quantities of corticotrophin. The administration of doses of corticotrophin which approach the minimal quantities required for stimulation of the adrenal cortex are valuable in determining the sensitivity of the gland. The procedure also has value in the biological assay of corticotrophin and its synthetic analogues, using man as the test animal. There appears to be no difference between the degree of adrenal stimulation produced by DW-75 and synacthen when given i.v. in nanogram amounts[10].

Résumé. L'activité de la corticotrophine porcine a une durée plus longue que celle du DW-75, quand on l'administre en quantités égales, basées sur un test biologique. Le DW-75 a une durée d'activité plus longue par injection intraveneuse que par injection intramusculaire. Une quantité nanagramme du DW-75 a la même action stimulante adrénocorticale que le synacthène.

M. FRIEDMAN

Paediatric Department, University College Hospital and Clinical Research Centre, London (England), 5 December 1968.

[10] I am most grateful to Dr. E. R. EVANS of Sandoz for supplies of DW-75 used in this study.

The Relationship Between Human Chorionic Somato-Mammotropine Hormone and Thyrostimuline in Biological and Radioimmunological Assays

A new protein hormone was isolated and purified in recent years from the placenta[1,2]. It was found in the blood of pregnant women and in cases of chorionic tumour[3,4]. This hormone, human chorionic somato-mammotropine (HCSM), has previously been called human placental lactogen (HPL), chorionic growth hormone, prolactine (CGP) and placental purified protein hormone (PPPH)[5].

A cross reaction between this hormone and the somatotropin STH was observed[6,7]; moreover the HCSM has a biological action similar to STH and also to prolactin action[1,2,6-8].

This placental hormone has a molecular weight between 36,000 and 38,000[9,10] and possesses some similar amino acid composition and a sequence with one part of the STH chain[10-12]. These last observations might explain the cross reaction existing between STH and HCSM and the high level of STH found both with radioimmunoassay and with biological assay during the pregnancy. This increase, however, is not due to STH but to HCSM. Indeed YEN et al.[13] have shown that STH is not increased during pregnancy; moreover, the growth hormone cannot interfere with the HCSM values, as these are about a thousand times more elevated than the STH values. A radioimmunoassay had been developed for the determination of HCSM in blood and biological fluids[3,4,14,15,21]. Since in the state of pregnancy an increased level of plasma TSH[16] was observed, it appears necessary to check also the possibility of an interference of the HCSM or of a cross reaction between HCSM and TSH in radioimmunological and biological assays. TSH could have some similar amino acid sequence with HCSM which would react with the antiserum in the radioimmunoassay, or could have some TSH-like biological activity. These problems are the purpose of this paper.

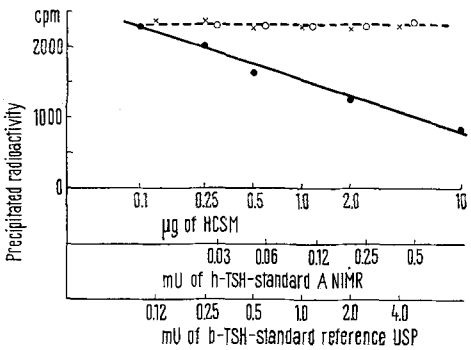
Methods. The radioimmunoassay of HCSM was described recently[4]. The radioimmunoassay for the TSH was established, as previously explained, using both bovine or human TSH antisera[17,18] and the biological assay of TSH according to MACKENZIE's method[19].

Results. The Figure shows the decreasing radioactivity of the antigen-antibody complex, as increasing quantities of unlabelled HCSM (kindly supplied by Dr. P. Neri, Isut Sclavo, Siena, Italy) are added to the incubation medium. When TSH, either bovine or human, is added, no change in the binding capacity occurs. No cross reaction is observed between HCSM and TSH in the radioimmunoassay of the HCSM. In a second experiment we have studied the possible influence of increasing quantities of HCSM on the TSH radioimmunoassay. No proportionality was observed and under a dilution of HCSM of 10 µg/ml of HCSM, no TSH was detected; but with a concentration of 100 µg/ml or more, a mean value of 0.27 mU of TSH per milligram of HCSM was detected, in the case of the radioimmunoassay using the United States Pharmacopae (USP) bovine TSH reference standard and bovine antibodies. With the human TSH standard A of National Institute of Medical Research (London) (NIMR), and human TSH antibodies a mean of 0.19 mU of TSH per milligram HCSM was found. If we suppose a mean value of 10 µg/ml of HCSM during pregnancy, it would indicate a contamination in TSH of 0.0027 or 0.0019 mU/ml of plasma for the bovine or

