HISTOLOGICAL, METABOLIC AND HISTOCHEMICAL STUDIES ON NORMAL HUMAN PLACENTA IN ORGAN CULTURE

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ISOLATED cells from normal human placenta can be readily grown in tissue (cell) culture (Guggisburg and Neuweiler, 1928; Friedheim, 1928; Stewart, Sano and Montgomery, 1948; Thiede, 1960), but these cells become dedifferentiated, morphologically and functionally (Levintow and Eagle, 1961). The organ culture technique appears more promising for study of the cellular metabolism of normal human placenta, since the structure of the chorionic villi is preserved and the different cell types can be identified confidently; furthermore it has been shown that such explanted tissue releases chorionic gonadotrophin into the culture medium and that certain of its constituent cells are synthesising DNA (Tao and Hertig, 1965; Gerbie, Hathaway and Brewer, 1968*a*, *b*). In these studies the culture fluid contained horse serum; this complex biological fluid cannot be standardised and may contain hormones or other substances capable of affecting the culture tissue. Clearly, it would be advantageous to maintain organ culture explants in a chemically-defined medium where the identity and concentration of each constituent is known.

We have already shown that explants of normal human placenta can be maintained in organ culture in a chemically-defined medium (Hou and Beck, 1967). This paper presents results of histological, metabolic and histochemical studies on normal human mid-pregnancy placentas maintained in organ culture with a chemically-defined fluid for periods up to 42 days.

MATERIALS AND METHODS

Human placentas.—Cultures were prepared from normal human placentas removed under sterile conditions from 31 patients undergoing therapeutic termination of pregnancy by hysterotomy for a variety of medical or psychiatric reasons. The gestational ages ranged from 10–22 weeks.

Organ-culture technique.—Explants of the placenta were prepared with full aseptic precautions within 2 hr. of removal from the patient. The placenta was first washed in sterile 0·15 M-NaCl. Masses of apparently normal placental tissue (approx. 1·0 cm. diameter) were transferred to Hanks' balanced salt solution where the membranes, decidual masses and blood vessels were carefully removed leaving mainly the villous part: the nature of this tissue was confirmed by stereo-microscope examination. Placental villi were then cut into small pieces (2–3 mm. diameter); these were maintained in organ culture with a modification of the method described by Hoorn and Tyrell (1965) in ventilated 2 in. diameter pre-sterilised, non-toxic and water-wettable polystyrene Petri dishes (Nunclon). Four ml. of chemically-defined culture medium (Glaxo T.C. 199) containing 100 i.u. sodium penicillin G, 100 i.u. mycostatin and 100 μg. streptomycin sulphate per ml. of medium were added to each dish. The explants were completely immersed in the culture medium and therefore in the fluid phase. To ensure maximum surface area in contact with the medium, the Petri dishes were shaken gently to separate the villi of the explants. The dishes were then incubated at 37° in a humidity chamber with a gas mixture of 95 per cent O_2 and 5 per cent CO_2. In the earlier experiments

the medium was changed twice weekly and in the later experiments it was changed daily. The explants were examined daily with the stereo-microscope.

Histological studies.—Representative explants from each placenta were fixed in 4 per cent neutral-buffered formaldehyde in saline after maintenance for 1, 3, 5, 7, 10, 12, 14 days and 3, 4 and 5 weeks *in vitro*. Some explants were fixed after 11 hr. culture. In addition several placentas were cultured for periods up to 78 days. After processing, paraffin sections (5 μ) were stained with haematoxylin and eosin.

Glucose consumption.—The glucose consumption of explants of 6 normal placentas (3 of 10 to 12 weeks' and 1 each of 15, 17 and 20 weeks' gestation) was estimated during culture for 35 days in T.C. 199 (Glaxo). Two or 3 Petri dishes were prepared from each placenta. The culture fluid was changed daily and samples of the discarded media and of the unused media were cultured in nutrient broth to ensure that there was no bacteriological or fungal contamination. These were then assayed for total reducing sugars with the alkaline ferricyanide reduction method using the Technicon Autoanalyser: it is probable that this technique was measuring glucose concentration in the medium since it had been shown previously in thyroid organ culture experiments that the Autoanalyser gave results closely similar to those of the glucose oxidase technique (Nicol and Beck, 1968).

^{3}H-thymidine studies.—Explants of 3 normal placentas of 10, 14 and 15 weeks' gestation respectively were maintained in T.C. 199. After 1, 3, 5, 7, 10, 14 and 22 days' culture, the medium was changed to T.C. 199 containing 2 μc ^{3}H-thymidine (thymidine-methyl-^{3}H, specific activity 5·0 mc/mM, Radiochemical Centre, Amersham, England)/ml. and incubation was continued for 8 or 18 hr. The explants were washed thoroughly with saline and then fixed in 4 per cent neutral buffered formaldehyde for autoradiographic study.

In another experiment, 2 μc ^{3}H-thymidine was added to the culture fluid, in which explants of 3 normal placentas of 10, 12 and 14 weeks' gestation were cultured, for 24 hr. either at the start of incubation or after culture in unlabelled T.C. 199 for 1 or 5 days; thereafter the explants were cultured in unlabelled T.C. 199 and each day tissue samples were fixed for autoradiographic study.

