern. "Pregnant" medium. Magnification, $\times 150$

fixed and native preparations growth was observed at the sites where the villous stroma had been transected. In such cases oblong cells with processes and an oval nucleus grew on radial direction. These proved to be fibroblasts, occurring also in other tissue cultures (*Fig. 1*).

A comparison of the growths in the two kinds of medium revealed that in that containing "non-pregnant" serum and plasma, fibroblastic proliferation occurred most often and was more marked than in the "pregnant" medium.

Langhans' cells showed a slower rate of growth in one plane. Like the other epithelial cells, these cells spread usually concentrically, more or less separating at the periphery. In unstained specimens they were lighter than the fibroblasts and had round and ballooned nuclei (*Fig. 2a and 2b*). As to the growth in the two kinds of medium, Langhans' cells showed a more marked proliferation in the "pregnant", than in the "non-pregnant" plasma.

The syncytial elements began to grow during the first 27 hours of cultivation (*Fig. 4*), but stopped growing after 3 days. In general, the arrangement of newly formed cells was typical for the syncytial pattern. In this case, the "pregnant" medium had a more favourable influence on growth than the "non-pregnant" one, though the proliferation of Langhans' cells was more marked than that of the syncytium in the "pregnant" medium. In some cases the incubation mixture contained no embryonic fluid but this made no appreciable difference.

Discussion

Placenta from early pregnancy was explanted in media containing plasma and serum from women in early pregnancy and non-pregnant women, and the growth of the single components of the trophoblast was studied.

As compared to the "non-pregnant" medium, the "pregnant" one significantly enhanced the growth of the epithelial elements of the trophoblast. Of the elements of the chorionic epithelium, especially the Langhans cells grew markedly, although syncytial buds sometimes appeared. On the other hand, fibroblastic growth was more marked in the medium containing serum and plasma from non-pregnant subjects.

Studies on the trophoblast from early pregnancy (LAJOS et al., [9,10]) have revealed that *in vitro* treatment with anterior pituitary extract and serum from pregnant subjects gives rise to chorionic gonadotrophin production. Anterior pituitary extracts and sera from non-pregnant subjects had no such effect. The factor influencing HCG output is detectable in the adeno-hypophysis from the earliest phase of gravidity until a few weeks after delivery. This suggested that the placenta, like the other endocrine organs, would be under pituitary control.

The above results support the view that the considerable epithelial proliferation taking place in the "pregnant" medium, and the fact that HCG is synthesized in the trophoblastic epithelium incubated in serum of pregnant women make it justified to claim that growth of, and HCG production by, the trophoblast is under pituitary control.

Summary

(i) In a medium containing serum and plasma from non-pregnant subjects ("non-pregnant" medium) the connective tissue elements of the placenta showed intense growth. No such rate of fibroblastic growth occurred in a medium containing serum and plasma from early pregnancy ("pregnant" medium).

(ii) The growth of epithelial elements was significantly enhanced in the "pregnant" medium.

(iii) Langhans' cells grew more intensively than the syncytial elements.

(iv) In "pregnant" serum the trophoblast synthesized HCG, whereas the "pregnant" medium significantly enhanced the growth of the epithelial elements of the explanted chorion.

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ОПЫТ ПО ЭКСПЛАНТАЦИИ В ЦЕЛЯХ ИССЛЕДОВАНИЯ УСЛОВИЙ РОСТА ТРОФОБЛАСТОВ

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В небеременной среде авторы наблюдали главным образом интенсивный рост соединительнотканевых элементов плаценты. В случае применения беременной среды не наблюдалось роста фибробластов в таком размере.

Далее авторы установили в беременной среде значительное повышение роста эпителиальных элементов.

Клетки Ланганса показали в опытах авторов более интенсивный рост, чем синцитиальные элементы.

При помещении трофобластов в сыворотку беременных произошел синтез HCG, в то время как в опытах по эксплантации беременная среда вызывала значительный рост эпителиальных элементов наружной оболочки зародыша.

EXPLANTATIONSVERSUCHE ZUR UNTERSUCHUNG DER WACHSTUMS-
VERHÄLTNISSE VOM TROPHOBLASTEN

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In nichtschwangerem Medium wurde ein intensives Wachstum der Bindegewebelemente der Plazenta beobachtet. Bei Anwendung von schwangerem Medium war das Wachstum von Fibroblasten geringer.

In schwangerem Medium konnte eine bedeutende Steigerung des Wachstums von Epithelialelementen wahrgenommen werden.

Die Langhans-Zellen zeigten in den Experimenten ein intensiveres Wachstum als die synzytialen Elemente.

Wurden die Trophoblasten in schwangeres Serum gelegt so erfolgte während der Inkubation HCG-Synthese, während in Explantationsversuchen das schwangere Medium ein beträchtliches Wachstum der Elemente des Chorionepithels hervorrief.

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