4877

Isolation of the Relaxative Hormone on the Corpus Luteum.*

H. L. FEVOLD, FREDERICK L. HISAW AND R. K. MEYER.

From the Department of Zoology, University of Wisconsin.

Pelvic ligaments of guinea pigs undergo very pronounced relaxation during pregnancy to facilitate the birth of young. While studying the physiology of this reaction, one of us discovered that it was under hormonal control and that the substance responsible was present in the blood of certain mammals during pregnancy.[1] This hormone is capable of producing relaxation of the pelvic ligaments of virgin guinea pigs in a fashion typical of pregnancy, if given while the animals are in or recovering from oestrum. Later it was found that this reaction could also be produced through the use of extracts of corpora lutea of sows' ovaries. General methods for the preparation of these extracts have been given elsewhere.[2] These corpus luteum extracts, in addition to relaxing the pelvic ligaments, also produced other physiological changes ordinarily attributed to the corpus luteum, such as inhibition of ovulation, vacuolation of the vaginal mucosa of rats,[3] production of pseudo-pregnancy in rabbits,[4] and production of premenstrual endometrium in the uterus of castrate monkeys.[5]

The opinion that more than one hormone was present in our corpus luteum extracts was expressed in some of our earlier papers. This has proven to be the case, since we have obtained a crystalline fraction which produces relaxation of the pelvic ligaments of the guinea pig, while the remaining fraction contains the active material which is responsible for the other physiological reactions. This paper deals with the preparation of the crystalline substance together with some of its properties.

The minced lutein tissue is extracted, for 48 hours, with twice its volume of acid alcohol, at room temperature. The alcohol is acidified by adding 2 cc. of concentrated HCl to 98 cc. of 95% alco-

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

[1] Hisaw, F. L., *J. Physiol. Zool.*, 1929, ii, 59.

[2] Hisaw, F. L., Fevold, H. L., and Meyer, R. K., *J. Physiol. Zool.*, 1930, iii, 135.

[3] Hisaw, F. L., Meyer, R. K., and Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 754.

[4] Hisaw, F. L., and Leonard S. L., *Am. J. Physiol.*, 1930, xcii, 574.

[5] Hisaw, F. L., Meyer, R. K., and Fevold, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 400.

hol. The extract is removed and diluted with one-third its volume of water. The fatty material, which separates out, is filtered off and discarded. The extract is neutralized to a pH of 6.8 whereupon a voluminous precipitate settles out. This is filtered off, redissolved in acid alcohol and reprecipitated. The precipitate, after the second precipitation, is discarded and the second extract is added to the first. The united extracts are evaporated to semidryness in a fan oven at 37° C. and the residue is extracted with 95% alcohol. The alcoholic extract is evaporated to semidryness as before. The evaporated extract is emulsified in water and an equal volume of acetone is added to precipitate the phosphotides. These are filtered off and discarded, while the extract is again evaporated to semidryness and thoroughly extracted with acetone or ether to remove any remaining fats. The residue is then extracted with 97% alcohol, 200 cc. for every kilogram of material. Any insoluble material is discarded.

The 97% alcoholic extract is evaporated to semidryness as before and the residue is dissolved in glacial acetic acid. The solution is permitted to evaporate slowly at 35° C. until the acid has been removed. Crystals of a definite form appear. These are purified by dissolving away the brown syrupy material by means of 99% alcohol. The crystals are insoluble in the alcohol and by repeated extractions they are obtained in a pure state. They can be redissolved and recrystallized from glacial acetic acid. The crystals contain the relaxative hormone, while the alcoholic extract is entirely inactive with respect to the relaxation reaction.

The crystals, containing the hormone, are very characteristic in form and are identical, no matter from what source they are prepared. They are composed of sodium chloride and nitrogenous organic material in the proportion of 4 to 1. This proportion is very constant, as shown by analysis of crystals prepared at different times, both before and after recrystallization.

The product is soluble in water, forming a water clear solution, which when injected, produces the characteristic physiological reaction. Less than one milligram of crystalline material or two tenths of a milligram of organic material is sufficient to produce relaxation of the pelvic ligaments of a guinea pig in full oestrum. This represents approximately one gm. of fresh lutein tissue.

The relaxative hormone forms water soluble salts of hydrochloric, sulphuric and acetic acids, while with picric acid it forms a salt which is insoluble in water, alcohol and ether. By means of this insoluble salt we have purified the hormone to the point where 0.07 of a milligram will give a positive reaction in the relaxation reaction. The hormone is very labile, being destroyed by

heat, alkalies, and oxidation. On exposure to air in dry form, the activity gradually decreases. In aqueous solution at pH of 3.2 it is stable. Such solutions have been kept in the icebox for eight to twelve months with no detectable decrease in activity.

4878

Purification of Hormone of Corpus Luteum Responsible for Progestational Development and Other Reactions.*

H. L. FEVOLD, FREDERICK L. HISAW AND R. K. MEYER.

From the Department of Zoology, University of Wisconsin.

While the relaxative hormone of sows' corpora lutea produces no other physiological reaction, as far as we know, other than relaxation of the pelvic ligaments, a second hormone is responsible for such reactions as inhibition of ovulation, production of pseudopregnancy in rabbits, vacuolation of the vaginal mucosa of rats and production of a premenstrual endometrium in the uterus of monkeys. The physiologically active material, which is responsible for these reactions, is present in the fractions from which the relaxative hormone has been removed. We have, therefore, 2 separate and distinct hormones elaborated by the corpora lutea of the sow. The following reports the separation of the 2 hormones, and the preparation of a highly purified extract, containing the second hormone of the corpus luteum. For convenience we shall, in this paper, refer to this hormone as hormone "B".

The extract is prepared in exactly the same manner as that described in the previous paper,[1] for the relaxative hormone, up to the point where the active principles are taken up in 97% alcohol, with one important exception: Hormone "B" is somewhat soluble in acetone so ether must be used to remove the last traces of fatty material. The hormone is insoluble or very slightly soluble in ether, consequently the fats may be removed with no significant loss of hormone. The 97% alcoholic extract is evaporated to semidryness leaving a residue, which contains both of the corpus luteum hormones. From this point, either of two methods may be used to separate the hormones.

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

[1] Fevold, H. L., Hisaw, Frederick L., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 604.