

Although the progesterone reaction, that is, the decidual transformation of the uterine mucosa, does not occur until about 100 hours have elapsed, even after 48 hours the hormone is no longer present in an active form. In this respect, progesterone is similar qualitatively to oestrone. Inactivation of oestrone, however, occurs much more rapidly and much more intensely than that of progesterone.

Control experiments were set up to prove that, with the help of the extractive method, progesterone which had been added to the mash could again be proved quantitatively.

BERNHARD ZONDEK.

Obstetrical-Gynaecological Department,
Rothschild Hadassah-Hospital,
Jerusalem.

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¹ Zondek, B., *Lancet*, 227, 356 (1934); *Scandin. Arch. Physiol.*, 70, 133 (1934); "Hormone d. Ovariums und d. Hypophysen-vorderlappens", Second edition (Vienna: Springer, 1935), pp. 124-147.

Determination of rH in Endocrine Extracts of a Protein Nature

We have begun to study systematically extracts of endocrine glands containing hormones of a protein nature, especially extracts of the anterior pituitary lobe (ALH) of cattle. For technical reasons very dilute extracts were used: 1 gm. of the dry powder, previously kept *in vacuo*, was shaken for 4 hours with 20 ml. of a 0.0001 N NH_4Cl solution and then filtered. The pH of the filtrate was kept at about 6.2.

Search was made for reducible substances in the solution and their reduction potentials were measured by means of Heyrovsky's polarographic apparatus with the dropping mercury cathode. All measurements were made with a voltage of 3 volts across the potentiometer. Before each experiment a stream of electrolytic hydrogen, thoroughly purified, was bubbled through the solution. The sensitivity used was generally 1/50 and 1/100 of the total sensitivity of the galvanometer.

We show first the current voltage curve obtained with an extract of powdered ALH, showing three steps corresponding with three reducible substances (groups), having well-defined reduction potentials: $rH_1 = -0.23$ volts, $rH_2 = -1.00$ volts, $rH_3 = -1.65$ volts. The second decimal figure has no great significance.

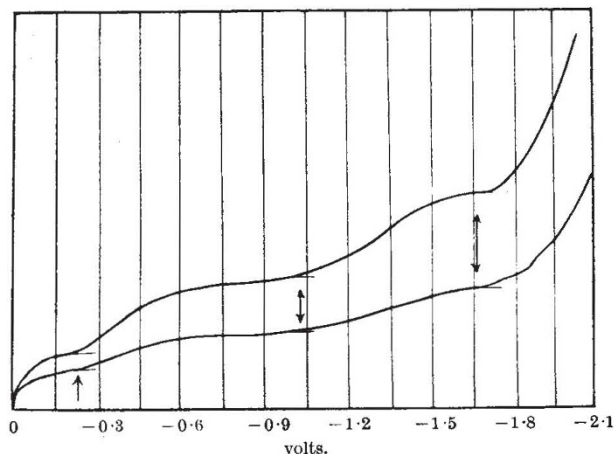
These three steps did not appear when the powder had not been kept *in vacuo*; they disappeared gradually as the extract was made more and more alkaline, and completely at pH 9.9. These substances (groups) are soluble in ether and in acetone. We were able to repeat these results with powders made from different fresh or frozen glands or glands kept for a time under acetone and also with different extracts prepared from the same gland.

Next, we wished to establish whether the three rH values corresponded with different anterior pituitary hormones. We first examined several purified commercial preparations. One of them had, as shown by tests on guinea pigs and rats, a thyreotropic and a gonadotropic action. This preparation, diluted with 0.0001 N NH_4Cl , showed reduction potentials at $rH = -0.23$ v. and $rH = -1.65$ v., and therefore corresponds with rH_1 and rH_3 of the total extract of the powdered ALH. Another preparation showed in our own tests a gonadotropic action, but no signs of thyreotropic activity, as determined by the usual guinea pig method. This preparation

showed $rH = -0.23$ v. and sometimes $rH = -1.00$ v. and therefore corresponded with rH_1 and rH_2 of the total extract.

Finally, we ourselves prepared extracts from the powdered ALH. One extract showed a gonadotropic action, but no definite thyreotropic effect or only very little. It had a reduction potential of $rH = -0.23$ v. only.

Another extract prepared by us showed some thyreotropic but no gonadotropic action and a reduction potential of $rH = -1.65$ v.; it therefore corresponded with rH_3 of the total extract.



CURRENT VOLTAGE CURVE OF A TOTAL EXTRACT OF POWDERED ALH. ABSCISSA: VOLTAGE, ONE DIVISION = -0.15 VOLTS. ORDINATE: INTENSITY OF THE CURRENT. UPPER CURVE: SENSITIVITY 1/50 (ORDINATE: 1 MM. = 2×10^{-7} AMP.). LOWER CURVE SENSITIVITY 1/100 (ORDINATE: 1 MM. = 1×10^{-7} AMP.).

We were not able to find these two substances ($rH = -0.23$ v. and $rH = -1.65$ v.) in extracts from other glands. There are, of course, reducible substances in such extracts; thus we found in some extracts from freshly prepared powdered ovaries from cows rH values of -0.17 v. and -1.35 v., but never values of -0.23 v. or -1.65 v.

The substance (or group of substances) characterized by $rH = -1.00$ v., the rH_2 of the total extract of the powdered ALH, described above, was also found in extracts of the pituitary posterior lobe, whether these extracts had both a hypertensive and an oxytocic effect, or only either of these effects alone.

We conclude from these experiments that in extracts from the freshly prepared powdered anterior lobe of the pituitary the gonadotropic hormone is always accompanied by a reducible group of $rH = -0.23$ v. and the thyreotropic hormone by a reducible group of $rH = -1.65$ v. Gonadotropic hormone prepared from urine of pregnant women does not contain this reducible group having an rH of -0.23 v. The physiological significance of these two specific reducible substances (groups) remains still to be investigated.

Pharmacological Department,
University,
Ghent.

H. HANDOVSKY.

Laboratoires Belges
d'applications biologiques,
Brussels.
Dec. 7.

L. HAUSS.