Serum HGH concentrations in pregnancy and in the post-partum period have not previously been recorded. Our findings indicate that the concentration is normal during pregnancy but increased in the post-partum period. This is of special interest because of the known requirement for growth hormone in mammary gland development and in milk secretion (Lyons *et al.* 1955) but more particularly because it is known that the administration of growth hormone to animals in declining lactation leads to a resurgence of milk production (Cotes *et al.* 1949).

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REFERENCES

Campbell H J (1959) *J. Physiol.* **149**, 394
Cotes P M, Crichton J A, Folley S J & Young F G (1949) *Nature, Lond.* **164**, 992
Lyons W R, Johnson R E, Cole R D & Li C H (1955) *In:* The Hypophyseal Growth Hormone. Nature and Actions. Eds. R W Smith jr, O H Gaebler & C N H Long. New York; p 461
McGarry E E, Fishman J, Lesnoff A, Simpson R, Lucius J, Ballantyne A & Beck J C (1960) *Acta endocr., Copenhagen.* Suppl. 51, 299
Raben M S (1959) *Recent Prog. Hormone Res.* **15**, 71
Read C H (1960) *In:* Clinical Endocrinology I. Ed. E B Astwood. New York; p. 598
Stavitsky A B (1954) *J. Immunol.* **72**, 360

The Immunological Assay of Growth Hormone [*Abstract*]

by M Hartog BM MRCP and
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The paper described the technique, its accuracy and the results obtained in 18 normal subjects, 11 patients with acromegaly and 7 hypophysectomized patients. This work has recently been published (*J. Endocrin.* **22**, 101).

In addition results in 5 patients with active acromegaly who had been treated by pituitary implantation were presented. Two of these patients experienced no improvement following implantation of ^{198}Au and their serum GH values were unchanged. Another patient whose serum GH was also unchanged experienced some improvement following ^{198}Au. Two patients had clear clinical improvement, one following ^{198}Au and the other following ^{90}Y implantation, and their serum GH values fell significantly from their pre-implant figures.

Immunological Study of Human Gonadotrophins

by W R Butt PhD ARIC, A C Crooke MD MRCP and F J Cunningham BSc (*Birmingham*)

There is an extensive literature on the early work on antibodies or antihormones to gonadotrophins which has been fully reviewed by Zondek & Sulman (1942) and by Evans & Simpson (1950). Interest in the subject has been renewed by the preparation of purer hormones and by more recent developments in immunological techniques (Henry & van Dyke 1958). Recently Wide & Gemzell (1960) and Brody & Carlström (1960, 1961) have claimed to have developed immunological assays for chorionic gonadotrophin as a test for pregnancy. We have been interested in cross-reactions between different gonadotrophic antigens and antisera which suggest that they may be unspecific (Butt *et al.* 1960).

Antisera have been raised against gonadotrophins from the urine of both postmenopausal (HMG, human menopausal gonadotrophin) and pregnant women (HCG, human chorionic gonadotrophin) and also from human pituitary glands (HPG). The extracts from the urine of postmenopausal women have been purified using ion exchange materials by methods described previously (Butt *et al.* 1959) and HCG was supplied by Leo Pharmaceutical Products Ltd (Copenhagen). Human pituitary preparations supplied by Dr A Korner of the Department of Biochemistry, Cambridge University, were fractionated on columns of carboxymethyl (CM) cellulose and diethyl aminoethyl (DEAE) cellulose and calcium phosphate (CP) (Butt, Crooke & Cunningham, in press).

The antigens were dissolved in saline and mixed with an equal weight of bentonite to which they adsorb strongly. 2·0 ml of the suspension, usually containing 0·5 mg protein were given intravenously to rabbits twice a week for three or more weeks. Biologically active antisera have been obtained against HMG, HPG and HCG using the following amounts of protein:

(1) HMG 20 mg of the DEAE fraction having a total potency equivalent to 300 mg of the International Reference substance, HMG24.
(2) HPG 5 mg of the DEAE fraction having a total potency equivalent to 1,300 mg HMG24
(3) HCG 2·75 mg having a total potency equivalent to 4,400 i.u.

The activity of the antisera was tested *in vivo* using mice or rats. Each serum was injected intraperitoneally and the next day the antigen was given subcutaneously. Antisera to both HMG and

to HPG inhibited both HMG and HPG in mice but failed to inhibit the activity of HCG in rats. Antiserum to HCG, however, inhibited all types of gonadotrophin, HCG, HMG and HPG in mice and rats. This confirms our earlier observations (Butt *et al.* 1960).

In more recent experiments using the agar gel diffusion technique we have observed at least two precipitin lines when antiserum to HMG reacted with either HMG or with HCG but HPG purified to the stage involving chromatography on DEAE cellulose gave only one precipitin line. Further purification of this material on the CP column produced a fraction which had a potency equivalent to 3,000 mg HMG24 and this gave no precipitin lines against antiserum to HMG. The antigen which was responsible for the reaction in the cruder fraction was adsorbed more firmly on to the CP column but was biologically inactive. Antisera to HPG and HCG gave no precipitin lines with any of the gonadotrophic antigens.

A method has now been developed whereby precipitation can be observed in agar gel between antiserum to HCG and gonadotrophic antigens. Nigrosine W.S. (George T Gurr) was first incorporated into the agar gel and after allowing time for the diffusion of antigen and antibody to occur the excess dye was washed out. A dyed precipitin line was now observed where previously no reaction was visible. At least two lines could be seen when antiserum to HCG reacted with HCG or with HMG but when the antiserum was first mixed with HMG to absorb antibody and the absorbed antiserum was allowed to react with HCG only one line was visible in the nigrosine-stained agar gel.

Recently we have applied the red cell hæmagglutination technique, as modified by Ling (1960), to these substances. The absorbed antiserum to HCG agglutinated cells which had been sensitized with HCG and this reaction was inhibited by the addition of microgram quantities of HCG. It was not specific for HCG, however, since the fraction from HPG which was predominantly luteinizing hormone (LH) was also found to be a powerful inhibitor of agglutination. The fraction which was predominantly follicle stimulating hormone (FSH) was much less effective and fractions which were free of gonadotrophic activity, including growth hormone, were without effect.

Conclusion: The evidence so far suggests that antiserum to human chorionic gonadotrophin reacts with not only its own predominantly luteinizing hormone antigen but also the LH from human pituitary glands. The immunological assay of HCG as a test of pregnancy should therefore be accepted with reserve because of this lack of specificity. The antiserum to HCG is relatively much less effective against the mainly FSH fraction from HPG. Antiserum to the DEAE fraction of HPG, which is relatively rich in FSH, is effective against HPG and against HMG but is ineffective against HCG. This suggests that an antiserum which is specific for follicle stimulating hormone can be developed, and this problem is now under investigation.

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REFERENCES

Butt W R, Crooke A C & Cunningham F J (1959) *Acta endocr., Copenhagen* **32, 509**
Butt W R, Crooke A C, Cunningham F J & Gell P G H (1960) *J. Endocrin.* **20, ix**
Brody S & Carlström G (1960) *Lancet* ii, 99 (1961) *Nature, Lond.* **189, 841**
Evans H M & Simpson M E (1950) *In:* The Hormones II. Eds. G Pincus & K V Thimann. New York; p. 351
Henry S S & van Dyke H B (1958) *J. Endocrin.* **16, 310**
Ling N R (1960) *Biochem. J.* **77, 12P**
Wide L & Gemzell C A (1960) *Acta endocr., Copenhagen* **35, 261**
Zondek B & Sulman F (1942) The Antigonadotrophic Factor. Baltimore