Hibernation, therefore, interferes with spermiogenesis by a retardation of cell differentiation. Cell proliferation seems to be independent, because no difference in testis growth between the 2 groups of animals was observed. In general, an increase in the testis weight can be due either to cell proliferation or to a liquid retention, which may be attributed to a rise in the osmotic pressure in the tubules. Swelling of the latter kind, however, is only seen when the production of spermatozoa is maximal and when the seminal vesicles are enlarged. Such a state of spermiogenic activity was only found in the 2 control hamsters with the heaviest testes (hamsters Nos. 27 and 47). As was already shown, such a maximal activity cannot be reached during hibernation (WELLS[1]).

The discrepancy between cell proliferation and cell differentiation in the germinal epithelium of the testis, with regard to the influence of hibernation, possibly expresses a different endocrine balance. An analysis of hypophyseal and thyroidal microscopical states is in progress now in an attempt to reveal differences between control and hibernating hamsters at the endocrine level.

Zusammenfassung. Während einer Periode von Dezember bis März wurden von Goldhamstern im Winterschlaf und gleichzeitig von Kontrolltieren, die bei Zimmertemperatur gehalten wurden, die linken Testes untersucht. Aus der Korrelation zwischen dem histologischen Bild und dem relativen Organgewicht ging hervor, dass der Winterschlaf keinen Einfluss auf die Proliferation der Zellen des Keimepithels hat, dass sie aber die Differenzierung dieser Zellen verzögert.

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Persistence of a Growth Hormone-Like Antigen in Organ Cultures of Human Placentas

The human placenta contains a substance (human placental factor, HPF[1]) that is antigenically related to human pituitary growth hormone (HGH). The active component in extracts of human placenta has been variously named 'placental lactogen'[2], 'chorionic growth hormone-prolactin'[3] and 'growth hormone-like substance'[4].

This paper reports our immunofluorescence (IF) studies on the persistence of HPF in organ cultures of 11 normal placentas (gestational ages 12–40 weeks). Normal villi were dissected under sterile conditions from 2 full-term placentas collected after spontaneous vaginal delivery and from the other placentas after caesarian section or hysterotomy. Explants (2–3 mm^3) of the placental villi were maintained in a chemically-defined medium (T.C. 199) with a simple organ culture technique[5]. Representative explants from each placenta were fixed in 4% neutral buffered formaldehyde in saline after culture for 1, 3, 5, 7, 10, 12, 14 days and 3, 4, 5 and 6 weeks in vitro. The histological appearances of the trophoblast in our organ cultures, although slightly modified, indicated that the tissue is living; this conclusion was supported by the report of DNA synthesis in cytotrophoblast nuclei in similar organ cultures[6] and by evidence of glucose consumption by the explants[5].

The indirect IF staining technique has been described previously[7]. In the first stage, the sections were treated with a goat antiserum to the RABEN[8] preparation of HGH or with non-immune goat serum and 0.15 *M* NaCl as controls; fluorescein-conjugated rabbit anti-goat IgG serum was used in the second stage. The IF staining of organ-cultured explants of human placenta was reproducible as consistent results were obtained on staining sections of each block on 3 separate occasions with anti HGH serum and the controls. The specificity of this anti HGH serum in IF staining of normal uncultured human pituitary gland and placenta has been characterized previously[1,7,9]. Nevertheless it was essential to demonstrate that staining of organcultured placenta was also immunologically specific: the experiments to determine this[10] were performed on sections of 3-, 5- and 7-day explants of 2 placentas from each trimester with the methods described previously[7]. The findings were in complete concurrence with those of previous experiments in which the immunological specificity of the IF staining system had been established[7].

