SECRETION OF HUMAN CHORIONIC GONADOTROPHIN BY THE EARLY PLACENTA *IN VITRO*

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Summary. The secretion *in vitro* of HCG and proteins was studied in fragments of placenta from women in the first trimester of pregnancy by a pulse-chase system. A 10-min pulse with [^3H]leucine was used. It was concluded that the approximate half-time of release for HCG was 150 min. Proteins precipitable with trichloroacetic acid had a bi-exponential pattern, the half-times of release being 100 and 270 min. These rates of release indicate that the HCG produced by the early placenta was rapidly passed into the circulation rather than stored.

Previous studies have established that HCG and proteins synthesized *in vitro* by placental tissue taken from women in the first trimester of pregnancy are secreted into the incubation media (Benagiano *et al.*, 1972; Patrino *et al.*, 1973).

The rate of release of these macromolecules is not known and difficult to study in women *in vivo*, because the amount of radioactive material to be administered may be hazardous (Lerner *et al.*, 1971). Moreover, the values may be a reflection of maternal or fetal rather than placental function (Klopper, 1969). Therefore we have used the *in-vitro* pulse-chase system developed by Meldolesi *et al.* (1972) and Samli & Lai (1973) to study the secretion of prolactin and growth hormone from rat pituitaries. The tissue is given a radioactive label for a short period of time, and the transport of a constant amount of radioactive molecule can be subsequently followed (Schramm, 1967).

Placentae were obtained from five women in the first trimester of pregnancy after therapeutic abortion induced by curettage. Tissues were collected in cold Ringer solution and used within 1 hr. Fragments of each placenta, weighing approximately 5 to 10 mg, were washed several times with cold Krebs–Ringer bicarbonate, pH 7.2 (Krebs, 1950). Aliquots of 100 mg were placed in 15-ml Ehrlenmeyer flasks containing 1 ml Krebs–Ringer bicarbonate and 8 μCi L-[4,5- ^3H]leucine (sp. act. 41.2 Ci/mmol) (New England Nuclear Corporation). The flasks were left in a cold water-bath for 15 min and then pulse-labelled for 10 min at 37°C in an atmosphere of 95% CO_2 /5% O_2 in a shaking bath at 1.5 cycles/sec. The fragments of placenta were then separated from the radioactive incubation media, washed three times with large volumes of warm in-

cubation medium containing 760-fold excess cold leucine (chase medium), and reincubated in the chase medium for 15, 30, 60, 120 or 240 min.

At the end of incubation, the media were separated from the tissues, which were then washed several times and homogenized with 1 ml chase medium. The media and homogenized tissues were centrifuged at 20,000 *g* at 4°C and the corresponding supernatants used for subsequent analysis.

The isolation of labelled HCG was performed as described by Patrino *et al.* (1973) using 100 i.u. HCG carrier and antiserum to HCG according to its titre. The precipitate obtained was washed twice with Krebs–Ringer bicarbonate, then dissolved in 0.1 M-NaOH, precipitated with the same volume of 10% of trichloroacetic acid (TCA) and finally washed as for total proteins.

Table 1. Effect of different preincubation periods on the incorporation and secretion of [³H]leucine into HCG and total proteins by fragments of placenta taken from women in the first trimester of pregnancy during incubation for 30 min

Preincubation time (min)	HCG (ct/min × 10 ⁻² /g)		Total protein (ct/min × 10 ⁻² /g)	
	Tissue	Medium	Tissue	Medium
0	144 ± 18*	244 ± 49	754 ± 54	1206 ± 140
30	126 ± 13	227 ± 51	697 ± 31	988 ± 122
60	119 ± 16	187 ± 38	618 ± 44	1131 ± 155
120	134 ± 11	202 ± 43	754 ± 38	1006 ± 98
240	110 ± 21	214 ± 55	707 ± 46	1115 ± 114

* Values are the means ± S.D. for three different placentae.

For the isolation of radioactive proteins equal volumes of 10% TCA were added to 0.2 ml of medium or tissue supernatants. The mixture was centrifuged at 3000 *g* for 20 min and the supernatants were used for determination of the radioactivity of TCA-soluble proteins. The precipitates were washed twice with 5% TCA containing 0.01 M cold leucine, dissolved with 0.1 M-NaOH, precipitated again with TCA up to 5% concentration, and finally washed with ether and solubilized in 0.2 ml 0.1 M-NaOH. Aliquots of 0.1 ml were incorporated into 10 ml dioxane containing 0.2% of 2,5-diphenyloxazol (PPO) and 10% naphthalene. The remaining aliquots were used for assessment of the protein content by the method of Lowry *et al.* (1951). A control sample was pulse-labelled in the same way, but with the addition of 20 mM cold leucine. The radioactivity obtained was subtracted from that of the samples.

