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.

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

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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.

Procedure I. The residue, from the evaporation of the 97% alcoholic extract, is dissolved in glacial acetic acid and the relaxative hormone crystallized and purified as previously described. Hormone "B" is removed from the crystalline fraction by the extraction with 99% alcohol while the relaxative hormone remains insoluble in the crystalline form as previously described. The alcoholic solution is diluted with 99% alcohol until no more precipitate forms. The precipitate is thoroughly washed with alcohol and discarded, since it is inactive. The diluted alcoholic extract contains hormone "B" in purified form. The alcohol is removed by evaporation at 37° C., the residue extracted with ether and the ether insoluble material taken up in water in which it dissolves readily. The aqueous solution may be concentrated to any desired concentration with no turbidity appearing. The extract is sterilized by filtering through a Berkefeld or Seitz filter into sterile ampules which are then stored in the icebox.

Procedure II. The residue, after evaporating the 97% alcoholic extract, is thoroughly extracted with a large amount of 99% alcohol. The insoluble residue contains the relaxative hormone, while the alcoholic solution is entirely free from this substance but contains all of Hormone "B". This extract is then evaporated to dryness, extracted with ether, dissolved in water and prepared for injection as in Procedure I. The alcohol insoluble material is used as a source of the relaxative hormone.

Hormone "B" is soluble in alcohol and water and has more tendency to dissolve in fatty solvents than the relaxative hormone. It is more stable toward heat than is the relaxative hormone, also more stable toward oxidation, but is very sensitive toward alkali. In slightly acid solution it is quite stable. With picric acid it forms a dark brown oily product which is insoluble in water. The purification of this oily material is being carried out at the present time.

Hormone "B" may be extracted from fresh lutein tissue by means of neutral alcohol as described by Corner.[2] This leaves the relaxative hormone and it may be obtained by subsequent extraction with acid alcohol. However, the extract thus obtained is not as active for the relaxation reaction as an acid alcohol extract of the fresh tissue. We have, therefore, found it more convenient to obtain both hormones in solution by means of acidified alcohol and separating the two by the methods described.

The methods of fractionating the corpus luteum hormones as described here, with slight modifications, have also been used in the

[2] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, lxxxviii, 326.

separation of the gonad stimulating hormones of the anterior lobe of the hypophysis.[3]

The purified extract of hormone "B" is almost entirely free from the oestrus producing hormone. This is of great importance since it has been found that the action of hormone "B" depends to a large extent on the presence of the oestrus producing hormone. [4, 5] It is therefore desirable to have an extract free from oestrin in order to study the relationship of the 2 hormones in certain physiological reactions.

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A Renal Lesion Following Plasmapheresis.

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Chronic nephritis with edema (nephrosis) is associated with a marked albuminuria and a lowered total serum protein as pointed out by Epstein.[1] The importance of the level of the albumin fraction in relation to the appearance of the edema, both clinically and experimentally, has been emphasized by Barker and Kirk.[2] In an attempt to study the effect of the low proteinemia on the kidneys, a similarly low serum albumin level (about 1 gm. per 100 cc. of blood serum) has been produced and maintained in a series of dogs by plasmapheresis. Renal tissue has been obtained from these dogs by nephrectomy or by destroying the animal at periods varying from 2 weeks to 6 months. Gross and microscopic studies have shown the beginning and rather rapid progression of a degenerative renal lesion.

The first changes were noticeable at the end of 2 weeks at which time the kidney appeared swollen. The cortex was relatively thickened and was a brownish-gray color. Microscopically, cloudy swelling was noted particularly in the convoluted tubules together with desquamation of the tubular epithelium and extrusion of many nuclei. There was some hyaline droplet formation and occasional shrinkage of the glomerular tufts. After about one month of plas-

[3] Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 29.

[4] Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 490.

[5] Hisaw, Frederick L., and Leonard, Samuel L., *Am. J. Physiol.*, 1930, xcii, 574.

[1] Epstein, A. A., *Am. J. Med. Sci.*, 1922, clxiii, 167.

[2] Barker, M. Herbert, Kirk, E. J., *New Eng. J. Med.*, 1929, 408.