^{3}H-leucine studies.—Explants of 3 normal placentas of 10, 12 and 14 weeks' gestation were maintained in T.C. 199. Either at the start of the experiment or after culture for 1 or 5 days, 2 μc ^{3}H-leucine (DL-leucine-4,5-T, specific activity 500 mc/mM, Radiochemical Centre, Amersham, England)/ml. was added to the culture fluid for 24 hr. Thereafter the explants were cultured in unlabelled T.C. 199 and each day tissue samples were removed, washed thoroughly in saline and fixed in 4 per cent neutral-buffered formaldehyde for autoradiographic study.

Autoradiography.—Autoradiographs were prepared from dewaxed paraffin sections, either with AR 10 stripping film (Kodak, England) or by dipping in L 4 nuclear tracking emulsion (Ilford, England). The preparations were exposed for 2 weeks before development and counter-stained with haematoxylin.

Histochemical studies.—Specimens were snap-frozen at -70° from 15 placentas (gestational ages 10–22 weeks) before culture and after 1, 3, 7, 10, 14 days and 3, 4, 5 and 6 weeks in organ culture. These specimens were stored at -20°; 5 μ cryostat sections were cut within 24 hr. of freezing the tissues.

Unfixed sections were stained histochemically for alkaline phosphatase with the calcium phosphate method (Gomori, 1952*a*), for acid phosphatase with the naphthol AS-TR phosphate method (Barka and Anderson, 1962), for cytochrome oxidase (Burstone, 1961), for naphthyl AS esterase (Gomori, 1952*b*) and for α-naphthyl esterase (Gomori, 1952*b*). Sections fixed for 12 hr. at 0° were stained for amino peptidase with the method described by Glenner, Burstone and Meyer (1959). For controls, adjacent sections were stained in the absence of essential substrates or in the presence of specific enzyme inhibitors (Table I).

RESULTS

Stereo-microscope appearances

During mid-pregnancy (12–22 weeks' gestation) the placental villi were semi-translucent and had primary, secondary and tertiary branches (Fig. 1). The primary villi were slightly larger in diameter and the secondary and tertiary villi were somewhat tortuous tubular structures; the tips of the tertiary villi were

TABLE I.—*Control Experiments to Establish Specificity of Histochemical Staining of Enzymes in Placenta Organ Culture*

Histochemical staining method for demonstration of	Control experiments: Essential enzyme substrate omitted from incubation mixture	Control experiments: Specific enzyme inhibitor procedure
Alkaline phosphatase	Sodium β-glycerophosphate	Pretreatment with 0·05 M-ethylene-diamine tetra-acetate (EDTA) (Freimann, 1953)
Acid phosphatase	Naphthyl AS-TR phosphate	0·005 M-NaF added to incubation mixture (Walker *et al.*, 1954)
Aminopeptidase	L-leucyl-β-naphthylamine	Pretreatment with 0·05 M-EDTA (Burstone and Folk, 1956)
Cytochrome oxidase	p-aminodiphenylamine	0·001 M-KCN added to incubation mixture (Burstone, 1962)
α-Naphthyl esterase	α-naphthyl acetate	Pretreatment with 0·002 M-NaF (Gomori, 1952*b*) *or* pretreatment with, and addition of, 0·001 M E 600 to incubation mixture (Pearse, 1960)

rounded. Blood vessels ran axially along primary and secondary villi and were more numerous in the tertiary villi at 20–22 weeks' gestation than at 12–16 weeks gestation.

After organ culture for 3 days small sessile projections were seen on the surface of the tertiary villi; these became more numerous until the 7th or 8th day *in vitro* (Fig. 2). Over the next 7 days these enlarged into taller sessile or polypoid projections (in general up to 200 μ tall and 80 μ diameter), but no new projections appeared at this stage. After 14 days' culture, no further micro-anatomical changes were noted in the villi. However, small opaque areas suggestive of degenerative changes and necrosis were observed in the villi during the 3rd week of culture; these enlarged and became more numerous during the remainder of the period of study. Blood vessels were visible up to about 7 days *in vitro* but could not be identified later due to lysis of erythrocytes.

Histological appearances

The histological changes in three 12-week, two 14-week, one 15-week and one 22-week placentas were studied in detail. A composite description will be given as there was very little individual variation.

All explants appeared histologically viable for the first 14 days in organ culture (Figs. 3 and 4), but thereafter foci of coagulative necrosis became larger and more numerous until little viable tissue was seen in 5-week explants: nevertheless explants retained their villous structure throughout the period of study. All surviving villi were formed of a core of stroma covered with trophoblast; outgrowths of fibroblasts or other cells were never seen.

Throughout the period of culture the outer layer of surviving trophoblast remained recognisable as syncytiotrophoblast (Figs. 5, 6 and 7). During the first 3 days in culture partial clumping of nuclear chromatin and replacement of cytoplasmic basophilia by eosinophilia were seen and after 3–10 days *in vitro* these minor cytological modifications were followed by the appearance of infrequent small focal areas of nuclear crowding, but mitotic figures were never seen. After

3 weeks or longer in culture, karyolysis of nuclei and granular eosinophilic coagulative change of the cytoplasm were prominent in areas of necrotic syncytiotrophoblast.

