In vitro Synthesis of an ACTH-Like Hormone and Human Chorionic Somatomammotrophin by Placental and Amniotic Cells

On the basis of adrenocorticotrophic activity found in placental extracts, several authors have previously suggested that the placenta could produce an ACTH-like hormone[1–4]. However, no definite evidence was found for the site of production of this hormone, as it could as well be brought in through the blood as directly produced in the placenta[5]. Previous studies from this group[6,7] have recently demonstrated the presence in plasma of pregnant women of a factor, different from pituitary ACTH, which cross-reacts with synthetic human ACTH in a homologous human-anti-human ACTH radio-immunoassay system[8].

A peptide or protein cross-reacting with pituitary ACTH both through its biological and its immunological activity[6,7] was extracted from placental tissue with acid-acetone according to LYONS[9], and partially purified with oxycellulose by the method of ASTWOOD et al.[10] and with glass powder according to RATCLIFFE and EDWARDS[11]. This factor was shown to stimulate the corticosterone production in hypophysectomized rats in the bioassay of LIPSCOMB and NELSON[12], and was called 'human chorionic corticotrophin' (HCC)[6,7]. It was also found in the incubation medium of culture of placental fragments.

The present research was carried out to demonstrate the in vitro synthesis and release of HCC by placenta and amniotic membrane, and to compare them to the synthesis and secretion of human chorionic somatomammotrophin (HCS).

Tissue cultures. Placental fragments of about 1 mm³ and amniotic membranes were cultured in roller tubes and in plastic culture dishes with 2 ml of McCoy's medium (Grand Island Laboratories, N.Y. USA) with the addition of 0.5 µg/ml hydrocortisone hydrogensuccinate and 50 µg/ml crystalline insulin.

For cultures in the roller tubes, the fragments (100–200 mg wet wt/tube) were distributed over the walls of the tubes and excess fluid allowed to drain off; 2 ml of culture medium were then added.

For cultures in the plastic dishes, the fragments were placed on a Millipore membrane (0.45 µm) supported by a piece of stainless steel mesh. The level of the 2 ml culture fluid was just at the level of the Millipore membrane. Cultures were incubated in a water-saturated atmosphere of 5% carbon dioxide in air.

In a first group of experiments, the tissues were cultured for 48 h at 37 °C. At the end of the culture period, the medium was collected, centrifuged and the supernatant analyzed. For comparison, other tubes with placental or amniotic membrane fragments were stored at 4 °C during the incubation period. The supernatant was analyzed after sonication and centrifugation.

In another group of experiments the tissues (placental fragments) were cultured for 72 h at 37 °C. Every 24 h the culture medium was removed for analysis and replaced by fresh medium. At the end of the incubation period, the remaining tissue was put in 2 ml medium, sonicated and centrifuged. The supernatant was analyzed. For comparison, other fragments of placenta were immediately frozen with 2 ml of McCoy's medium. At the end of the culture period, they were defrosted, sonicated and centrifuged. The supernatant was analyzed. McCoy's medium was used as a blank in all the assays.

Assays. The HCS radioimmunoassay was performed in the incubation media diluted in 0.04 *M* phosphate buffer, pH 7.4 containing 0.1% beef serum albumin according to previous description[13]. HCS SCLAVO was

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Immunoreactive HCS and ACTH levels measured in the incubation media of placental fragments and amniotic membranes cultures in vitro, and in the supernatant of the same tissues stored at 4 °C, sonicated and centrifuged (intracellular tissue content)

		Placental fragments		Amniotic membranes	
		I.R.HCS (µg/ml)	I.R.ACTH (pg/ml)	I.R.HCS (µg/ml)	I.R.ACTH (pg/ml)
McCoy's medium		0.0	0.0	0.0	0.0
Intracellular tissue content		12.5	48.8	0.045	39.7
		16.5	80.0	0.080	60.5
Incubation media					
	Roller tubes	40	151.0	0.272	125.0
		36	111.0	0.145	155.0
		29	340.0	0.450	175.0
		41	107.0	0.410	165.0
	Plastic dishes	20.5	172.0	0.145	125.0
		19	115.0	0.230	165.0
		16	125.0	0.320	101.0
		9.5	145.0		

