mgm.) of macerated anterior hypophysis into immature female mice led rapidly to the premature appearance of puberty, as judged by the ripening of follicles and ovulation, formation of corpora lutea, and an outpouring of œstrin, demonstrated by the opening and cornification of the vagina and the enlargement and hyperæmia of the uterus. Similar results were independently obtained by Smith and Engle[95, 96, 97] with rats, the observed superovulation being especially remarkable. None of these effects could be produced in oöphorectomized animals.

These findings have been confirmed repeatedly.[17, 18, 20, 41, 49, 70] The active principle appears to be present in the anterior hypophysis at all ages[88, 89, 92, 93, 94, 66] and in male animals and castrates to an extent greater than in normal females;[42] in pregnancy, the amount present decreases.[10, 81] It is present also in other human tissues, as in the decidua and the placenta (implants of 0.1 gm.), especially in the first half of pregnancy,[81] in the corpora lutea of pregnancy, and in the blood and urine from an early stage in pregnancy till some days after parturition.[3] It is not demonstrable in blood or urine at other times, except occasionally in infants,[21, 80] nor regularly in amniotic fluid,[21, 81] nor in cerebrospinal fluid during pregnancy.[48, 99, 31] These results led Aschheim and Zondek[4, 8, 9] to suggest the injection of one or two c.c. of urine into immature mice as a test for pregnancy. They describe three reactions which may follow: (1) development of follicles and œstrous changes in the vagina and uterus; (2) hæmorrhages into the follicles, usually visible macroscopically: (3) formation of corpora lutea, chiefly atretic. These reactions are not specific for pregnancy, as they may be obtained with urine from cases of various endocrine disorders, tumours, or inflammatory lesions,[6] or in amenorrhœa, or at the menopause.[32] Reactions 1 and 2, however, permit a diagnosis of pregnancy from the second month onwards, and give correct results, positive or negative, in about 97 per cent of the cases, as other workers have also found.[69, 30, 63, 106] Positive results have also been obtained with the urine in pregnancy in apes[30] and monkeys, but not in the cow, sow, rabbit, or mouse.[30] Active extracts have been prepared from the anterior hypophysis, and from the human placenta or urine of pregnancy.[118, 17, 85, 41] It is frequently impossible to discover from the writings of the German workers the source of the material used, and it has been universally assumed that the active principle is the same in each case. Evans and Simpson[43] however, point out that while the increase in size of the ovary is roughly proportional to the amount of hypophyseal material implanted, no such linear relation can be discerned with injections of varying amounts of urine of pregnancy.

Injections of such extracts into immature mice (3 to 4 weeks, 6-8 gm.) or rats (4 to 5 weeks, 30-35 gm.) produce effects similar to those of the solid implants, except that ovulation is seldom if ever observed,[37, 123] and the tendency to atretic luteinization and hæmorrhage is greater. The outpouring of œstrin leads to the uterus becoming distended and hyperæmic; the vaginal smear shows cornified cells and an absence of leucocytes, and the blood cholesterol probably rises.[86, 119] The unit is usually taken as the amount which, divided into six doses, produces signs of full œstrus in about 100 hours in such immature rodents. Mice are absolutely as well as relatively less sensitive than rats.[119] The absence of œstrin must be demonstrated by negative results with oöphorectomized animals; the weight of evidence[93] is against the view that an œstrin effect may be obtained with anterior hypophysis. Copulation may take place during such premature œstrus,[96] but the age at which the animals first become pregnant is not reduced.[33] If the injections are continued, the ovary enlarges to ten times the normal size, and consists almost wholly of corpora lutea, the majority of which are atretic and often hæmorrhagic. The cyclic changes in the vagina may persist for some time[18] but tend to disappear, and the epithelium passes into a secretion phase with a high mucous layer, while the uterus is no longer distended, but its muscular layers are hypertrophied.[121, 73]

These effects may be ascribed to the internal secretion of the corpora lutea,[26, 111] the activity of those produced by this treatment having been demonstrated by the deciduoma reaction.[103, 74, 19, 44] This hormone itself may be present in the placenta.[61] The luteinization and enlargement of the ovaries are also conspicuous in mature animals, though superovulation may still be produced by implants.[96, 34] In the ovaries of pregnant mice[117]