Likewise, the cytotrophoblast remained recognisable throughout the period of study. After 3 days in culture the cells were more closely packed as a single layer beneath the syncytiotrophoblast while their nuclei became slightly denser and their cytoplasm somewhat more eosinophilic. There were very few heaped areas of " altered cytotrophoblast " (Tao and Hertig, 1965) and mitotic figures were never seen.

By contrast, the stroma of the villi underwent great modification during organ culture (Figs. 5, 6 and 7). Plump spindle cells with large open nuclei (probably fibroblasts) were present after 1 day in culture and these increased in numbers until the 3rd or 7th day, when they replaced the normal stroma completely and Hofbauer cells could only be recognised with difficulty (Fig. 6). Thereafter the spindle cells disappeared progressively and the nuclei of the surviving stromal cells were small, round or oval, and had densely clumped chromatin after 14 days in culture. Later the stroma was gradually replaced by loosely arranged fine fibrillary eosinophilic material. Despite the great proliferation of stromal cells, mitotic figures were seen only in the 11-hr. cultures. Karyorrhectic granules were seen in the stroma after 3 days' culture and these were very numerous after 7 days' culture, but frank necrosis was not seen at this stage. Pyknotic nuclei were seen in the blood vessels after 1–2 days' culture and the erythrocytes became lysed during the first week. Concurrent with this, the blood vessels collapsed and their endothelial cells disappeared in the proliferated stromal spindle cells (Fig. 6); it was not possible to determine whether the endothelial cells degenerated or were transformed into stromal cells.

Glucose consumption

The average glucose consumption of explants of the three 10–12 weeks' placentas was 12·8 mg. glucose/g. w.w. placenta/24 hr. (range 9·8–16·4 mg. glucose/g. w.w. placenta/24 hr.) on the first day of culture, it rose to 16·9 mg. glucose/g. w.w. placenta/24 hr. (range 14·4–21·3 mg. glucose/g. w.w. placenta/24 hr.) on the 3rd day and then fell to 8·4 mg. glucose/g. w.w. placenta/24 hr. (range 6·8–11·4 mg. glucose/g. w.w. placenta/24 hr.) on the 7th day (Fig. 8). Thereafter there was a progressive fall in glucose consumption ($P < 0{\cdot}001$) and the slope of this curve of glucose consumption/day was $-0{\cdot}203$ mg. glucose/g. w.w. placenta/24 hr.

The glucose consumption of explants of the individual normal 15, 17 and 20 weeks' placentas was similar to that of explants of the 10–12 weeks' placentas.

^{3}H-thymidine studies

In the first experiment, ^{3}H-thymidine was given to cultures of various ages for 8 or 18 hr. before fixation. Stromal cell and cytotrophoblast nuclei labelled with heavy overlying deposits of silver were seen in histologically-preserved tissue of all explants given ^{3}H-thymidine after periods of up to 7 days *in vitro*, although these were more frequent in 1-day and 3-day cultures than in 7-day cultures (Fig. 9): labelled stromal cell nuclei were also seen occasionally in older cultures. Although labelled stromal cells were often concentrated at the edge of the villi close to the

cytotrophoblast, they were also scattered throughout the core of the villus; most of these cells were fibroblasts, but a few were identified as Hofbauer cells. The nuclei of scattered cytotrophoblast cells were labelled in the earlier cultures but these cells were not labelled in explants maintained for more than 7 days *in vitro*. The nuclei of syncytiotrophoblast were never labelled in these experiments.

In the second experiment ^{3}H-thymidine was given for 24 hr., either at the start of incubation or after culture for 1 or 5 days in T.C. 199; thereafter the explants were maintained in unlabelled medium and sampled daily. In the stroma, labelled nuclei were most numerous immediately after culture in ^{3}H-thymidine; during subsequent culture in unlabelled T.C. 199 they became progressively less numerous

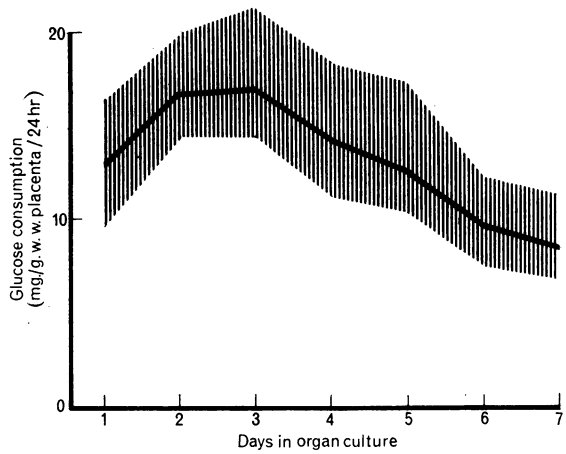


FIG. 8.—Glucose consumption of 10–12 weeks normal human placentas in organ culture. Mean consumption shown with solid line and range by hatched area.

but they were quite commonly seen after 7 days in T.C. 199. Labelled nuclei were first seen in the histologically-intact syncytiotrophoblast 3–4 days after 24 hr. culture in ^{3}H-thymidine; these labelled nuclei were never common and always widely scattered. A high proportion of the cell nuclei in the foci of " altered cytotrophoblast " were labelled, but labelled nuclei were never seen in the overlying syncytiotrophoblast until 3 or 4 days after culture in ^{3}H-thymidine for 24 hr.