[1] J. B. JOSIMOVICH and J. A. MCLAREN, Endocrinology *71*, 209 (1962).
[2] H. COHEN, M. M. GRUMBACH and S. L. KAPLAN, Proc. Soc. exp. Biol. Med. *117*, 438 (1964).
[3] S. L. KAPLAN and M. M. GRUMBACH, J. clin. Endocr. *25*, 1360 (1965).
[4] A. R. GENAZZANI, M. AUBERT, M. BENUZZI-BADONI and J.-P. FELBER, Int. Symp. Foeto-placental Unit (Milan, Italy, September 1968).
[5] P. FIORETTI, A. R. GENAZZANI and P. NERI, Riv. ital. Ginec. *52*, 331 (1968).
[6] J. B. JOSIMOVICH and B. L. BRANDE, Trans. N.Y. Acad. Sci. series 2, *27*, 161 (1964).
[7] M. M. GRUMBACH and S. L. KAPLAN, Trans. N.Y. Acad. Sci. Series 2, *27*, 167 (1964).
[8] I. A. FORSYTH, J. Endocr. *37*, 35 (1967).
[9] P. NERI, G. CANALI, F. BENVENUTI and A. DE GORI, Boll. Soc. ital. Biol. sper., in press (1968).
[10] J. R. FLORINI, G. TONELLI, C. B. BREUER, J. COPPOLA, L. RINGLER and P. H. BELL, Endocrinology *79*, 662 (1966).
[11] K. J. CATT, B. MOFFAT and O. H. NIALL, Science *157*, 321 (1967).
[12] C. H. LI and B. STARMAN, Biochim. Biophys. Acta *86*, 175 (1964).
[13] S. C. C. YEN, N. SAMAAN and O. H. PEARSON, J. clin. Endocr. *27*, 1341 (1967).
[14] A. G. FRANTZ, M. Y. RABKIN and H. FRIESEN, J. clin. Endocr. *25*, 1136 (1965).
[15] K. J. CATT and G. W. TREGEAR, Science *158*, 1570 (1967).
[16] TH. LEMARCHAND-BÉRAUD and A. VANNOTTI, Acta Endocr. *60*, 315 (1968).
[17] TH. LEMARCHAND-BÉRAUD and A. VANNOTTI, Experientia *21*, 353 (1965).
[18] TH. LEMARCHAND-BÉRAUD, B. R. SCAZZIGA and A. VANNOTTI, Schweiz. med. Wschr. *96*, 718 (1966).
[19] J. M. MCKENZIE, Endocrinology *62*, 865 (1958).

human TSH respectively (as determined by the radioimmunoassay).

By the TSH biological assay a mean TSH-like activity of 0.30 mU/mg of HCSM was found. This would give, during pregnancy, a TSH-like activity of 0.003 mU/ml of plasma. This value would be undetectable by the biological assay. With HCSM preparation, previously incubated with TSH antiserum before the biological assay, or when incubated with HCSM antiserum, no TSH-like activity was present. These results suggested that the TSH-like activity found in the HCSM preparation could be due to a slight contamination with a TSH-like material isolated from placenta. This contamination would represent only about 0.5 µg/mg of HCSM and would not interfere either with the HCSM or with the TSH radioimmunoassay. The TSH increase during pregnancy was about a threefold elevation (from 0.19 mU/ml to 0.45 mU/ml) and this occurred as early as after the sixth week. This level remains elevated during pregnancy with a further increase around the 34th week. The presence of a high level of HCSM would then increase the TSH level by only $^1/_{100}$ and could not be responsible for the high TSH level observed during pregnancy. The reason for this blood TSH increase during pregnancy remains unknown. The possible role of HCG was tested and was found not to interfere with TSH.



