

Gonadotrophic Substances Of Extra-Pituitary Origin

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THE literature that has grown up during the past twelve years dealing with the nature and actions of the gonadotrophic substances found in tissues and body fluids of animals has become one of the most bewildering chapters in endocrinology. No attempt will be made to review this vast and controversial topic but merely to indicate some of the highlights of discovery and to state the position of the problem as it would appear to be today.

In 1926, Professor P. E. Smith, of the department of Anatomy, Columbia University, discovered the striking effects on the ovaries of rats and mice of the implantation of fresh pituitary tissue. The ovaries assumed remarkable proportions; there was a ripening of a large number of Graafian follicles, ovulation and formation of corpora lutea. This was shortly followed by the no less remarkable observation of Aschheim and Zondek in Germany that anterior pituitary-like substances were to be found in the urine and blood of pregnant women. The injection of a few ml. of urine of pregnancy into rats and mice brought about growth of follicles, ovulation and corpora lutea formation and it was from this finding that the now famous Aschheim-Zondek test for pregnancy developed. Evans and Long of California had earlier (1921) shown that certain extracts of anterior pituitary caused massive luteinization of ovaries and this coupled with the work of Smith, and Aschheim and Zondek led many to the view that there were two gonadotrophic hormones. That the pituitary is actually the source of the gonadotrophic substances controlling the reproductive organs was unequivocally shown by Smith in 1927 by the removal of the pituitary from rats. Removal was followed by atrophy of the gonads and also of the accessory organs, the atrophy of the latter being a secondary effect through the gonads. Hypophysectomy has now been done on all species of common laboratory animals and the early reports of Smith amply confirmed.

Evidence that possibly two pituitary-like principles occurred in urine of pregnancy was presented by Aschheim and Zondek in 1927. They discovered that a substance was present in urine of menopause and also in urine of female castrates which produced predominately follicular growth in the ovaries of rats and mice whereas the urine of normal pregnancy contained a factor or factors which promoted both follicular growth and luteinization. Fluhmann of California also made the observation that blood of women in the post climacteric period, following castration and in certain cases of amenorrhoea contained mainly follicle-stimulating substances.

Laboratories everywhere took up the problem of the separation of these various fractions from pituitary material, and from blood and urine

of pregnancy. Numerous claims have been made for a separation of two distinct gonadotrophic substances from pituitary tissue. The bulk of work on the fractionation of anterior hypophysis in America has been done by a group of workers led by Professors Fevold and Hisaw. These investigators report on two separate principles—one designated with the monogram FSH, which is the follicle-stimulating hormone and a second, LH, the luteinizing hormone.

The problem of the pituitary gonadotrophic substances will not be considered in this article and the discussion limited as far as possible to gonad-stimulating substances of extra-hypophyseal origin.

Classified according to their biological source two groups of such substances are found:

(1) Those substances which occur only in the presence of living chorionic tissue. This includes:

- (a) the gonadotrophic principle found in the blood and urine of pregnant women and in the placenta, referred to jointly in this paper as PU. (The active substance from placenta is often designated by the monogram A.P.L.)
- (b) the substance which appears for a short time in the blood serum of pregnant mares and often referred to as PMS.

(2) Those substances found in highest quantities in urine in conditions of dysfunctioning or absent ovaries, e.g. in castrate urine, menopausal urine and in certain conditions such as amenorrhoea. This is referred to as CU. Small amounts of CU are to be found throughout life in the urine of both men and women and in certain pathological states.

Occurrence of PU and PMS. The observation of anterior pituitary-like substances in urine of pregnancy by Aschheim and Zondek was confirmed and extended by a host of other workers. It has been found in all body fluids during pregnancy, saliva, cerebrospinal fluid, sweat, amniotic fluid, oedema fluid and gastric juice and in placental tissue. Its concentration during pregnancy has been studied quantitatively by a number of workers among whom may be mentioned Browne and Venning, and Evans *et al* on this continent. Browne and Venning from a study of a number of cases found that the excretion of material (tested on immature rats) rose rapidly from about the 40th to 50th day counting from the last **experienced** menstrual period to reach a maximum between days 50 to 60 and fell sharply again around the 60th to 85th day. Small amounts were found throughout gestation. Evans and coworkers report a peak near the 30th day counting from the last missed period. Other workers although using different modes of assay have obtained results which in the main support the above findings—i.e. a high urinary excretion of gonadotrophic hormone during the second month of pregnancy. Since implantation of the egg in the human occurs within two weeks the appearance of the hormone might in some way be related to the stage of development of the chorion.

