PART I. GONADOTROPHINS

Chapter I. *The Nature and Functions of Gonadotrophins*

Introductory remarks. Inactivation, excretion, sources and distribution of gonadotrophin. Are the pituitary gonadotrophins identical with those produced by the placenta? Are FRH and LH distinct compounds? The action of gonadotrophins on the ovary and testis. The interstitial-cell-stimulating hormone. Puberty and the awakening of sexual activity.

Introductory Remarks

THE gonadotrophins are so called because they govern the development and biological activities of the gonads.* Cushing & Goetsch (1915) showed that pituitary deficiency, whether naturally or artificially produced, is accompanied by atrophy of the reproductive and adrenal glands. Noble (1938*a*) has described the progressive atrophy of these organs and of some of the accessory genital structures which follows hypophysectomy in male and female rats (Table 1).

TABLE 1. Progressive atrophy of reproductive organs after hypophysectomy in male and female rats (Noble, 1938*a*)

Males

Intervening time (weeks)	Number	Average weights of organs in mg.			
		Testes	Prostate	Seminal vesicles	Adrenals
1	12	1,204	120	52	12
2	12	854	109	56	12
4	8	366	87	42	8
6	5	273	54	34	5

Females

Weeks	Number	Average weights of organs in mg.		
		Ovaries	Uterus	Adrenals
1	4	24	142	28
2	6	22	129	23
4	4	17	117	14
6	3	15	82	8

The changes in the gonads and adrenal cortices after removal of the pituitary include shrinkage of nuclei and cytoplasm with arrest of secretion. These results can be prevented, or if already present can be reversed, by injecting extracts made from the anterior lobe of the pituitary of other animals into the muscles or subcutaneous tissues.

The chemical nature of the gonadotrophins has not been exactly determined. They are soluble in water, give the general reactions of proteins, and are precipitated without denaturation by ethyl alcohol. According to Askew & Parkes (1933) the ovulation-producing hormone of pregnancy urine is inactivated by heating to 100° C. in water, but loses none of its activity if kept at that temperature for 1 hour when dry; the results are unaffected by the exclusion of oxygen. From

* γονή = gonad, τροφή = nourishment.

these and other observations it seems that the gonadotrophins are proteins or are so closely associated with proteins that their activity disappears when the latter are destroyed. The gonadotrophins also contain carbohydrate in the form of mannose and galactose. Among gonadotrophins from different sources Gurin (1942) has detected differences in the carbohydrate content.

The effects of proteolytic enzymes on gonadotrophin. Evans, Simpson & Austin (1933*b*) found that the gonadotrophin of pregnant mares' serum was but little affected by pepsin during 4 hours at 37° C. when the *p*H was between 4 and 5, though at a *p*H of between 1·8 and 2 its activity was destroyed. They note that other samples lost their potency when subjected to this *p*H in the absence of pepsin. Trypsin at a *p*H of 8·5 inactivated the hormone. Bates, Riddle & Lahr (1934) obtained FRH from beef pituitaries and digested it with trypsin at 37° C. and *p*H 8·0 for 2 hours. The material after this treatment was injected in four equal daily doses into immature ring doves whose testes were weighed 96 hours after the first dose, and compared with those of untreated birds of the same age. The results show that the hormone had been destroyed by the tryptic digest (Table 2). (See also Riddle, Bates, Lahr & Moran, 1936.)

TABLE 2. The effect of trypsin on a gonadotrophic extract rich in FRH (Bates, Riddle & Lahr, 1934)

Material injected	Dose (mg.)	Average weight of doves' testes 96 hours after the first injection mg.	
		Uninjected control doves	Injected doves
Untreated FRH	4	8·7	49·2
	4	6·5	34·0
FRH after digestion with trypsin	4	6·5	6·8
	4	7·8	10·7
	8	6·3	6·6
	8	6·3	8·8