^{3}H-leucine studies

After explants had been cultured in ^{3}H-leucine for 24 hr. there was diffuse labelling of the stroma with increased activity at its periphery (Fig. 10). This activity diminished slowly during subsequent culture in unlabelled T.C. 199 and it was still present 7 days later. The labelling of stroma was more marked in tertiary villi than in secondary villi. Histologically-preserved syncytio- and cytotrophoblast were never labelled, but moderately intense labelling was seen in foci of " altered cytotrophoblast ". The labelling detected in these experiments was not diffusible and had been fixed by formaldehyde: the ^{3}H-leucine had been, presumably, protein bound.

Histochemical studies

The results of histochemical staining for 5 enzymes in organ-cultured explants of normal human placentas (gestation ages 10–22 weeks) are shown in Table II. Explants of 3 of these placentas were also stained for naphthyl-AS-esterase but no activity was found in either the trophoblast or the stroma.

Trophoblast.—The enzymes listed in Table II were demonstrated by histochemical staining in the cytoplasm of the syncytiotrophoblast of mid-pregnancy placentas (Figs. 11, 13 and 15). When these enzymes were detected in histologically-viable trophoblast of organ-cultured placenta, they were always localised to the cytoplasm (Figs. 12, 14 and 16): necrotic trophoblast was not stained.

The intensity of histochemical staining of trophoblastic enzymes diminished progressively during organ culture (Table II). Nevertheless there was little change in the activity of alkaline phosphatase (Figs. 11 and 12) during the first 14 days and of acid phosphatase (Figs. 13 and 14) and aminopeptidase during the first 7 days in culture. Although the activity of cytochrome oxidase (Figs. 15 and 16) and of α-naphthyl esterase fell after 3 days, these enzymes were detectable at a lower level during the remainder of the first week *in vitro* in most experiments. After 28 days culture, only alkaline phosphatase was detectable in trophoblast.

Thus we have shown that the activity of the enzymes we have studied remains virtually unchanged for the first 3 days of culture and that the only change in the

TABLE II.—*Histochemical Staining of Enzymes in Organ Cultures of Normal Human Placentas*

Enzyme	No. of placentas examined	Histochemical staining: Tissue	Intensity	No. of cultures showing various enzyme activities after given no. of days in culture: 0 (pre-cultured)	1	3	7	14	21	28	35	42
Alkaline phosphatase	14	Trophoblast	++	14	14	14	12	9	6	2		
			+				2	5	7	11	13	12
			−						1	1	1	2
		Stroma	+			2						
			−	14	14	12	14	14	14	14	14	14
Acid phosphatase	14	Trophoblast	++	14	14	14	12	3	1			
			+					10	5	3		
			−					1	8	11	14	14
		Stroma	++		7	9	10	7	3	1		
			+	12	7	5	4	7	9	6	2	
			−	2					2	7	12	14
Aminopeptidase	14	Trophoblast	++	14	14	14	6					
			+				8	9	1			
			—					5	13	14	1	14
		Stroma	++		11	13	13	9	2	1		
			+	13	3	1	1	5	12	9	4	1
			—	1						4	10	13
Cytochrome oxidase	14	Trophoblast	++	14	13	7						
			+		1	7	11	1				
			−				3	13	14	14	14	14
		Stroma	−	14	14	14	14	14	14	14	14	14
α-naphthyl esterase	3	Trophoblast	++	3	3	2						
			+			1	2	2	2			
			−				1	1	1	3	3	3
		Stroma	+	3	3	3	3	2	2	2		
			−					1	1	1	3	3

++ intense staining; + weak staining; − no staining.

remainder of the first week is a slight fall in cytochrome oxidase and α-naphthyl esterase between the third and seventh days.

Stroma.—The stromal cells of most mid-trimester placentas contained acid phosphatase, aminopeptidase and α-naphthyl esterase, but histochemical staining for alkaline phosphatase, cytochrome oxidase and naphthyl-AS-esterase was usually negative. The intensity of histochemical staining of acid phosphatase (Figs. 13 and 14) and aminopeptidase increased markedly during the first few days of organ culture, but it started to fall by the end of the 4th week *in vitro*: α-naphthyl esterase activity started to fall at the end of the first week and had disappeared by the 5th week. Alkaline phosphatase, cytochrome oxidase and naphthyl-AS-esterase were not demonstrated in significant quantities in stromal cells of any of the organ cultures (Figs. 15 and 16). We have thus shown that there are dramatic changes in the enzymic activity of stromal cells during organ culture.

EXPLANATION OF PLATES

FIG. 1.—Stereomicroscopic appearance of uncultured explant of a normal human placenta (14 weeks' gestation) immersed in culture fluid. The tertiary villi are semi-translucent and granular, and have a smooth surface: they arise from the secondary villus. Indirect illumination. ×33.

FIG. 2.—Stereomicroscopic appearance of explant of placenta shown in Fig. 1 after culture for 8 days in T.C. 199. There are numerous sessile, or polypoid, projections on secondary and tertiary villi: the villi remain semi-translucent and granular. Indirect illumination. ×33.

FIG. 3.—14 weeks' normal human placenta before culture. H. and E. ×38.

