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Placental hormones

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The intermediary function of the placenta, between the maternal organism and the foetus, has been known since early history. However, it has been only within the last three decades that another function has been ascribed to this organ, namely, that of a gland of internal secretion. The first expression of this view was made in the year 1905 by Halban,(I) a german physician.

Halban's hypothesis, which was based upon the interpretation of clinical facts and almost entirely from his own experiences, has stood the test of time and research. It has been only within the last decade that experimentors have comfirmed and proven most of the deductions of Halban and placed his hypothesis on a firm basis.

It will be of interest to give some of the conculsions (I)(2) Halban obtained from his clinical work, merely to show his comprehension of the subject before any experimental work had been done. He considered the placenta as a gland of internal secretion, and whose secretion, caused thegrowth and hyperemia of the uterus and breast through out pregnancy. This influence was removed with the expulsion of the placenta, and the mammary glands were able to secrete milk. However, if the placenta remained with a circulatory communication with the uterine wall the activated breasts could not secrete milk. In addition, Halban noticed that after the early period of gestation had passed, the ovaries could be removed with out interrupting

pregnancy or interfering with milk secretion postpartum. Although Halban did no experimental work to prove his theories, his conculsions acted as a stimulus for the work which followed.

The experimental work on placental hormones may be divided into three different periods. The first and earliest period was one in which aqueous extracts and press juices were used. In the second, the lipoid soluble subltances were investigated. In the third period, which takes us up until the present time, different fractions and different solvents were used with most encouraging and definite results.

The first period, dating from about 1905 till 1913, consisted of a series of experiments conducted by Clayton, Starling and Basch.(3). These men used aqueous extracts of the rabbit placenta, and injecting it into rabbits thought they were able to produce milk secretion in both the pregnant and nonpregnant rabbit. Basch even went so far as to market one of his extracts as a useful therapeutic agent. These extracts were soon proven to be inert and discarded. Nothing more need be said of this period except that it was the beginning of experimental work on placental hormones.

The second period of investigation began with the work of Iscoresco(4) which was published in 1912. His work dealt with a lipoid extract from the placenta. This extract when injected into mature rabbits, produced marked hyperplasia and congestion of the uterus and vulva, as well as of the breasts. These changes were so striking and definite that they could easily be recognized.

Frank states (I)-(P.63) regarding the technic of Iscoresco. "Here it will suffice to say that the fresh minced tissue was extracted in alcohol and that further extractions were performed with other lipoid solvents according to a definite technic some part of which has formed the basis of practically all of the work performed since then."

Following Iscoresco's work, Schickele(5), Fellner(6), Aschner (3), Hermann(4), Seitz(3), Wintz(5), Fingerhut(6), and Frank(5), between the years 1912-1915 experimented with extracts obtained with lipoid solvents. These men all agreed that unmistakable stimulation of the genital and mammary systems of females could be obtained with their extracts of the ovary and placenta.

Probably the most complete investigations of the above men were carried out by Fellner and Herrmann. (I)(P63) Fellner's extracts were prepared by extraction with alcohol, petroleum ether and again alcohol were emulsified with water. This extract, when injected into virgin and castrated rabbits or gguinea pegs, produced marked uterine and breast reactions. From this they concluded: first, in the placenta and membranes, are contained materials which, by subcutaneous or intraperitoneal injections, produce growth of the breasts, nipples, and uterus, also estrus and pregnancy phenomena in the uterine mucosa, and in the vagina. Second milk secretion was not noted. Third, the substance goes into saline solution, is alcohol, ether and acetone soluble, and may be a lipoid. Fourth, excised

guinea pig uterus responds to aqueous alcohol-ether extracts of the placenta with a strong protracted contraction.

In regard to Herrman's work, Daisy (3) states: "Herrman's paper constitutes the most complete systematic, chemical, and physiological study of the placental hormone prior to I920" Proceedure resembling Herrman's in many respects have been used by Frank, Daisy, Dickens, Dodds and Wright, Faust, and Hartman. Faust and Hartman, (3) (P6I0) apparently following in the foot steps of Herrman, have improved the proceedures.

Herrman's product was probably the most pure of any product up until I920 and he was able to obtain a patient for his extract. By his proceedure he was able to remove all sulphur, nitrogen, and phosphorus. This product however was not chemically pure as proven by Frank (I) (P67) and was therefore discarded.

The work of these men has definitely shown that a hormone exsists in the placenta, one which has a specific action on the genital and mammary tracts of the female. Therefore, the next step to be accomplished is the isolation and purification of the hormone.