Van Dyke (1936) says that the gonadotrophin of pregnancy urine is rendered inert by boiling, ultra-violet light, hydrogen peroxide or trypsin, but not by pepsin, though according to Fevold (1937) it is destroyed by pepsin. Collip (1937) states that pituitary gonadotrophin is inactivated by prolonged boiling and is sensitive to alkali, losing its potency at a little beyond *p*H 8. Thyrotrophic hormone, he says, shows approximately the same reactions. Using extracts of sheep's pituitary McShan & Meyer (1938, 1939) found that LH* is largely if not entirely destroyed by trypsin when exposed to it for $3\frac{1}{2}$ hours at 37° C. and *p*H 8·0, and is relatively resistant to ptyalin, whereas FRH is resistant to trypsin and destroyed by ptyalin. Ch'en & Van Dyke (1939) found that tryptic digestion abolished most of the luteinizing action of extracts of sheep or horse pituitary, but large doses of such digested extracts still caused some luteinization in the ovaries of hypophysectomized immature rats, showing that the destruction was not complete.

* For brevity and ease of discussion follicle ripening and luteinizing hormones will be referred to as FRH and LH respectively, as though their separate identities had been established.

Chow, Greep & Van Dyke (1939) incubated extracts of fresh pig pituitaries at 37° C. with various proteolytic enzymes; some of their results are given in Table 3, and show that the gonadotrophin used was inactivated by trypsin, chymotrypsin and pepsin, but not by papain or carboxypeptidase.

TABLE 3. The effect of proteolytic enzymes on pituitary gonadotrophin (Chow, Greep & Van Dyke, 1939)

Enzyme	*p*H of digest	FRH	LH	Thyrotrophin
Pepsin	4·57	+		+
Papain	4·57	−	−	−
Trypsin	8·69	+ −	+	+
Chymotrypsin	8·69	+ −	+	+
Carboxypeptidase	8·69	−	−	−

NOTE. + = inactivated; − = not inactivated; + − = partially inactivated.

Abramowitz & Hisaw (1939) have also investigated the action of proteolytic enzymes on three different gonadotrophins, namely purified FRH and LH extracted from the pituitaries of sheep and a chorionic gonadotrophin derived from the urine of pregnant women. Their findings suggest some differences in the proteolytic reactions of the extracts which were tested (Table 4).

TABLE 4. Proteolysis of pituitary and chorionic gonadotrophins (Abramowitz & Hisaw, 1939)

		Pituitary gonadotrophin		
Enzyme	*p*H of digest	FRH	LH	Chorionic gonadotrophin
Papain	7·1	+ −	−	+
Trypsin	7·1	+	+	+
Chymotrypsin	7·6	+ −	+ −	+
Crude ptyalin	7·1	+	−	+

NOTE. + = inactivated; − = not inactivated; + − = partially inactivated.

Li (1940) ground and treated the pituitaries of gonadectomized rats with trypsin at *p*H 9·6 and incubated the material for 2 hours at 38° C., after which it was assayed on 21-day-old female rats, eight doses being given in 4 days, and the findings were compared with those obtained by pituitaries which had not been treated with trypsin (Table 5). His results show that the extract used which was rich in FRH was to a large extent inactivated by trypsin; the high degree of alkalinity of the digest will be noted.

TABLE 5. The effect of trypsin on the gonadotrophic potency of the rat's pituitary (Li, 1940)

Sex of pituitary donor	Total dose (mg.)	Pituitary treated by trypsin	Mean weight of ovaries in test rats (mg.)	Mean weight of uterus in test rats (mg.)
Female	5	−	69·76	82·10
Female	10	+	23·73	63·77
Male	5	−	66·65	82·89
Male	10	+	22·81	40·35

The results of proteolysis which have just been mentioned, though they are not all in complete agreement, suggest that the gonadotrophins, if not themselves

protein, are dependent for their activity on a close association with protein. Spielman & Meyer (1937), having examined the electrophoretic properties of chorionic gonadotrophin, believe that it probably consists of a specific principle combined with a non-specific carrier. They arrived at this conclusion by observing that the hormone may be still active biologically in spite of a change of its isoelectric point.