The effect of human and bovine TSH on the reaction between HCSM-I^{131} and HCSM antiserum. Note the absence of cross reaction between HCSM and TSH either from bovine or human origin. HCSM antiserum 1/5000 with increasing quantities of unlabelled HCSM (●—●), bovine TSH (×---×), human TSH (○---○). mU of human TSH expressed according to the reference of human TSH standard prepared by the National Institute for Medical Research (NIMR), Mill Hill, London. mU of TSH expressed according to the bovine reference standard established by the United States Pharmacopae (USP).

Discussion. In conclusion no cross reaction occurs between HCSM and TSH in radioimmunoassay. The HCSM tested in the TSH radioimmunoassay shows a slight TSH-like activity of 0.27 mU of TSH/mg of HCSM in the bovine radioimmunoassay and of 0.19 mU/mg of HCSM in the human TSH system, and of 0.30 mU/mg of HCSM in the biological assay. This slight TSH-like activity is not responsible for the increased TSH level found during pregnancy. This latter could be due to a TSH-like activity secreted by the placenta[20]. As this TSH-like activity was suppressed by TSH and HCSM antibodies, it may be due to traces of TSH-like material in HCSM preparation. Further investigation on this point is being made.

Résumé. Les auteurs montrent qu'il n'existe pas d'interférences et de réaction croisée entre la thyréostimuline et l'hormone placentaire, la HCSM (human chorionic somato-mammotropine hormone) lors des déterminations respectives de ces hormones par test radioimmunologique. L'HCSM n'est donc pas responsable de l'augmentation de la TSH plasmatique observée au cours de la grossesse.

A. R. Genazzani
and Th. Lemarchand-Béraud

Clinica ostetrica e ginecologica, Siena (Italy) and Clinique médicale universitaire, 1011 Lausanne (Switzerland), 7 November 1968.

[20] P. Hennen and J. G. Pierce, in *Protein and Polypeptide Hormones* (Ed. M. Margoulies; Excerpta Medica Foundation ICS 161, part 2, 1968), p. 511.

[21] M. Aubert, A. R. Genazzani and J. P. Felber, Acta Endocr., in preparation (1969).

Determination of Human and Bovine Growth Hormones in the Physiological Range by the Immunoelectroadsorption Method

The new and rapid immunoelectroadsorption method (IEA) for measuring antigen-antibody reactions has been described in previous articles[1-4] and the results obtained with this method for the determination of circulating bovine and human growth hormones are presented in this note.

In brief, the IEA test consists in carrying out 2 successive electroadsorptions on a chromium coated glass slide with the help of a small electric current (300 µA) with the proper polarity. The antigen is deposited in the first and the antibody in the second adsorption. When the immune serum used in the second adsorption is homologous to the antigen, the adsorbed layer is thicker than that adsorbed from the same antiserum when no antigen was present in the first adsorption. For all technical details see the method by A. Rothen et al.[5].

The following procedure was adopted for testing the applicability of the IEA method to the determination of the concentration of human growth hormone in a serum. Known amounts of growth hormone[6] were dissolved in a 0.001 *M* buffer solution of veronal containing 2% normal human serum. The pH of this solution (7.7) was much above the isoelectric point of the hormone (4.9). This was the reason for having the slides positively charged during the electroadsorption.

[1] C. Mathot, A. Rothen and J. Casals, Nature *202*, 1181 (1964).
[2] C. Mathot, A. Rothen and S. Scher, Nature *207*, 1263 (1965).
[3] C. Mathot, P. A. D'Alesandro, S. Scher and A. Rothen, Am. trop. Med. Hyg. *16*, 443 (1967).
[4] C. Mathot and A. Rothen, Am. J. trop. Med. Hyg. *17*, 756 (1968).
[5] A. Rothen and C. Mathot, Immunochemistry, in print 1969.
[6] We are greatly indebted to the National Pituitary Agency for providing us with many samples of human growth hormone and antihuman growth hormone sera as well as to Dr. Hao-Chia Chen of Rockefeller University, who most kindly provided us with a generous supply of very pure bovine and human growth hormones. We also wish to thank Dr. H. Demura of Cornell Medical School for a sample of a potent rabbit antiserum against human growth hormone.