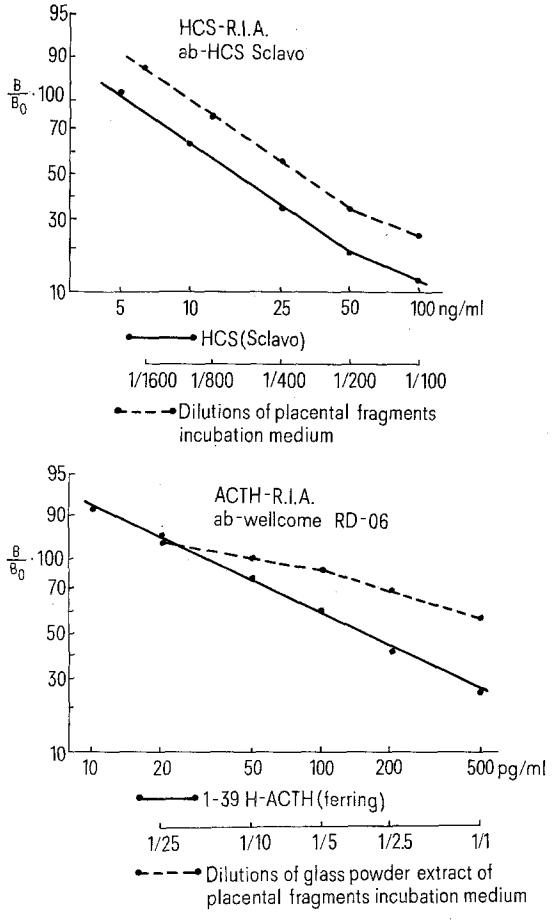

Fig. 1. Upper part: HCS radioimmunoassay. Dilution curves of standard HCS and of incubation medium of placental fragments culture. Lower part: ACTH radioimmunoassay. Dilution curves of standard synthetic 1-39 h-ACTH and of glass powder extracts of incubation medium of placental fragments culture.

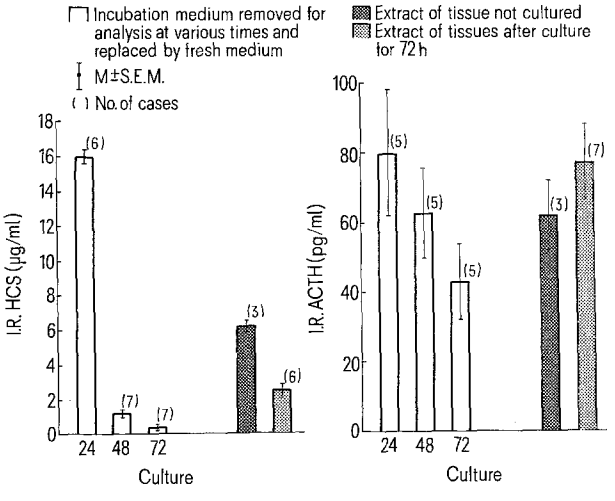

Fig. 2. Placental fragments culture in vitro: immunoreactive HCS (left side) and immunoreactive ACTH (right side) levels in the medium removed every 24 h and replaced by fresh medium, in noncultured tissue and at the end of 72 h culture.

used as standard[14]. HCC was detected and partially measured by means of its partial cross-reaction in the radio-immuno-assay for human ACTH.

The ACTH radioimmunoassay was performed using anti-human ACTH rabbit serum (Wellcome RD 06), synthetic 1–39 human ACTH from Ferring A.B. as a standard and for labelling, and goat-anti-rabbit gamma-globulin serum (11-ab, Sclavo) for precipitation[8,15]. ACTH was labelled according to GREENWOOD et al.[16] with I^{125} and purified on a short dry cellulose column (Whatmann no 1, Chromedia CF 11)[17]. The biological fluids were extracted before the assay. Extraction was performed with porous glass according to RATCLIFFE and EDWARDS[11] with minor modifications[15]. The results were corrected for loss during extraction procedure. The statistical analysis of the results was made according to the Student's *t*-test.

Results. The incubation media of placental or amniotic membrane fragments tested for their immunoreactive HCS and ACTH contents showed high amounts of substances measurable by radioimmunoassay (Table). The incubation media from cultures performed in roller tubes always gave higher immunoreactive HCS contents than those from tissues cultured in plastic dishes. The immunoreactive HCS levels found in the cultures of placental fragments were found to be much higher than those in cultures of amniotic membranes.

The immunoreactive ACTH levels, on the contrary, failed to show any significant difference between the different incubation procedures or the different tissues tested. For both hormones the contents in the tissue prior to culture were lower than the amount recovered in the incubation media (Table).

In the HCS radioimmunoassay (Figure 1) the dilution curve of the incubation media of the placental fragments demonstrated a very close similarity and parallelism with the standard curve for HCS. In contrast to this, in the ACTH radioimmunoassay the dilution curves of the glass powder extracts of the same culture media did not show any parallelism with the standard curve for ACTH.