FIG. 4.—Explant of placenta shown in Fig. 3 after organ culture for 14 days in T.C. 199. There is no evidence of necrosis. H. and E. ×38.

FIG. 5.—Normal human placenta before culture (14 weeks). Each villus is covered with trophoblast and its core is formed of loose mesenchymal cells and occasional Hofbauer cells. H. and E. ×180.

FIG. 6.—Explants of placenta shown in Fig. 5 after organ culture for 3 days. The trophoblast has remained intact but stromal cells are more numerous and have large open nuclei. Blood vessels show early degenerative changes. H. and E. ×210.

FIG. 7.—Explants of placenta shown in Fig. 5 after organ culture for 7 days. The trophoblast is still intact; the stromal cells have small dense nuclei and are much less numerous than at 3 days. H. and E. ×210.

FIG. 9.—Explant of 14 weeks normal human placenta maintained in organ culture in T.C. 199 for 3 days before exposure to 2 μc ^{3}H-thymidine/ml. T.C. 199 for 1 day. Autoradiograph showing labelling of nuclei of many stromal cells and occasional cytotrophoblast cells. Nuclei of syncytiotrophoblast were not labelled. Haematoxylin. ×210.

FIG. 10.—Explant of 14 weeks' normal human placenta maintained in organ culture in T.C. 199 for 5 days before exposure to 2 μc ^{3}H-leucine/ml. T.C. 199 for 1 day. Autoradiograph showing diffuse labelling of stroma with some concentration at its periphery. The trophoblast is not labelled. Darkground. ×90.

FIG. 11.—Histochemical staining of alkaline phosphatase in 12 weeks normal human placenta. Activity is intense and restricted to the trophoblast. Gomori. ×210.

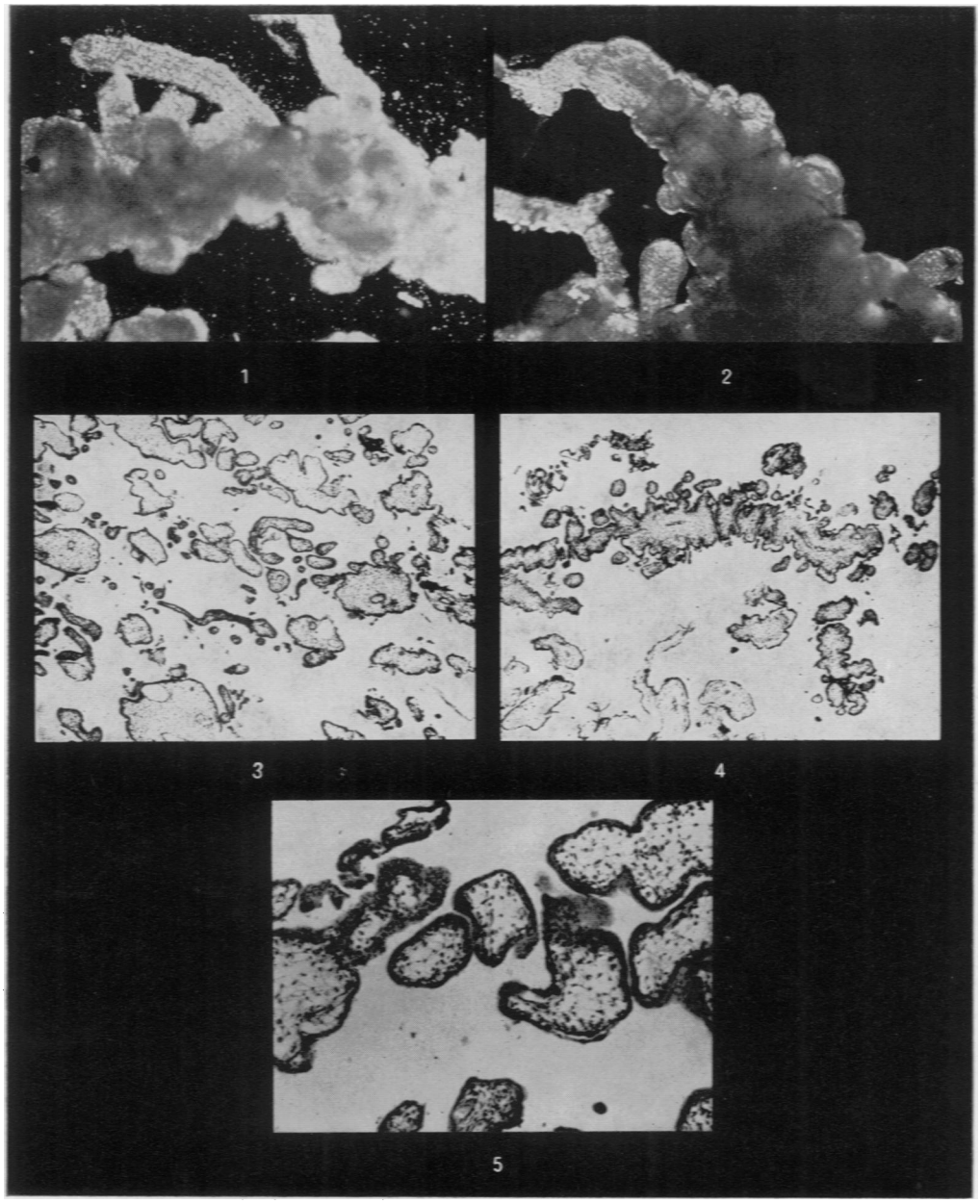
FIG. 12.—Histochemical staining of alkaline phosphatase in explants of placenta shown in Fig. 11 after organ culture for 7 days. Activity is still intense in the trophoblast. Gomori. ×210.

