

the anterior pituitary gland has been confirmed by Fels,<sup>4</sup> Brouha and Simonnet,<sup>3</sup> Loewe,<sup>6</sup> and Siegmund.<sup>7</sup>

Dr. Wiesner,<sup>10</sup> of the Department of Animal Breeding of Edinburgh University, has recently succeeded in preparing potent aqueous extracts of the ovary-stimulating hormone from human placenta. Wiesner's method consists in extracting the fresh placenta or placental press-juice with sulphosalicylic acid. This has the advantage of giving almost immediately a practically protein-free extract, which, after neutralization and clarifying by adequate filtration, may be injected directly. It has the disadvantage, however, that it still contains sulphosalicylic acid, or its salts, and does not readily lend itself to concentration processes. Dr. Wiesner visited the writer's laboratory in September, 1929, and demonstrated in a single but convincing experiment the effectiveness of the sulphosalicylic acid extract of fresh human placenta in producing premature maturity in the young rat. At Dr. Wiesner's very urgent request the writer took up the problem of the concentration of the premature maturity producing principle in the placental extract. A considerable number of experiments were therefore undertaken with sulphosalicylic acid extract of placenta and Dr. Wiesner's claims for the effectiveness of this extract were amply confirmed. At times, an immature female rat could be made to develop full oestrus and ovarian hypertrophy (confirmed by microscopic sections) on the fifth day following the injections of an amount of extract equivalent to 1/3 gm. of placenta. It was found best in practice to give two injections, one on the first and one on the second day, and to sacrifice the test rat on the fifth day.

It soon became apparent that the presence of the sulphosalicylic acid in the extract presented a most formidable barrier to further concentration and purification of the active principle. A variety of methods of extraction and fractionation were therefore resorted to and as a result it has been found possible to attain great concentration of the active principle. It has been obtained free from protein, salt, lipid and oestrin, and in the form of an aqueous solution it may be administered either subcutaneously or orally. It is possible to place the equivalent of 100 gm. of placenta in 1 c.c. of water with a relatively small loss of the active principle con-

tained in the original tissue. The potency of any extract may be ascertained fairly accurately in terms of rat units by making use of the slightly modified method of Zondek and Aschheim referred to above. The minimal amount given in two injections on successive days to produce full oestrus in immature rats three to five weeks old on the fifth day may be taken as the unit. It is also essential that the extract should be without effect on castrates.

Whether, as it has apparently been assumed, the ovary-stimulating hormone which has been demonstrated in anterior pituitary implants, in anterior pituitary extracts, in the blood and urine of pregnant women, and in the placenta, is the same in each instance remains for the present an academic issue. Why the pituitary gland should liberate this secretion so promiscuously during pregnancy, so that tens of thousands of rat units are lost in the urine, and why the placenta should act as a storehouse, are questions which must remain unanswered for the present. The writer prefers to hold the theory that in the case of the placenta the hormone with which we are dealing is actually elaborated therein.

Smith<sup>8</sup> has stated that anterior pituitary gland is ineffective by the oral route. A chance laboratory technical error gave a clue to the fact, later established, that the placental hormone under course of study was quite effective when administered by mouth. Certain control immature animals which were caged with injected animals were observed to show oestrus. Since there is nearly always an appreciable leakage of the injected fluid at the point of injection, and as rats are very prone to lick one another's coats, it seemed that this was the obvious explanation for the observed result in this case. Systematic feeding experiments were carried out. It was found best to administer the extract by mixing it with finely ground lean meat, which absorbs it almost completely, and which the animals eat ravenously. The amount of extract thus effective was surprisingly small. The equivalent of one gram of fresh placenta fed daily as an aqueous oestrin-free extract has produced oestrus on the fifth day. Much larger amounts produce the same effect.

In order to ensure the conviction that the efficiency was unimpaired by digestive juices, the oestrin-free extract has been treated with gastric