Between the years I920 to I923 very little was accomplished in the way of isolating or purifying the hormone. Up until this time there had been no satisfactory test for the standardization of the substance. It had been necessary to sacrifice the life of the animal for the test, and consequently it took considerable

time and expense to carry out any tests. But with the introduction of the vaginal smear test by Allen and Doisy (7) this difficult portion of the experiment was solved. This test, which was published in 1923, was a great advancement in the isolation and purification of the hormones because of the ease of application and interpretation which it afforded.

Since it will be necessary to refer to this test later a short discription of it will be given here. The test consists in the injection of an immature or castrated mouse with some potent extract which is to be tested. After forty eight hours there are definite estrual changes consisting of thinning of the uterine walls with thickening and cornification of the vaginal walls. The test is read by the amount of cornification and thickening which occurs. The amount of extract which will produce the greatest change in an animal weighing 140 grams is designated as a rat or mouse unit, depending upon the animal used.

The third period of investigation may be said to have started with the work of Zondek (1) (P141) in 1925 and continues up until the present time. Doisy (3) (P610) speaking of this period stated; "Though Saqueur, Zondek and Glimm mention the solubility of the hormone in water, the work of Saqueur and his associates in showing that the hormone may be obtained in a refined condition by aqueous extraction must be regarded as one of the real advances in the progress in the isolation of the hormone".

From this time on the work consisted in the isolation and purification of the hormone by such men as Frank, Zondek, Glinn and Daisy and his coworkers. The hormone, however, was not completely isolated till about 1929 by Daisy (8) and his coworkers, using an improved technic of the above men. He has just recently placed on the market a hormone which he calls "theelin".

We may state here that there are numerous names given to this hormone, however we will limit them to that of "oestrin" and "theelin" in this paper.

The amount of knowledge obtained from the work of isolating theelin led investigators to believe that the ovary did not regulate its own periodicity. Consequently they began to investigate to determine the factors which enfluenced the ovary. The first work of that nature was carried out by Zondek and Aschheim (6) in 1927.

Their experiments consisted in the implantation of placental and anterior pituitary tissue into mice. From this work they concluded: first, that the implantation of these substances into the immature mouse brings about precocious oestrus, including the ovarian changes; and second, that it is the stimulation of the ovaries, which undergo maturation and elaborates the oestrus producing hormone or theelin.

In an effort to isolate this substance Zondek (6) (P 152) found that it was present in the blood, urine, and feces of pregnant women in very large amounts. This resulted in the Aschheim-Zondek test for pregnancy.

There was no further attempt to isolate this hormone from the placenta until Weisner (9) in 1929 attempted but did not obtain it in a pure form. However his proceedures, with some modification, were adopted by Collip (10)(11) in 1931 who was able to obtain the placental hormone in a pure state.

Collip (12)(15) began his work early in 1930 as a result of the influence of Weisner. He first endeavored to isolate the anterior pituitary-like substance from the placenta, but was unsuccessful at this time. However at this time he isolated a substance which he thought was the anterior pituitary-like hormone until he had carried out some experimental work with it. He found from further investigation that it was an entirely new substance having different physiological and chemical properities from either that of the anterior-pituitary like hormone or theelin. Collip named this hormone "Emmenin".

The significance of the occurrence of these hormones in the placenta has not been completely worked out. Men such as Aschheim, Daisy, Allen and Weisner (13) beleive that the placenta secrets the hormones as well as storing them, while Parkes, Lillie, and Bellerby (13) beleive that the placenta only acts as a protector of the foetus by filtering the hormones liberated by the anterior pituitary and the ovary, since these hormones are present in the blood during pregnancy in rather large amounts.

The men in favor of the secretion of the hormones by the placenta base their conclusions on the fact that there is an abundance of such in the placenta, and that the hormones have been isolated from the cord and amniotic fluid. The men on the opposite side base their conclusions on the fact that injection of theelin during pregnancy leads to reabsorption or abortion, therefore its secretion by the placenta would be a definite anomaly.

The placenta contains as much hormone weight for weight as the ovary, and weighs about five hundred times as much, thus containing five hundred times as much theelin as the ovaries. The hormone has been isolated from the cord and amniotic fluid thus rather disproving the fact that the placenta acts as a filter. In view of these facts it would seem that the evidence obtained was more in favor of the secretion of theelin by the placenta.