FIG. 13.—Histochemical staining of acid phosphatase in 14 weeks normal human placenta. Activity is strong in the trophoblast and weak in the stromal cells. Barka and Anderson. ×210.

FIG. 14.—Histochemical staining of acid phosphatase in explants of placenta shown in Fig. 13 after organ culture for 7 days. Activity is still intense in the trophoblast, but it is now stronger in the stromal cells. Barka and Anderson. ×210.

FIG. 15.—Histochemical staining of cytochrome oxidase in 12 weeks normal human placenta. Activity is moderately intense and restricted to the trophoblast. Burstone. ×210.

FIG. 16. Histochemical staining of cytochrome oxidase in explants of placenta shown in Fig. 15 after organ culture for 7 days. Activity is reduced but it is still present in the trophoblast. Burstone. ×210.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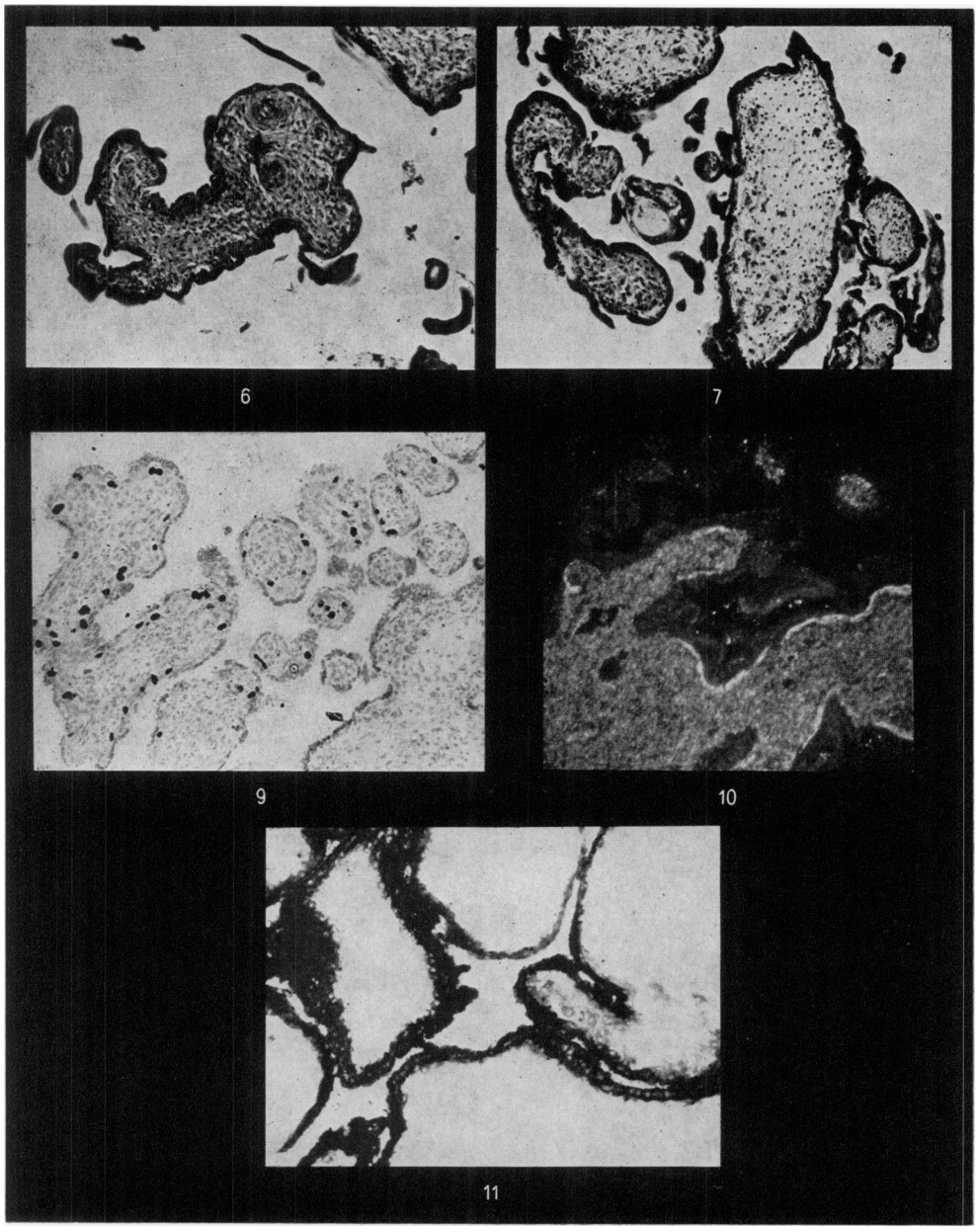

