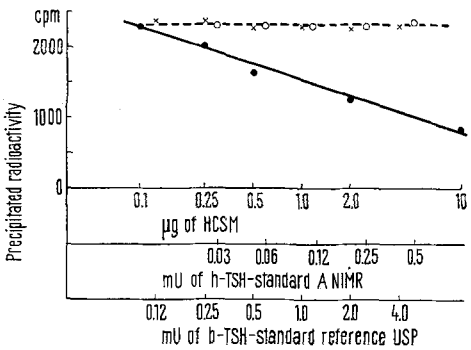
human TSH respectively (as determined by the radioimmunoassay).

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.

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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.

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[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.

Tests were made in the range of concentrations from 0.1 ng/ml to 1000 ng/ml. Therefore, in all antigenic solutions tested there was an overwhelming number of protein molecules of the serum as compared to the number of growth hormone molecules present. Hence good controls could be made with the veronal solution containing 2% normal human serum used for dissolving the hormone. Such a solution was called the carrier. For each test made with growth hormone plus carrier for the antigen adsorption, a simultaneous test was made using the carrier only. The same solution of immune rabbit serum was then adsorbed on both slides. The immune serum used was diluted 1–50 in veronal buffer 0.03 *M*, pH 7.7. Any difference in the thickness adsorbed from the antiserum on the 2 slides gave an indication of an immunological reaction. Thus the specific adsorption could be separated from the non-specific one.

Human growth hormone. A few representative results obtained in experiments made as just described have been summarized in Table I. The numbers Δ A represent in Å units the difference in the thickness of the layers adsorbed from the immune serum on 2 slides, one coated in the first adsorption with units of growth hormone plus carrier and the other with carrier only. The thickness adsorbed on slides coated with the carrier was of the order of 20 Å.

A very striking fact is immediately evident. The difference in thickness (Δ A) adsorbed from the antiserum is independent of the concentration of growth hormone, except for very low concentrations. Under the most favorable conditions, using a very potent antiserum, the limit of sensitivity reached was 0.1–0.2 ng/ml, which is adequate for clinical determinations. This compares favorably with the values 0.07 ± 50% ng/ml obtained by HUNTER and GREENWOOD[7] with the radioimmunoassay. It should be said that the thickness of the layer specifically adsorbed varied greatly depending on the immune serum, values as high as 35 Å have been observed. With the IEA method cross-reactions do not seem to occur between human and bovine growth hormone as illustrated by the results summarized in the Table II, where the symbols NRS, AHGH, and ABGH stand for normal rabbit serum, antihuman growth hormone, and antibovine growth hormone, respectively. The figures in the last column represent the thickness in Å observed after normal or immune sera adsorption which lasted 30 sec. 72 ng/ml of BGH and 70 ng/ml of HGH in 0.002 *M* veronal without carrier were used. When the concentration in bovine growth hormone was as high as 360 ng/ml no cross-reaction could be observed with an antihuman growth hormone serum.

Bovine growth hormone. All bovine growth hormone solutions were made in 0.005 *M* veronal buffer containing 2% normal rabbit serum. The results were similar to those obtained with human growth hormone except for the fact that the thickness adsorbed from the immune serum increased with the concentration in growth hormone up to 14 ng/ml where a plateau was reached. The maximum thickness specifically adsorbed was about 73 Å. As seen in Table I in the case of human growth hormone the maximum was reached for a concentration of 0.5 ng/ml.

Evaluation of the concentration in growth hormone of human sera. Tests were conducted as follows to determine the concentration in growth hormone of human sera. Slides were treated for the first adsorption with the unknown serum at successive dilutions. For the second adsorption, one part of the slide was treated with a dilute rabbit antiserum and the other part with a normal rabbit serum at the same dilution. With increasing dilutions of the unknown human serum used in the first adsorption, a dilution was obtained for which the thickness observed was the same on the 2 parts, one treated with the immune the other with the normal rabbit serum. The corresponding dilution of the human serum is called the limiting dilution. It permits, with the help of such data as shown in Table I to estimate the growth hormone concentration in the undiluted human serum. In a few experiments with sera from a hypopituitary and an acromegalic subject, 0.8 ng/ml and 12 ng/ml of growth hormone were found respectively.

It can be easily calculated from the data given in Table I, the dimensions of the immersed part of the slide and the known size of antibodies molecules that at least 200 molecules of antibodies have been immobilized by one molecule of antigen when the concentration in growth hormone was 0.5 ng/ml.

The results presented in this report should encourage clinical investigators to try and perfect the assay of immunoelectroadsorption, especially so since this assay can be performed in a very few minutes[8].

Table I. Specific adsorption from an antihuman growth hormone rabbit serum diluted 1/50 in veronal as a function of the concentration in growth hormone of the solution used for the adsorption of the antigen

GH ng/ml	0	0.1	0.2	0.34	0.5	1	5	16	55	200	1000	20,000 (in water only)
Δ A	0	0	3	10–15	17	15	20	18	18	18	19	21

Table II. Differentiation of bovine and human growth hormone by IEA

Antigen	Sera	Thickness in Å
BGH	NRS	74
BGH	AHGH	72
BGH	ABGH	115
HGH	AHGH	110
HGH	ABGH	60
HGH	NRS	74

NRS, AHGH, and ABGH stand for normal rabbit serum, antihuman growth hormone and antibovine growth hormone. The concentrations of the antigen solutions were 72 ng/ml for bovine growth hormone and 70 ng/ml for human growth hormone in 0.001 *M* veronal. The antisera were diluted 1/20 in veronal buffer 0.03 *M*.

Résumé. La méthode dite d'immunoélectroadsorption a été adaptée à la détermination des concentrations en hormones de croissance présentes dans les sérums humain et bovin. La limite de sensibilité atteinte est de 0.1 à 0.2 ng/ml, amplement suffisante pour une application clinique.

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