The gonadotrophins undergo bacterial decomposition, and cannot be given very effectively by the mouth, though some degree of response may follow oral administration (Goetsch & Cushing, 1913; Goetsch, 1916).

As a rule gonadotrophins are not stored appreciably in the body; they are inactivated when introduced into the bloodstream and are excreted with the urine. An exception to the last rule will be mentioned presently (p. 5).

Inactivation, Excretion, Sources and Distribution of Gonadotrophin

Inactivation in the living body. Geist & Spielman (1934) collected blood from the two ends of the severed umbilical cord of a baby and identified gonadotrophin in blood from the placental end in a concentration of 165 r.u. per litre, while none was recognizable in the blood obtained from the foetal end. (See also Sklow, 1942.)

It was shown by Lipschütz & Vivaldi (1934) that human chorionic gonadotrophin when given intravenously to a rabbit disappears rather rapidly from the blood. Six to 8 hours after the intravenous injection of 100 r.u. only 20 per cent could be recovered from the blood, and 10 hours after injection only 10 per cent could be recovered.

Friedman & Weinstein (1937) assayed 24-hour specimens of urine from normal men for gonadotrophin and found a daily excretion of about 6 r.u. Oral ingestion of human chorionic gonadotrophin did not increase the amount excreted. Intramuscular injection of 600 or 750 r.u. was followed by an excretion in the urine of between 5 and 15 per cent of the amount given.

Stamler is quoted by Zondek (1940*c*) as having given intravenous injections of gonadotrophin to dogs and found 3 hours later only 38·4 per cent of the amount injected still present in the circulation. The hormone was recognized in the urine within 1 minute of its injection, and continued to be present until 20 hours later, the total amount excreted being 11·2 per cent of the original dose. In the gelding Stamler recovered from the urine only 5 per cent of a dose of gonadotrophin given intravenously. Zondek (1940*c*) made a detailed inquiry into the inactivation of gonadotrophin within the body. First he killed young rats weighing about 30 g. and minced them. He then added to the mince a known amount of chorionic gonadotrophin together with a phosphate buffer of *p*H 7·9, and placed the material in the incubator. Later he was able to extract from the mashed and incubated tissues all the gonadotrophin which had been added. If however 1,000 r.u. were injected subcutaneously into rats of the same age and the animals were killed 24 hours later, only 10 per cent of the injected gonadotrophin could be extracted from their bodies. By other experiments he showed that inactivation of the hormone did not take place in the liver, spleen or muscles.

It is conceivable that the gonads are partly concerned in the inactivation of gonadotrophin. This possibility is suggested by analogy, for Loesser (1934) in-

jected 3,000 units of thyrotrophin into normal rabbits and was unable, by tests on guinea-pigs, to recognize any of the hormone in the rabbits' blood after the lapse of 1 hour, whereas if the same dose was given to a rabbit whose thyroid had been removed thyrotrophin could be detected in its blood 7 hours later. Loesser suggested, therefore, that the disappearance of thyrotrophin from the blood after intravenous injection might be, in part at least, effected by the thyroid gland. Pursuing this idea, Rawson, Sterne & Aub (1942) tried the effects on thyrotrophin of various tissues *in vitro*. They discovered that thyrotrophin is inactivated by thyroid gland, and to a slight extent also by thymus and lymph glands, but not by other tissues.

Excretion of gonadotrophin. In man the excretion of chorionic gonadotrophin by the kidneys was demonstrated by Aschheim & Zondek (1927, 1928) and is the foundation of their test for pregnancy. As will be shown presently, chorionic gonadotrophin may be formed apart from pregnancy; in the presence of chorionic tumours, whether in women or men, the urinary output of gonadotrophin is often very large and its recognition is a valuable diagnostic aid.