The sections were examined independently by both of us, without prior knowledge of the serum used, and HPF

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[10] *Firstly*, we showed that the staining capacity of the anti HGH serum diluted $^1/_{16}$ in saline (as used throughout this investigation) was removed by absorption with acetone-dried powders of human pituitary and placenta (approximately 100 mg/ml), with RABEN[8] preparation of human pituitary growth hormone (4 mg/ml) and with FRIESEN[11] extract of human placenta (4 mg/ml); absorption with acetone-dried powders of other human organs (adult liver, heart, kidney and spleen, and foetal brain, liver, spleen and skin) (approximately 100 mg/ml), purified human pituitary adrenocorticotrophic hormone (ACTH) prepared by the method of CURRIE and DAVIES[12] (4 mg/ml), purified human urinary gonadotrophins (4 mg/ml) or human IgG (4 mg/ml) did not remove the staining capacity. *Secondly*, the anti HGH serum was fractionated by chromatography on DEAE Sephadex and various fractions were used separately as the first stage in the IF staining; bright staining was obtained with the IgG fraction only. The active principle is, therefore, an immunoglobulin. *Thirdly*, it was shown in absorption experiments (50 mg absorbant/ml) that the staining capacity of the fluorescein-conjugated anti-goat IgG serum could be removed by treatment with goat IgG (prepared by DEAE Sephadex chromatography of normal goat serum) but it was not affected after treatment with other fractions of goat serum, human IgG or rabbit IgG.
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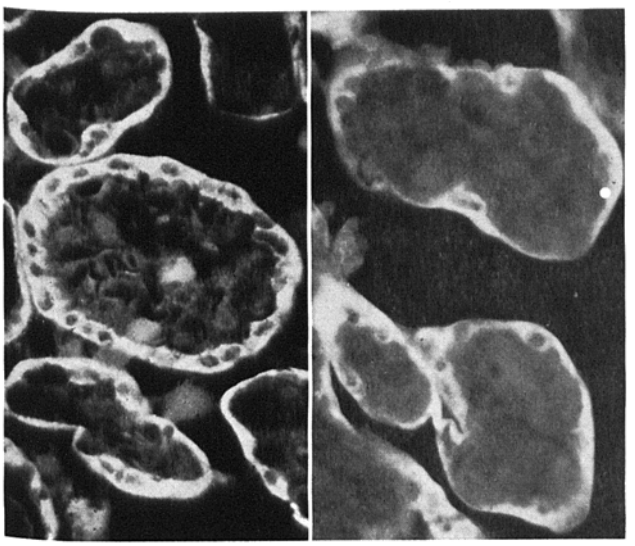
Immunofluorescence staining with anti HGH serum in organ-cultures of human placentas of various gestational ages

Gestation (weeks)	No. of placentas cultured	No. positive on culture at day 0 (uncultured)	3	7	10	14	21	28
12	3	3	3	3	3	3	0	0
14–16	3	3	3	3	2	0	0	0
20–22	2	2	2	2	2	0	0	0
38–40	3	3	3	3	2	1	0	0



A B

Fig. 1. IF staining with anti HGH serum of syncytiotrophoblast cytoplasm in chorionic villi from a 40-weeks human placenta. × 400. (A) uncultured tissue. (B) 4 days in vitro.

was considered to be present when a clear distinction could be made between specific fluorescence in sections stained with anti HGH serum and the autofluorescence of the controls. The results are summarized in the Table. Organ cultures of all 11 placentas show specific staining of HPF in the cytoplasm of the syncytiotrophoblast up to 10 days in vitro, and the explants of placentas of 12-weeks and 38-40-weeks gestation still show specific staining up to 14 days. After 21 days in vitro, HPF cannot be detected in any of the placenta explants. Specific staining is bright in uncultured tissue and 1-day cultures, but it becomes progressively less intense during culture until it is very weak at 10 days. This fall in intensity of fluorescence was noted with all cultures, irrespective of gestation age.