In preliminary experiments, the viability of placental cells was checked by determining their rate of incorporation of [³H]leucine into HCG and total proteins for periods of incubation similar to those in the chase assays. The fragments were first preincubated for 30, 60, 120 or 240 min with Krebs–Ringer bicarbonate without radioactive leucine. The tissues were then incubated for 30 min with the radioactive leucine and the amount of synthesized HCG and total proteins measured in tissues and media. As shown in Table 1, the amount of radioactivity remaining in the tissue and secreted into the medium was similar for all the preincubation periods.

The distribution of pulse-labelled HCG and total proteins during the chase incubation is shown in Text-fig. 1. The radioactivity decreased in the tissue and increased in inverse proportion in the medium during incubation. The ct/min of tissue plus medium for each period of incubation was considered as 100%, and the half-times for release of labelled HCG and proteins were calculated as shown in Text-fig. 2. The approximate half-time of HCG release was 150 min. There appeared to be a bi-exponential release of total proteins into the medium: a fast phase during the first 60 min of chase with a half-time of release of 100 min, and a slow phase with a half-time of release at 270 min of chase.

TEXT-FIG. 1. The distribution *in vitro* of pulse-labelled (a) TCA-precipitable protein and (b) HCG between tissue and medium, as a function of minutes of chase, in early human placenta. Vertical bar represent the S.D. for five placentae. ●, Total labelled protein or HCG in tissue plus medium; ●, total labelled protein or HCG in tissue; ○, total labelled protein or HCG in medium. The numbers on the graph represent the % of total proteins/HCG in tissue plus medium at the corresponding time of the chase, when compared with the value of the pulse (10 min) as 100%.

The incorporation of labelled amino acid into HCG and total proteins after the pulse-labelling was checked by determining its specific activity in ct/min in the tissue plus medium/g of tissue. There was no change during the chase period, but a consistent drop of 10 to 20% as compared with the pulse label was found by the end (Text-fig. 1).

The radioactivity (ct/min/mg protein) of the TCA-soluble, and the TCA-precipitable proteins in the tissue and in the tissue plus medium, respectively, remained unchanged during the chase periods. There was a parallelism between the values for TCA-insoluble and TCA-soluble proteins, but the levels of the latter were lower.

The present study shows that, during a short pulse period, fragments of early human placenta incorporated [³H]leucine into HCG and other TCA-precipitable proteins which were later released at different rates into the chase medium. The bi-exponential pattern of release of total proteins indicates the existence of at least two different pools of material in the placenta.

TEXT-FIG. 2. Percentage of pulse-labelled (a) TCA-precipitable protein, and (b) HCG in tissue and medium in a chase experiment on human placental tissue after a pulse of 10 min. ●, In tissue; ○, in medium. Vertical bars represent the S.D. for five placentae.

The possibility of re-synthesis of HCG and total proteins after pulse-labelling, leading to an overestimate of release, was avoided by using an excess of cold leucine in the chase medium. Unmodified specific activity during the chase indicated that re-synthesis had not occurred. The viability experiments showed the integrity of the cellular system. The fairly constant values of radioactivity obtained in tissue and media at different times suggest that the synthesized HCG and total proteins were not leaked into the chase medium.

The significance of the short time of release obtained for HCG under the experimental conditions described supports the idea (Patrino *et al.*, 1973) that the hormone produced was not accumulated in the cells, but was rapidly secreted.

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REFERENCES

- BENAGIANO, G., PALA, A., MEIRINHO, M. & ERMINI, M. (1972) Biosynthesis of human chorionic gonadotrophin *in vitro*: incorporation of [^{14}C]L-leucine. *J. Endocr.* **55**, 387–396.
- KLOPPER, A. (1969) The assessment of placental function in clinical practice. In *Fetus and Placenta*, p. 472. Eds A. Klover & E. Diczfalusy. Blackwell Scientific Publications, Oxford.
- KREBS, H.A. (1950) Body size and tissue respiration. *Biochim. biophys. Acta* **1**, 249–269.
- LERNER, U., SAXENA, B.N. & DICZFALUSY, E. (1971) Extracorporeal perfusion of the human foetus, placenta and foeto-placental unit. In *Perfusion Techniques*, p. 311. Ed. E. Diczfalusy. Bogtrykkeriet Forum, Copenhagen.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- MELDOLESI, J., MARINI, D. & DEMONTE MARINI, M.L. (1972) Studies on *in vitro* synthesis and secretion of growth hormone and prolactin. I. Hormone pulse labeling with radioactive leucine. *Endocrinology* **91**, 802–808.
- PATRINO, L.C., FLURY, A., ROSATO, J. & MARTÍN, A. (1973) Biosynthesis *in vitro* of chorionic gonadotrophin from human placenta. *Hoppe-Seyler's Z. physiol. Chem.* **354**, 1129–1132.
- SAMLI, M. H. & LAI, M.-F. (1973) Protein synthesis in the rat anterior pituitary. III. The fate of total protein, prolactin and growth hormone labeled in an *in vitro* incubation. *Endocrinology* **93**, 767–776.
- SCHRAMM, M. (1967) Secretion of enzymes and other macromolecules. *A. Rev. Biochem.* **36**, 307–320.