SUMMARY

Highly purified urinary FSH, LH and HCG were prepared from extracts of menopausal urine and urine of pregnant women respectively.

The HCG preparations were obtained from starting material with a biological potency of about 1500 IU/mg by using a fractionation with ethanol, CM-Sephadex chromatography, gel filtration with Sephadex G-100 and final isoelectric focusing. The final preparations had the following properties:

- The concentration of contaminants was very low as judged by starch-gel electrophoresis and immunodiffusion.
- The biological potencies ranged from 1700 to 18000 IU/mg and the immunochemical potencies as determined by radioimmunoassay from about 3000 to about 9000 IU/mg. The results of chemical, physical-chemical and immunochemical studies of these preparations show: HCG is not a single molecular entity but consists of a number of components that have identical amino-acid compositions and are immunochemically strongly related to each other. They differ, however, in NANA content, biological potency and isoionic point. Most observations can be explained by differences in the number and position of the NANA groups in the different components.

The FSH and LH preparations were obtained by successive fractionation with ethanol, chromatography with DEAE-cellulose and CM-Sephadex, immunochromatography with an insolubilized anti-HCG serum and final gel filtration. The final FSH preparations obtained had the following properties:

- The biological FSH potencies varied from 1120 to 4720 IU/mg and the immunochemical FSH potencies as estimated by radioimmunoassay from 860 to 1680 IU/mg.
- They had different i/b ratios.
- FSH/LH ratios up to 1770 as determined by radioimmunoassay were found, reflecting a good separation between FSH and LH.
- In two preparations with biological FSH potencies of 2190 and 4720 IU/mg the NANA contents were found to be 6.5 and 6.2% respectively. These results and those of other investigators do not indicate a correlation between the NANA content and the biological potency, such as is observed with HCG.
- Isoelectric focusing showed the presence of immunochemical FSH activity in at least four discrete zones in the pH-ranges 3.7 - 4.0; 4.0 - 4.1; 4.1 - 4.4; 4.4 - 7.0.
- Starch-gel immunoelectrophoresis showed the presence of several immunochemically different materials.
- Immunodiffusion demonstrated that the preparations still contained contaminants.
- The amino-acid compositions of the various final FSH preparations were dissimilar. They were also different from those found by other investigators for highly purified pituitary and urinary FSH.

These findings suggest that urinary FSH is not a single molecular entity but that it consists of a number of molecules that have different biological and immunochemical potencies, and different protein moieties. All observations can be explained by postulating that the urinary FSH molecules are degradation products of FSH from the pituitary.
The most potent urinary LH obtained by immunochromatography had a biological LH potency of 9200 IU/mg and an immunochemical LH potency of 9100 IU/mg. The FSH/LH ratio as estimated by radioimmunoassay was 0.028. Starch-gel immunoelectrophoresis showed one precipitation line against an anti-HCG serum and no line against an antiserum raised to several more or less purified FSH preparations.

The amino-acid composition of this preparation differed from those reported for highly purified urinary LH and highly purified pituitary LH.

The FSH and LH preparations obtained have the highest biological and immunochemical potencies ever found for these urinary hormones.