The presence of the gonadotrophic factor in the serum of the pregnant

mare bears out this supposition. Its occurrence in mares' serum was reported as early as 1930. Some, but only a limited amount, occurs in the urine. The serum shows a maximum content of hormone between the 50th and 80th days of pregnancy (gestation period approximately 330 days); it then falls gradually and is practically absent by the 180th day. Implantation of the blastocyst in the mare occurs about the 40th day and as in the human the appearance of the hormone might be related to the development of the chorion.

Reports of gonadotrophic substances during pregnancy in primates other than man are fragmentary and work along this line might profitably be extended in laboratories where the keeping of a colony of large animals is possible. Zondek reported a positive Aschheim-Zondek test in an orangutan and it has been reported for a short time during pregnancy in the chimpanzee and in the macaque monkey.

Reports on urine during pregnancy in the goat, sheep, dog, cat and guinea pig have been universally negative. Irregular positive tests have been claimed for urine of cows and positive tests for the foetal part of the pig placenta but the majority of reports are negative for these species.

In conclusion, the hormone appears definitely to be associated with chorionic tissue—it is found in the placenta at all periods and continues to be produced when fragments of placenta are retained after incomplete abortion. It is excreted in large quantities in the condition of chorionepithelioma and hydatidiform mole.

Occurrence of CU. Fluhmann and Zondek (see above) early reported on the presence of follicle-stimulating substances in blood and urine of castrated and menopausal women. Zondek at first was of the opinion that the excretion of this substance was associated with genital carcinomas, a supposition which has not been supported by later work. In conditions of carcinoma the excretion of the hormone would appear to be related to ovarian activity—natural or induced menopause—and not to the pathological state. The principle has been shown in the serum but not the urine of castrated rats and in the urine of castrated rabbits (one test).

The hormone has also been reported in the urine of castrated men and in small amounts at all ages in both sexes but its most constant appearance, and in the highest titer, is in cases of gonadal absence or atrophy.

Origin of PU and CU. Zondek considered the blood and urinary gonadotrophic substances from healthy pregnant and castrate women as of hypophyseal origin and called them along with material from pituitary "Prolan". (The word "Prolan" has been used widely in the literature, especially in Germany, to refer to substances with gonadotrophic activity; it too, is the patented name of a commercial product.) This position was immediately questioned and succeeding workers pointed out clear differences between the gonadotrophic substances of chorionic origin and the hypophysis.

These strong indications that the gonadotrophic principle of pregnancy urine was not the same as that of the pituitary (see below) coupled with

the latter's dearth of activity during pregnancy made it rather doubtful as the source of urinary material.

The placenta was early looked to as the origin and one might even be prejudiced to consider it as such from our classification, although there is no conclusive evidence that such is the case. The relative concentrations of gonadotropin in the decidua and the placenta points to the latter as the source, e.g. if the placenta is merely a storehouse for the hormone a sharp contrast might be expected between it and the decidua because of its selective absorption. Such is not found and furthermore the decidua near the placenta shows a higher concentration than that which is remote. The simplest explanation is that the hormone is elaborated in the placenta and not merely stored there. More positive evidence of a possible placental rôle in hormone production comes from experiments in which pieces of human chorionic villi were grafted in the anterior chamber of the eye of a rabbit. The ovaries of the host responded to a positive Friedman test (akin to an Aschheim-Zondek test) and furthermore the urine of the animal brought about a second positive test when administered to a second rabbit. The amount of tissue by itself contained insufficient hormone to produce a positive reaction when implanted intramuscularly or when extracted and the extract injected intravenously. The obvious conclusion is that it manufactured the hormone.

The substance found in castrate and menopause urine is closely related in its action to anterior pituitary and the hypophysis has universally been accepted as its source.

Certain differential responses to Gonadotropins in Animal Tests. That differences exist between gonadotrophic PU and pituitary extracts has been indicated above. Evans and his colleagues, for example, found that pregnancy urine extracts, no matter what quantity is administered, produce only ovaries of a limited size, whereas pituitary extracts and implants bring about a far greater ovarian response. This difference has been substantiated by many investigators. It is evidence that PU contains only the luteinizing principle and is not a stimulator to follicles; after luteinization of the follicles present in the ovary its action ceases and the ovary no longer increases in weight. Pituitary implants on the other hand with both follicular and luteinizing actions produce much larger ovaries.