Gonadotrophin derived from the pituitary, like that from chorionic tissue, is excreted by the kidneys. Though the activity of the pituitary in producing gonadotrophin varies with changing sexual activities, in many species the hormone is formed and excreted to some extent at all ages. In women during the reproductive period of life there is a maximum excretion during each menstrual cycle at or shortly before the time of ovulation. Kurzrok, Kirkman & Creelman (1934) studied the renal output of gonadotrophin in ten young non-pregnant women and detected a suddenly enhanced output at about the middle of the menstrual cycle, apparently just preceding ovulation. The continued formation and excretion of gonadotrophin after the menopause was shown by Österreicher (1933), who assayed the urine of 149 women aged between 50 and 93 years and detected the presence of gonadotrophin in 65 per cent. He also verified its presence in the urine of five women whose ovaries had been removed (p. 33).

In the pooled urine of normal men Evans & Gorbman (1942) detected between 1 and 4·5 r.u. or between 6 and 20 m.u. of gonadotrophin per litre.

The experiments just quoted show that the fate of gonadotrophin in the body, whether derived from the pituitary or chorionic tissues of the host, or artificially introduced by injection, is of two sorts; some is inactivated within the body and some is excreted by the kidneys in an active form. Parkes & White (1933), using male and female rabbits which had been deprived of their gonads, performed the following experiment. Under anaesthesia the bladder was emptied and its outlet obstructed by ligature. Gonadotrophin was then injected into the ear vein and at various intervals afterward each animal was killed, its bladder removed and the contained urine assayed. In this way it was found that about one-third of the injected hormone could be recovered from the urine during the first 9 hours.

Pituitary and chorionic gonadotrophins may not be excreted with equal ease by the kidney. Catchpole, Cole & Pearson (1935) showed that in the pregnant mare, though much gonadotrophin is present in the blood, none is detectable in the urine. Human chorionic gonadotrophin, after injection into the pregnant mare's blood stream, appears in the urine. Gonadotrophin, from the blood of a preg-

nant mare, when injected into the circulation of the gelding, monkey, rabbit or rat, does not appear in the urine. For example, 3,000 r.u. of gonadotrophin prepared from the blood of a pregnant mare were given intravenously to a rabbit. After the lapse of 24 hours, assays of the blood showed that about half of the original dose was still present in the circulation. At this time none was found in the uterus, spleen, lungs, kidney or liver, and the missing hormone was not present in the urine. Gonadectomy had no influence on the rate of disappearance. Two facts seem to be indicated by these experiments. The first is that gonadotrophin is inactivated in the circulation though we do not know how or where; the other is that the gonadotrophin of the pregnant mare is different from that of the pregnant women inasmuch as the former is not excreted by the kidney, perhaps, as Zondek has suggested, because its molecules are too large. It has been thought that the production of gonadotrophin throughout gestation in the mare may perhaps take place in the pituitary and not in the placenta.

Sources of gonadotrophin. (i) *The anterior lobe of the pituitary* is the chief source of gonadotrophin, and hypophysectomy is followed by atrophy of the gonads and the accessory genital organs (Cushing & Goetsch, 1915; Smith, 1927*b*, and many others). After hypophysectomy the reproductive system can be maintained, or if already atrophied can be restored to a functional condition, by repeated subcutaneous or intramuscular implantations of pituitary tissue or by the injection of extracts made from pituitary glands.

(ii) *The placenta.* Collip (1930) and Collip, Thomson, McPhail & Williamson (1931) tested extracts of human placenta on immature and adult rats, and obtained positive gonadotrophic responses; confirmatory experiments have been reported by Philipp (1931), Collip, Selye & Anderson (1933) and Collip, Selye, Thomson & Williamson (1933).

Apart from direct evidence of this kind other observations have indicated that the placenta forms gonadotrophin. Evans & Simpson (1929*b*) noted that although the pituitary is enlarged in pregnancy and although the output of gonadotrophin is large in this condition, the gonadotrophic potency of the pituitary as tested by implantation into immature animals is not increased during gestation, as might have been supposed. Subsequently it was shown that the pituitary has little or no gonadotrophic potency during pregnancy, and it became obvious that the large amounts excreted in the urine, which are the foundation of the Aschheim-Zondek test for pregnancy, must arise elsewhere than in the pituitary. The stage of gestation at which increased amounts of gonadotrophin begin to appear in the urine is somewhere about the time when the ovum becomes attached to the uterus (Crew, 1936*b*; Evans, Kohls & Wonder, 1937), that is to say when a placenta is formed; and the amount falls rather abruptly after parturition and expulsion of the placenta. Crew (1936*a*) examined the urine by the Aschheim-Zondek test in fifty cases at periods extending from $\frac{3}{4}$ of an hour to 144 hours after delivery, and his results are given in Table 6 in which the rapid diminution of gonadotrophin in the urine after expulsion of the placenta is clearly shown.

