of dosage, or[13] of the initial stage of the ovary. Amongst those[21] who believe that two distinct hormones are concerned, Wiesner[110, 112] has taken a leading position. He distinguishes "Rho-one", which stimulates the secretion of the œstrin, the maturation of follicles and the formation of corpora lutea, from "Rho-two" which maintains the corpus luteum in activity and stimulates it to internal secretion.[111, 73] Extracts of either anterior hypophysis[110] or placenta,[112] made with sulphosalicylic acid, contain both principles (an ingenious hypothesis is offered to explain the predominance of one or the other, according to dosage), but as "Rho-one" is totally destroyed by heating to boiling for one minute, while "Rho-two" in part survives such treatment,[112, 74] a clear separation can be obtained.

Mucification of the vagina in immature rats is the test by which "Rho-two" is assayed; this appears in sections, but is not detectable by the smear technique. Claus[23] has obtained from the anterior hypophysis a microcrystalline substance insoluble in absolute alcohol which promotes premature puberty and ovulation in female rodents; the potency is not stated but a great loss of activity is apparently involved in the preparation. A fraction soluble in absolute alcohol produced luteinization; the physiological effect of unresolved mixtures is dependent on the dose. Aschheim[7] pointed out that certain urines gave reaction 1, *i.e.*, œstrus, but never gave reactions 2 or 3, *i.e.*, hæmorrhagic follicles and luteinization. Zondek[123, 124] has now accepted the view that two hormones are concerned, "Prolan A", which causes œstrin secretion, and "Prolan B", which is assayed by the appearance of corpora lutea in the ovaries of immature mice; he claims, also, to have obtained "A" free from "B" from the urine of non-pregnant women, especially after natural or artificial menopause. The significance of the follicular hæmatomata is obscure; Fellner[46] regards the phenomenon as due to a non-specific, irritant impurity.

The relation of the placenta to the anterior hypophysis in this system remains obscure. Placental extracts induce in the hypophysis histological changes[1, 15, 65] similar to those seen in pregnancy,[38] but this may be due merely to œstrin.[11] Zondek,[122] like Philipp,[81] is inclined to believe that the placenta does not merely collect and store the hormone produced by the anterior hypophysis, but takes an active part in the production; but he maintains that the flooding of blood and urine with the hormone takes place so early in pregnancy that it must be ascribed to hypophyseal, not to placental, activity.

METHODS

Sulphosalicylic acid was employed in the first instance as an extracting agent, and it was used in the manner described by Wiesner.[110] Fresh or recently collected human placentæ were used. It was also found that frozen placentæ could be used and that the process of freezing did not materially affect the yield of active extract.

It was found that the injection of dilute sulphosalicylic acid extract into immature rats consistently produced the phenomenon of premature maturity. While attempts were being made to concentrate this extract and at the same time remove the sulphosalicylic acid without loss of potency (a result which was not achieved), it was decided to make some preliminary trials with acetone and alcoholic extracts of the fresh placenta. Dr. Wiesner had told us that he had had no success in the use of alcohol or acetone in the making of potent extracts, and in his recent paper he has emphasized this fact. However, it has been our experience that extraction of the material with neutral or faintly acidulated alcohol or acetone yields at once an extract which is invariably potent. When this fact had been thoroughly established it was decided to abandon the sulphosalicylic acid extraction process, and to develop a standard technique based upon the preliminary treatment with acetone or alcohol. Numerous attempts were made to fractionate these simple extracts and to recover the maximum amount of active substance in some one fraction. It should be added here that we had convinced ourselves at the outset that we were not dealing with œstrin. This factor has been carefully controlled by repeated extraction of our extracts with ether before submitting them to assay, and also by the use of proven oöphorectomized rats. These latter animals have been shown to be reactive to œstrin both before and after treatment with the œstrin-free placental ex-