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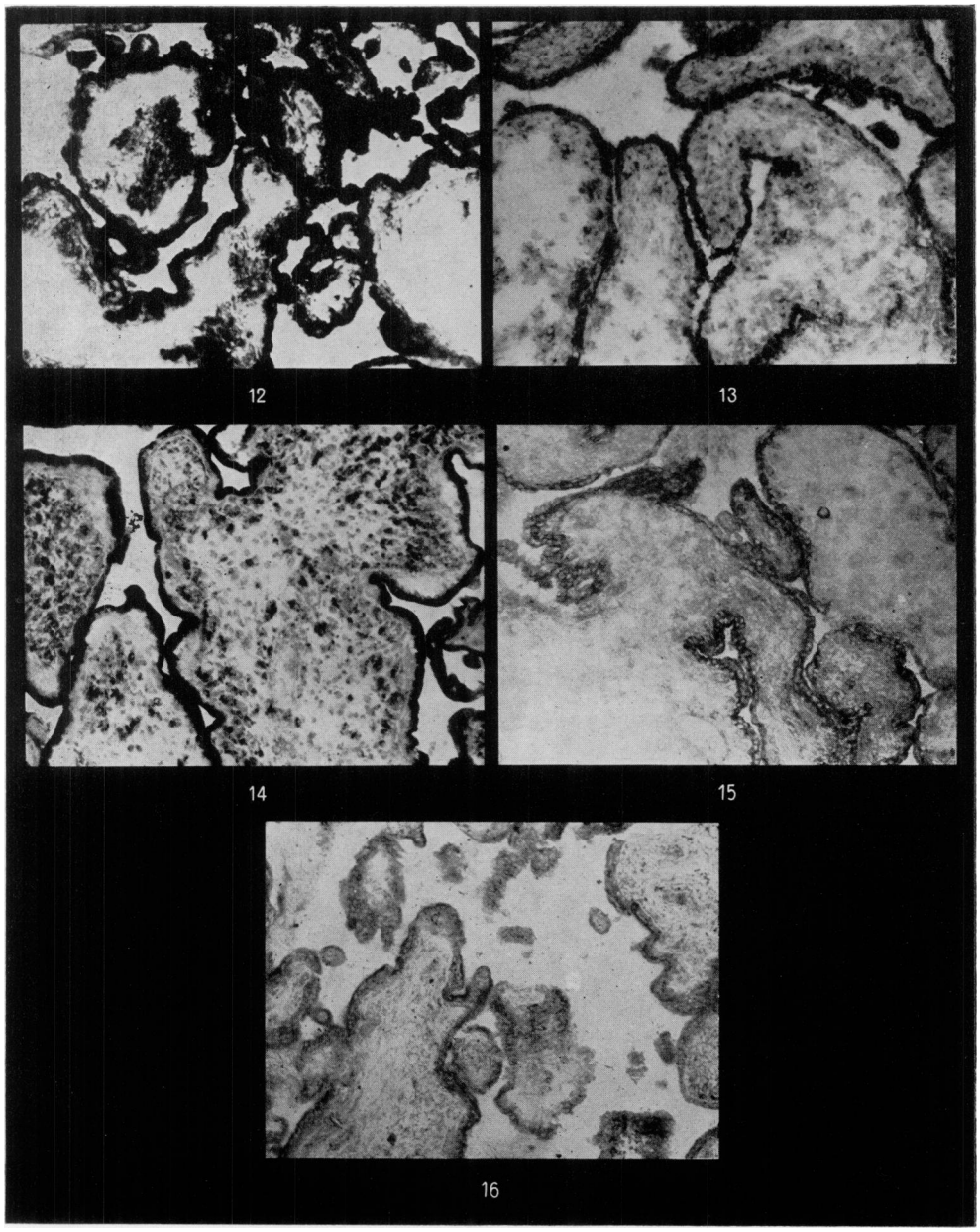

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DISCUSSION

Histology

Successful organ culture of normal human placenta has been reported from 2 laboratories using a serum-supplemented culture fluid (Tao and Hertig, 1965; Gerbie *et al.*, 1968*a*, *b*). We have reported previously that normal placenta can be maintained in organ culture in a chemically-defined medium and shown that the syncytiotrophoblast retains a growth hormone-like antigen during the first 7–14 days of culture (Hou and Beck, 1967). We have now shown that explants will survive for 14 days before the onset of focal necrosis and that areas of histologically viable tissue will persist for much longer.

The aim of the organ culture technique is to maintain small fragments of living tissue *in vitro* so that the constituent cells retain their *in vivo* relationship to each other. The descriptions of normal human placenta organ cultures in serum-supplemented medium (Tao and Hertig, 1965; Gerbie *et al.*, 1968*a*, *b*) show that this ideal was only partly achieved since collections of " altered cytotrophoblast " cells accumulated beneath the syncytiotrophoblast. Our results with a chemically-defined medium were little better since we found extensive proliferation of stromal cells; however, it must be emphasised that in our experiments the trophoblast showed little histological change during the first week of culture.

Metabolic studies

These have shown that the explants of normal human placenta were alive and were capable of performing at least some of their *in vivo* functions.