Evans, Kohls & Wonder (1937) made repeated assays of gonadotrophin in 24-hour specimens of urine from six pregnant women. In each instance the greatest concentration, which ranged from 75,000 to 1,040,000 r.u. per litre of

TABLE 6. The reduced excretion of gonadotrophin in the urine in 50 cases after parturition (Crew, 1936 *a*)

Specimen of urine	Hours after parturition	Positive to A-Z test	Negative to A-Z test
1	¾–7	45	5
2	24	26	24
3	48	15	35
4	72	2	48
5	96	0	50
6	120	0	50
7	144	0	50

urine, was observed 1 month from the beginning of the first expected but missed menstruation. This rise was followed by an abrupt fall, so that by the 65th day the hormone concentration in the urine was below 10,000 r.u. per litre and remained so till the end of pregnancy. Boycott & Rowlands (1938) by a different method found that the concentration of gonadotrophin in the urine rose rapidly from the 6th week of pregnancy, reached a maximum between the 56th and 84th days, and then declined to a fairly constant level which was maintained to the end of pregnancy. Browne & Venning (1936) found the highest concentration on the 60th day after the 1st day of the last menstrual period.

A curious observation bearing on the formation of gonadotrophin by the placenta was made by Ware, Main & Taliaferro (1938), who assayed the urine for gonadotrophin in a woman with abdominal pregnancy. The child was removed by laparotomy, the placenta being left *in situ*. The excretion of gonadotrophin in the urine continued for 47 days, during which mammary engorgement and lactation were absent. In this case the excess of gonadotrophin excreted in the urine seems attributable to the continued presence of the placenta; the foetus, at any rate, could not be regarded as the source.

Direct evidence of the formation of gonadotrophin by placental cells has been obtained by Jones, Gey & Gey (1943) who maintained *in vitro* cultures of cells from human placentae and hydatidiform moles. Assays were made when the media had been changed several times and the explants consisted entirely of new cells. Gonadotrophic responses were obtained in 20 of 29 tests made with this material on immature rats, whereas negative results followed in 18 control experiments.

Hitherto we have discussed gonadotrophin in general terms as though that which is formed by the placenta and excreted in the urine in pregnancy were identical with that normally produced by the pituitary. It is by no means sure, however, that placental and pituitary gonadotrophin are the same. Collip and his colleagues using a gonadotrophic extract of placenta found that its activities were like those of pregnancy-urine extracts, but differed from pituitary gonadotrophin inasmuch as it merely caused thecal luteinization in the ovaries of hypophysectomized, immature rats and guinea-pigs and did not bring about maturation of follicles or formation of corpora lutea; that is to say it consisted mainly of LH. This question will be discussed in more detail later on (p. 10).

(iii) *Chorionepithelioma*, whether in woman or man, is accompanied by a high output of gonadotrophin in the urine, as revealed by the Aschheim-Zondek

pregnancy test (Zondek, 1930; Heidrich, Fels & Mathias, 1930; Ferguson, Downes, Ellis & Nicholson, 1931; Evans, Simpson, Austin & Ferguson, 1933; Montpellier & Herlant, 1933). In either sex this fact may be of use to the diagnostician.