Occasionally follicle stimulation with pregnancy urine extracts is obtained in animals with intact pituitaries, a response which is probably one from the animal's own pituitary, mediated through the ovary. Several workers have been emphatic in denouncing the practice of testing for pituitary substances in intact animals where there is the possibility of an action from the animal's own gland. The removal of the pituitary in animals has aided materially in work of this kind. In the ovary of the hypophysectomized animal PU extracts form corpora lutea (if mature follicles are present at the time of administration) or bring about a histological change known as thecal luteinization; in no experiment has follicular development been found in the pituitaryless animal. Anterior hypophysis

on the other hand has been found much more effective in replacement therapy in hypophysectomized animals.

In the male (rat and monkey) qualitative differences between pituitary extracts and PU are not so marked.

In immature birds, however, PU has no action on the tests, while anterior pituitary causes hypertrophy of interstitial tissue and growth of accessory organs.

To generalize, one might state that the gonadotrophic principles of the placenta and of blood and urine of pregnancy are identical and differ from anterior pituitary extracts in that they contain the luteinizing factor but not the follicle-stimulating substance. Their origin may be in chorionic tissue. The principle appearing in functional or actual absence of the ovaries is considered to be follicle-stimulating only and to be elaborated by the pituitary.

Although such a concept aids in fitting many findings into a single picture the state of affairs is not quite so simple as one might be led to believe.

The substance from pregnant mares' serum fits but indifferently into this scheme of things. It appears to be a curious blend of both the pregnancy urine principle and anterior lobe substance. In the hypophysectomized female rat it undoubtedly causes follicular growth and subsequent luteinization. Ovulation in the ewe has also been reported. Again in the monkey administration results in follicular growth and the development of sex skin, a reaction noted for the principle from castrate urine and from pituitary but not from urine of pregnancy.

Furthermore there are many curious and unexplained species differences; differences that not only appear when the same substance is administered to different animals but also when gonadotrophic substances of different animals are tested by the same method. For example: the pituitaries of rabbit, rat and guinea pig promote follicular growth in guinea pigs but those of sheep and ox mainly follicular atresia. Admittedly the hypophysis is outside the realm of this discussion but the example above is introduced merely to illustrate the intricacy of this whole subject.

It is obvious that the problem is still in a very confused state. One of the greatest difficulties, apart from the intrinsic complexity of the subject itself, in the work has been the lack of a proper standard. Such a standard I believe now exists and it should be possible for workers to compare their fractions with this standard. Up to the present nearly all animal workers in this field have presented determinations in very variable animal units and it has not been possible to make proper comparisons between assays from different laboratories. Another difficulty has been that investigators are often imbued more with the spirit of competition than with that of co-operation and where their results differ slightly from those of another laboratory they tend to stress the differences, overlook the similarities, and claim that they have either a new substance or a product different from one already described.

Progress has also been delayed by lack of dependable methods for the purification of the gonadotropin. The best chemical work has been done on pregnancy urine fractions. Recent experiments in one laboratory have shown the most highly active fractions to be a polypeptide-polysaccharide complex and to have the properties of a mucoprotein. And especially significant may be a statement from the same laboratory that these highly active fractions appear homogeneous both from ultracentrifuge examination and from studies with the electrophoresis apparatus of Tiselius.

To date no reports have appeared on the biological action of this homogeneous gonadotrophic substance but such work is being awaited with keen interest.

Without theory practice is but routine of habit. Theory alone can bring forth and develop the spirit of invention. It is to you alone not to share the opinion of those narrow minds who disdain everything in science which has not immediate application. You know Franklin's charming saying? He was witnessing the first demonstration of a purely scientific discovery, and people round him said: "But what is the use of it?" Franklin answered them: "What is the use of a new born child?"—*Pasteur*.

Remarkable achievements are never unique achievements in nature. Even the greatest man rests on the shoulders of a multitude of small ones who have preceded him, and epochal discoveries emerge out of a period of intellectual restlessness that affects many minds.—*Flexner*.

Dogmatism is a deep seated vice of human nature not confined to theologians.—*Woodger*.

Doctors like royalties should keep aloof as much as possible, or their prestige will suffer; we all look our best in somewhat subdued light.—*Axel Munthe*.

All biological necessities have to be made respectable whether we like it or not.—*Shaw*.