In uncultured normal villi, HPF is present in, and restricted to, all viable syncytiotrophoblast where it is uniformly distributed in the cytoplasm (Figure 1-A). After culture for 3 days most of the syncytiotrophoblast contains HPF; thereafter the antigen is progressively lost from focal areas but this loss is apparently unrelated to cytological modification or degeneration or to micro-anatomical locality. In stained areas of cultured villi, although HPF is sometimes diffused throughout the cytoplasm of the syncytiotrophoblast (Figures 1-B and 2), it is often, particularly after 3–5 days in vitro, distributed focally in the syncytiotrophoblast as granules in the cytoplasm around the nuclei or in the basal part of the cell (Figure 3). In 4 explants of 12-14-weeks placentas HPF was detected in the cytoplasm of some of the cytotrophoblast in addition to the syncytiotrophoblast (Figure 2); it appeared in the cytotrophoblast only after 3 days culture and subsequently disappeared at the same rate as that in the syncytiotrophoblast.

It has been shown previously that the HPF is a characteristic antigen of viable uncultured trophoblast since it is present in all samples of normal syncytiotrophoblast[1,18] and its benign tumours (hydatidiform moles)[1], and in syncytiotrophoblast of a proportion of choriocar-

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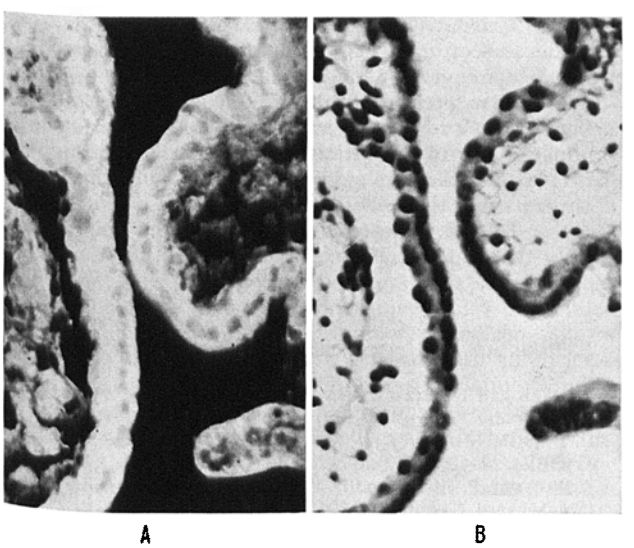
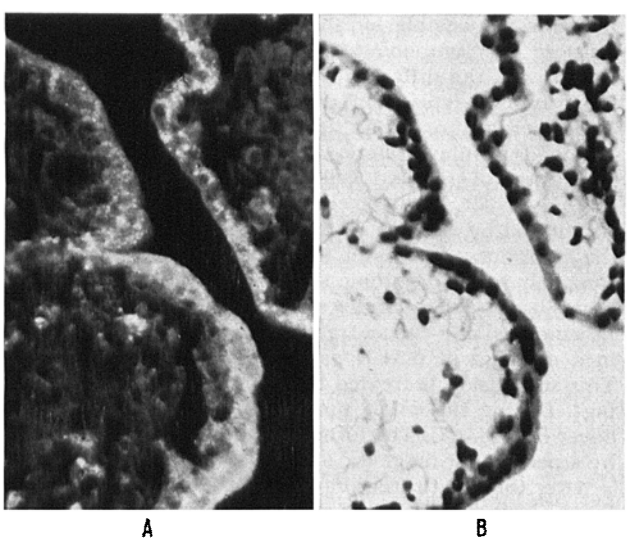


A B

Fig. 2. 14-weeks human placenta cultured for 3 days in vitro. × 400. (A) diffuse IF staining of syncytiotrophoblast and cytotrophoblast with anti HGH serum. (B) same area after restaining with haematoxylin and eosin.



A B

Fig. 3. 12-weeks human placenta cultured for 14 days in vitro. × 400. (A) granular IF staining around nuclei and in basal part of cytoplasm of syncytiotrophoblast with anti HGH serum. (B) same area after restaining with haematoxylin and eosin.

cinomas of the uterus[1] and malignant trophoblastic teratomas of the testis[14], but it is always absent from necrotic trophoblast[1,13]. In uterine trophoblast, HPF is restricted to the cytoplasm of the syncytiotrophoblast[1] but, in some malignant teratomas of the testis, it is present in the cytoplasm of occasional groups of cells identified as cytotrophoblast. Our finding of HPF in the cytotrophoblast of some cultured placental villi is therefore not surprising, and it possibly indicates a minor incoordination of differentiation in the organ cultures, as there is evidence that normal cytotrophoblast differentiates into syncytiotrophoblast[6,15].