In women, a positive test, continuing after the uterus has been apparently emptied of the products of conception, may establish the diagnosis at a time when remedial measures will be effective and from this standpoint the test may be of the greatest value. At an earlier stage, when an attempt is being made to distinguish a normal pregnancy from a hydatidiform mole, the test is not so useful, for, as Boycott & Smiles (1939) have found, the output of gonadotrophin in cases of chorionepithelioma is not necessarily greater than that in normal pregnancy, though perhaps an excessive excretion of gonadotrophin would rather suggest the presence of chorionepithelioma.

In men the test, being free from the possible complication of pregnancy, is of the greatest aid in making a differential diagnosis. Heidrich, Fels & Mathias (1930) were the first to report the use of the pregnancy test in a male. The patient was 35 and had a tumour of the testis, and among other symptoms had gynaecomastia with the secretion of colostrum. A positive Aschheim-Zondek test was obtained with both his blood and urine before death, one litre of urine containing 35,000 m.u. of gonadotrophin. After death implants of the primary tumour into immature mice also produced positive reactions, though implants of the normal testis or of the pituitary did not.

The test may be positive though the primary tumour in the gonad is so small as to be overlooked: in fact the affected testis may appear atrophic (Craver & Stewart, 1936).

Attempts to distinguish chorionepithelioma from other kinds of testicular tumour by means of the Aschheim-Zondek test have not been so successful as had been hoped.

According to Ferguson (1933 *a*, *b*; see also Ferguson, Downes, Ellis & Nicholson, 1931) an excess of gonadotrophin in the urine may accompany tumours of the testis which might be described as teratomata rather than chorionepitheliomata. It seems possible that in these cases chorionic tissue may form some small part of the teratoma.

Montpellier & Herlant (1933) reported a case of seminoma of the testicle in which large amounts of gonadotrophin were present in the urine. They believe that the nature of the gonadotrophin may to some extent reflect the nature of the testicular tumour. The urine from a patient with seminoma, they say, when injected into immature rats or mice, causes ripening of the follicles only, whereas the urine from cases of chorionepithelioma of the testis provokes the appearance of haemorrhagic follicles and corpora lutea.

Fortner & Owen (1935) think that quantitative estimations of the urinary output of gonadotrophin might help in distinguishing clinically between teratoma and chorionepithelioma and give approximate figures (Table 7).

Ferguson (1933 *a*) has noted the presence of gynaecomastia with secretion of colostrum in five among 117 cases of testicular tumour in which he has examined the urine for gonadotrophin. Further, he calls attention to the fact that the

TABLE 7. Urinary output of gonadotrophin in cases of testicular tumour (Fortner & Owen, 1935)

Condition	Mouse units of gonadotrophin per litre of urine
Normal	50
Teratoma	50–10,000
Chorionepithelioma	10,000–150,000 or more

pituitary in cases of chorionepithelioma shows histological changes similar to those which accompany pregnancy. These observations have been confirmed by Entwisle & Hepp (1935) and Solcard, Le Chuiton, Pervès, Berge & Pennanéac'h (1936). According to Evans, Simpson, Austin & Ferguson (1933), the gonadotrophin present in the urine in cases of testicular tumour shows predominantly an FRH activity, although presumably it has been formed by chorion-like tissue and therefore by analogy might have been expected to show a pronounced luteinizing action.

The presence of gonadotrophin in chorionepitheliomatous tissue was confirmed by Philipp (1931), who implanted fragments of tumour from a human case into immature female mice and obtained pronounced positive responses, as Heidrich, Fels & Mathias (1930) had already reported.

(iv) *Sources outside the animal body*. Apparently the production of gonadotrophin is not confined to animals. Hisaw, Greep & Fevold (1936) extracted from brewers' yeast a water-soluble substance which had some gonadotrophic properties. In immature rats it prevented atrophy of the testes after hypophysectomy, though it did not prevent some degeneration of the interstitial glandular cells of the testis nor atrophy of the accessory generative organs. In hypophysectomized adult rats treated with the extract the testes remained in the scrotum and spermatogenesis was maintained; the accessory glands, though smaller than normal, continued to secrete.

Friedman (1938) obtained from young oat plants a substance which when given intravenously to rabbits caused ovulation. One rabbit unit of this material was extracted from between 30 and 80 g. of the dried plants.