Glucose consumption.—In our experiments, the glucose consumption was higher during the first few days of culture than at the end of the first week (Fig. 8); thereafter there was a progressive fall in glucose consumption throughout the remainder of the period of study. It seems probable that the initial rise in glucose consumption is related to the great proliferation of stromal cells at that period and that the consumption at the end of the first week should be regarded as the " resting " level. Clearly, it has not been possible to determine the absolute glucose consumption of the different cellular components of the explants. We have not found any previous record of studies of glucose consumption by human or animal placenta in organ culture. It is, however, noteworthy that the " resting " level of glucose consumption by our organ cultures of normal human placenta (6·8–11·4 mg./g. w.w./24 hr.) was of the same order as that of non-proliferating hyperplastic human thyroid gland (2·9–8·0 mg./g. w.w./24 hr.) maintained with a similar technique (Nicol and Beck, 1968).

^{3}H-thymidine studies.—In our experiments where placenta explants were exposed to ^{3}H-thymidine for 8–18 hr. before autoradiographic examination we found labelled nuclei in cytotrophoblast and stromal cells but never in syncytiotrophoblast. This confirms previous observations in short-term experiments on cultures maintained in serum-supplemented medium (Tao and Hertig, 1965; Gerbie *et al.*, 1968*a*, *b*). It is noteworthy that, in the present study, DNA synthesis in stroma cells appears to be most active in the first 3 days of culture since this is the period of rapid proliferation of these cells. Mitotic figures were not infrequent in placenta explants cultured in serum-enriched medium (Gerbie *et al.*, 1968*b*) but these were seen only in our 11-hr. cultures; it is possible that T.C. 199 lacks ingredients necessary for mitosis or that the conditions were deficient in some

other respect since mitotic figures were not seen in DNA-synthesising organ cultures of hyperplastic human thyroid gland maintained with a similar technique (Nicol and Beck, 1968).

When we sampled explants at intervals after culture in ^{3}H-thymidine, we found that labelled nuclei had migrated from histologically normal and " altered " cytotrophoblast into the syncytiotrophoblast after 3 or 4 days: in this respect explants cultured in T.C. 199 behave similarly to those cultured in a serum-supplemented medium (Tao and Hertig, 1965; Gerbie *et al.*, 1968*a*, *b*). A large number of stromal cell nuclei are labelled when the explants are cultured in ^{3}H-thymidine for 1 day but the number falls when the explants are cultured subsequently in unlabelled T.C. 199. This suggests that there is a rapid turnover of stromal cells during the first week of organ culture: this conclusion would be compatible with the histological appearances.

^{3}H-leucine studies.—The uptake of ^{3}H-leucine by organ cultures of placenta has not been studied previously. We have now shown that the stroma incorporates this amino-acid from the medium at a time when we know from histological and ^{3}H-thymidine studies that there is rapid turnover of stromal cells: ^{3}H-leucine is also taken up by the proliferating " altered cytotrophoblast ".

The histologically-preserved syncytio- and cyto-trophoblast did not show any evidence of labelling after culture in the presence of ^{3}H-leucine. This suggests that these tissues are not synthesising large amounts of protein. It is therefore probable that the progressive loss of a growth hormone-like antigen during organ culture of placenta (Hou and Beck, 1967) might be explained by a failure of synthesis of this substance in the trophoblast under these conditions of culture.

Histochemical staining

Although the enzyme content of normal human placenta has been investigated extensively with histochemical staining methods, these techniques have not been applied previously to organ-cultured placenta. Our investigations on 5 hydrolytic and 1 oxidative enzymes have shown that during the 1st week of culture there is little change in the activity of these enzymes in the trophoblast whereas the stromal cells show great changes. Later the activity of these enzymes in histologically-viable trophoblast and stroma falls progressively. The activities of the enzymes in the trophoblast diminish at different rates—for example, cytochrome oxidase activity starts to fall after 3 days and has disappeared completely by 21 days, whereas alkaline phosphatase activity starts to fall after 7 days and has not disappeared completely even at 42 days. This loss of enzymatic activity cannot yet be explained but it appears likely that this results from failure of synthesis, due possibly to metabolic adaptation to the conditions of culture or to cellular degeneration: we consider it unlikely that the enzymatic depletion could be explained solely by diffusion into the culture fluid. Our experiments suggest that during the first week the trophoblast has retained at least some of its differentiated metabolic pathways but that the stromal cells have undergone considerable modification of their metabolism.

CONCLUSIONS

In this study we have shown that normal human placenta removed at mid-pregnancy by hysterotomy can survive for periods longer than 1 week in organ culture with a chemically-defined culture fluid. There is considerable histological

and histochemical evidence that the trophoblast retains its differentiation almost completely during the 1st week of culture but that the stroma undergoes great modification within 3 days. It is therefore obvious that these preparations are quite unsuitable for study of the normal function of the stroma cells. Furthermore the results of biochemical studies on whole explants would need to be interpreted with caution.

The preparations should prove useful as a model system for study of the function of normal trophoblast with qualitative or quantitative cytological methods, provided the period of culture is limited to 1 week.

SUMMARY

Explants of 31 normal human placentas (gestational ages 10–22 weeks) survive in organ culture with a chemically-defined medium for at least 14 days before the onset of focal necrosis; during this period the explants consume glucose and synthesise DNA. There was little change in the histological structure or enzyme content of trophoblast during the first week of culture, but there was extensive proliferation of stromal cells at this stage. In the first week of culture the preparations should prove useful for the study of trophoblast function.

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