The HPF antigen persists in organ cultures of human placenta for about 2 weeks although these cultures seemingly remain viable for at least 6 weeks. This is in marked contrast to the behaviour of an organ-specific microsomal antigen of human thyroid gland which persists in organ culture as long as the cells remain histologically viable[16]. It must be remembered, however, that the thyroid antigen is microsomal, whereas the placental antigen is a protein that is believed to be synthesized in and secreted by the syncytiotrophoblast in vivo[2]. If this is so, the depletion of the HPF antigen from the cytoplasm of the syncytiotrophoblast must result from an imbalance between the rates of synthesis in and loss, either by secretion or simple diffusion, from the organ-cultured tissue[17].

Zusammenfassung. Auswüchse menschlicher Placenten (12.–14. Woche Schwangerschaft) wurden in Organkultur gehalten. Obwohl sich die histologische Struktur langfristig erhalten lässt, bleibt ein dem menschlichen Wachstumshormon ähnliches Antigen, das in unkultiviertem Syncytiotrophoblast vorhanden ist, nur 10–14 Tage lang am Leben. Dasselbe erscheint vorübergehend in Cytotrophoblastzellen der Placentaauswüchse während der ersten 3 Monate.

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Study of the Lymphocyte Fractions in the Mixed Lymphocyte Culture in vitro

The culture of 2 lymphocyte populations from non-related individuals makes it possible to obtain the transformation of the lymphocytes into large blastic cells[1–4]. The % of these transformations is in proportion to the presence of incompatibility for some leucocyte antigens[5–7]. It is important to precise the nature of the substance which is responsible for these transformations. For this purpose the lymphocytes from one individual are disrupted and the different fractions are cultured with the living lymphocytes from another individual.

Our procedure is as follows: the lymphocytes are obtained from peripheric defibrinated blood after sedimentation of the red cells thanks to the addition of plasmagel (R. Bellon, Paris). The polynuclears are eliminated by sticking to the glass of Roux bottles while the lymphocytes remain in suspension. To disrupt the lymphocytes a hypotonic shock in distilled water for 30 min is used, which fragilizes the cells. Afterwards they are resuspended at a concentration of 3×10^6 lymphocytes/ml in a solution of $0.34 M$ saccharose and $0.0018 M$ CaCl. This suspension is treated by 4 successive freezing-thawings. During the whole procedure the temperature must never exceed 4 °C. The formation of aggregates is avoided by a permanent magnetic stir.

After the fourth freezing-thawing, a differential centrifugation at 600 *g* for 10 min, 17,000 *g* for 15 min and 105,000 *g* for 30 min produces 3 pellets: nuclei, mitochondria, and ribosomes; in addition the supernate remains. This procedure of fractionation is a modified version of that by RAPAPORT et al.[8]. The control under an electronic microscope shows the purity of our fractions.

The 3 pellets and the supernate are diluted in order to produce the equivalent of 1×10^6 cells/ml. All the fractions and the supernate are first tested in cultures with the autologous lymphocytes and then with the lymphocytes of one other individual. Four control cultures are carried out on the same day: (1) mixed lymphocyte culture (MLC) with the 2 living lymphocyte populations; (2) MLC with the killed lymphocytes of the donor of the fractions and the living lymphocytes of the other individual; (3) negative control culture (NCC) with the living lymphocytes of one of the individuals; (4) NCC with the living lymphocytes of the other individual.

The results, presented in the Table, show that only the pellet of the third centrifugation at 105,000 *g* is active. It produces the transformation of the isologous lymphocytes into blastic cells. Its activity is very similar to that observed when the killed lymphocytes are cultured in its place under the same conditions. Moreover, this demonstrates that the antigens responsible for the stimulation

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