In this connection it must be remembered that ovulation in the rabbit may be induced by the intravenous injection of material which has no gonadotrophic potency.

The distribution of gonadotrophin in the body. From the organs in which it arises, namely the pituitary, placenta and tumours of the type known as chorionepitheliomata, gonadotrophin passes into the blood and some is excreted in the urine. Because of its elimination by the kidneys together with its gradual inactivation in the body there is a falling gradient in the bloodstream. This has been demonstrated in the foetus, for blood collected from the placental end of the divided umbilical cord is rich in gonadotrophin whereas little or none is detected by ordinary means in the blood returning from the foetus (Geist & Spielman, 1934). The site of inactivation has not yet been determined. In the foetus the process appears to be rapid, for Parker & Tenney (1940) found that, in normal pregnancy, although gonadotrophin is present in about equal concentration in the maternal blood, urine and placenta, none is evident in the foetal organs,

blood or amniotic fluid.* Heim (1931) reported that by means of the Aschheim-Zondek test gonadotrophin could be detected in colostrum, and that it disappeared from the milk on the 5th day after parturition. At autopsies on a man and a woman who had died with chorionepitheliomata Ewald found relatively high concentrations of gonadotrophin in the cerebrospinal fluid. Gonadotrophin has been detected in the cerebrospinal fluid during normal pregnancy, though its concentration is low. Aschheim & Zondek (1927) detected gonadotrophin in the ovaries, placenta and blood of pregnant women and in blood from the umbilical cord.

Are the Pituitary and Placental Gonadotrophins identical?

Evans & Simpson (1929*b*) and Engle (1929*a*) seem to have been the first to report a difference between pituitary and placental gonadotrophin. The former found that the weights of ovaries precociously developed in rats of 26 days under the influence of implanted pituitary gland were, within limits, nearly proportional to the amount of tissue implanted. With 4 times the minimal dose the ovaries were increased approximately 4 times in weight. With an extract of pregnancy urine if 4 times the minimal effective dose had been given the ovaries were not appreciably larger than after a minimal dose; and it was found that with 150 times the minimal dose the ovarian tissue was barely trebled. The difference lay in the number of follicles stimulated. Pituitary implants caused a much more general follicular development than that which followed the administration of extracts of pregnancy urine (see also Evans, Meyer & Simpson, 1931). Engle (1929*a*) noted a difference between the effects on immature female mice of pituitary implants and extracts of pregnancy urine. The former caused extensive follicular maturation and ovulation while the latter caused follicular growth and luteinization without ovulation. In an adult macaque Engle (1932*a*, 1933) noted that pituitary gonadotrophin induced changes in the sexual skin like those which follow the administration of oestrin, whereas no effect on the sexual skin nor stimulation of ovarian follicles was caused by placental gonadotrophin. Hamburger (1933*a*) has made observations like those of Evans & Simpson. He compared gonadotrophin extracted (*a*) from the urine of men and women who had been castrated and of women who had passed the menopause with (*b*) gonadotrophin obtained from the urine of pregnant women. Hormone (*a*) is derived from the pituitary, while hormone (*b*) probably is derived mainly or entirely from the placenta because the human pituitary during pregnancy has been shown to be nearly or quite free from gonadotrophin. When tested on immature female mice or rats, hormone (*a*) caused a large number of ovarian follicles to ripen at the same time, whereas hormone (*b*) affected only a few follicles, causing them to ripen and to become very large and protuberant while the remainder were not brought to maturity. Gonadotrophin prepared from patients with testicular teratomata resembled in action that obtained from the urine of pregnant women (cf. p. 9).

Reichert, Pencharz, Simpson, Meyer & Evans (1931, 1932), Evans, Meyer & Simpson (1932) and Smith & Leonard (1933) found that, in dogs and rats, prolan prepared from the urine of pregnant women failed to preserve the normal de-

* On several occasions the author, collaborating with Douglas H. MacLeod, has obtained a positive A-Z reaction in mice with human amniotic fluid.