These results suggest the presence of a peptide or protein, other than pituitary ACTH, having immunological similarities to this hormone. To verify that HCS and this ACTH-like peptide are neosynthesized by the placental cells, another experiment was performed in which the incubation medium was replaced and assayed every 24 h until the 72nd h. The hormonal contents of the tissues were tested after their culture for 72 h and compared with that of tissues not cultured. The results (Figure 2) demonstrate in vitro synthesis of both HCS and an ACTH-like peptide by the placental fragments. The HCS produced by the cells was significantly higher than the intracellular contents; the placental synthesis in vitro was maximum during the first 24 h of the test and significantly reduced in the 48th and 72nd h samples. Compared to the values found before culture, the intracellular contents of HCS at the end of the experiment was significantly reduced.

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The immunoreactive ACTH intracellular contents did not change significantly after the 72 h incubation time. High immunoreactive ACTH concentrations were measured in all the incubation media samples changed at the various periods (Figure 2, right side). However, the ACTH-like protein immunoreactivity of the contents of the samples decreased successively with the increase of the incubation time and a significant difference was found between the 24 and the 72 h samples.

Moreover, the total ACTH-like immunoreactivity measured in the medium was nearly three times that found in the placental fragments before initiating the culture ($P < 0.001$).

Discussion. The results demonstrate the production, by human placental cells and amniotic membranes, of an ACTH-like peptide or protein which cross-reacts in a homologous human-anti-human ACTH radioimmunoassay. These observations confirm those of the previous studies on the presence of an ACTH-like substance in pregnancy plasma and in human placental extracts[6,7]. Moreover, they demonstrate that this substance is synthesized in the placenta itself, rather than coming from maternal blood.

The different results reported here and in the previous studies, and based both on the immunological activity of this ACTH-like substance, and demonstrating its placental origin, justify its proposed name: human chorionic corticotrophin (HCC). The use of a partial cross-reaction in the radioimmunological system does not permit an exact evaluation of the concentration of the substance. However, the observation that the total immunoreactive ACTH secreted in the medium corresponds to three times the intracellular content before culture strongly suggests that HCC synthesis is, in the experimental conditions used, very active. The demonstration of amniotic membrane content and secretion capacity of both HCS and HCC suggests a model for the study of the role of these cells in the protein composition of amniotic fluid.

In conclusion, the present data demonstrate the capacity of the placental and amniotic cells to synthesize and secrete in vitro, besides HCS, human chorionic corticotrophin. The importance and the role of the latter in human reproduction is open to much speculation and must be investigated by further research[18].

Résumé. La synthèse de la somatomammotrophine (HCS) et d'un facteur présentant une réaction immunologique croisée avec l'ACTH a été démontrée dans des cultures de fragments de placenta et de membrane amniotique. Ce facteur est comparable à la corticotrophine chorionique humaine précédemment décrite dans le plasma de femme enceinte et les extraits placentaires.

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Effects of Thyroidectomy on Ovarian Compensatory Hypertrophy in Rats

Several investigators have studied the effect of thyroidectomy (or propylthiouracil-induced hypothyroidism) on the hypertrophy of various organs following unilateral removal of the partner organ. ZECKWER[1] showed that thyroidectomy reduces the weight of the remaining adrenal of unilaterally adrenalectomized rats below that of intact controls but does not affect the compensatory response of the remaining kidney in the unilaterally nephrectomized rat (ZECKWER[2]). Also, PROULX and GORSKI[3] demonstrated that in unilaterally adrenalectomized rats rendered anovulatory by administration of androgens, the weight of the remaining adrenal is significantly reduced by administration of propylthiouracil. CONTOPOULOS and KONEFF[4] revealed that removal of the thyroid in intact female rats caused a reduction in ovarian weight and recently SAIDUDDIN[5],

Effect of thyroidectomy and/or hemicastration on body and ovarian weights and corpora lutea production

Group	Condition	No.	Body weights (g) (Mean ± S.E.)	Left ovarian weights (mg) (Mean ± S.E.)	Relative ovarian weight[d]	Number of corpora lutea (Mean ± S.E.)
I	Intact	46	224.2 ± 2.1	39.8 ± 1.1	0.178 ± 0.0045	13.1 ± 0.65
II	Hemicastrated	49	218.5 ± 2.6[a]	58.9 ± 1.6[c]	0.269 ± 0.0059[c]	18.5 ± 0.76[c]
III	Hemicastrated + thyroidectomized	46	202.3 ± 3.2[b]	49.9 ± 1.4[b]	0.247 ± 0.0067[b]	15.6 ± 0.93[b]

[a] Significantly different ($p < 0.05$) from group III. [b] Significantly different ($p < 0.05$) from groups I and II. [c] Significantly different ($p < 0.05$) from groups I and III. [d] Left mg ovarian weight/g body weight.