After having isolated the hormone Emmenin Collip (I4) states regarding the placenta as a gland of internal secretion: "Up to this time we had tentatively accepted the hypothesis that the maturity provoking factor of the placenta was identical with the anterior-pituitary gonad-stimulating principle. As the investigation proceeded however, it became apparent that a theory according to which the placenta is considered as the ductless gland of pregnancy, producing by an active process a pregnancy hormone with both physiological and chemical properities peculiar to itself, would fit the observed facts much better. While we do not feel

that we have as yet sufficient evidence available to prove this theory conclusively, we are nevertheless of the opinion that there are many observations which support it" He bases his theory on the facts obtained from Emmett which are:

I. Extracts which have been prepared from anterior pituitary lobes by use of acetone have been found to be non-oestrogenic.

2. It has been shown that the placental oestrogenic hormone is effective by the oral route.

3. There has been no decrease in potancy after treating the placental hormone with pepsin or trypsin.

4. With the placental hormone there has been no effect on the menstrual cycle, impregnation or lactation. There has been no increase in the size of the ovaries or seminal vesicles and lastly the hormone will not produce abortion.

From the facts listed above it would seem that the evidence in favor of the secretion of hormones by the placenta is by far themost convincing, yet they are not conclusive, and as stated by Collip the proof must await further investigation.

A discussion of the placental hormones will next be taken up with respect to their preparation; chemical, and physiological properties and clinical results.

The most recent proceedure in the preparation of theelin by Daisy(8) is its isolation from the urine of pregnant animals.

However he had previously isolated it from the placenta (I5). It consists in the adding of butyl alcohol to urine, the butyl alcohol acting as a solvent for the hormone. The solution is distilled to separate the urine from the butyl alcohol and is then leached with benzene. It is next changed to alkaline by the use of ether, alcohol and petroleum and sodium hydroxide. This proceedure is carried out several times until the product is pure then by crystallization theelin is formed. Theelin in this form contains .00I m.gm.of solids per mouse unit. Previous to this the purest contained .I m.gm. of solids per mouse unit. Although this is a very condensed description of the method of preparation it will serve to show the difficult technic and chemical reactions necessary to carry out the preparation of the hormone.

Theelin is a white crystalline substance soluble in alcohol, ether, acetone, benzene, chloroform and water(I6). The active substance is resistant to comparatively high temperature though the exact temperature at which destruction takes place appears to vary between 170 degrees and 360 degrees (centigrade) according to the solvent.(6P90) It does not contain nitrogen phosphorous, sulphur or cholestrol. Theelin is readily oxidized, undergoing slow inactivation from atmospheric oxygen, and rapid destruction by oxidizing agents. The hormone is non volatile and does not sublime. It is readily absorbed by dialysing

membranes and filter paper. it loses its activity when exposed to light and more rapidly in ultra-violet rays or sun light.

The physiological properities (6)(PII5) of theelin are best shown by its injection into animals. When it is injected into a normal animal just before oestrus occurs, there is an intensification of the normal changes in the genital organs without alteration of the ovarian cycle. If theelin is injected while oestrus is in abeyance it results in the appearance of extra ovarian oestrus. In an animal where the functional corpora lutea are present in the ovary, considerable dosage is required to bring about this effect. There are no ovarian changes characteristic of oestrus occuring during these induced periods.

The injection of theelin in a normal pre-pubertal animal has briefly been described under the vaginal smear test and will need no further discussion.

The injection of a pregnant animal with theelin may bring about two results. (6)(PII8) First, if injected in the earlier stages of pregnancy the effort of the uterus to assume an oestrus condition may result in an abortion. Second the injection may override the action of the persistent corpora lutea, and bring about a condition analogous with that produced by the removal of the corpora lutea during pregnancy.

In senile animals Parkes (6)(PII9) reports complete rejuvenation

including the recommencement of follicular maturation, an ovulation with the use of theelin.

Male animals show a degeneration of the testis and some "anti masculine reaction" according to Saqueur and his coworkers. However Parks (6)(PI2I) believes it is hard to reconcile this conclusion with the presence of oestrus like substance in the male urine.

Many of the articles on the clinical use of theelin are not available. However Parkes (6)(PI20-I2I) summarized the work very well up until I929.

He states: "Owing to the difficulty of adminstering adequate doses of the oily extract to women, clinical research with oestrin has not progressed far. With the new water-soluble preparations it should be possible to make a great advance in this direction. Even so however it is far from obvious what part oestrin may be able to play in the correction of reproductive disorders in the human. The premenstrual growth of the uterus is an effect of the Corpus luteum and at least a part of the ensuing menstration is pseudo pregnant degeneration. Frankel in particular has emphasized the improbability that oestrin plays a dominant part in the human cycle. Nevertheless, a variety of positive results have been reported. Wintz and Seitz, Wintz and Fingerhut record a number of chinical tests of corpus luteum

preparations "lipanin" and "luterlipoid", some of which appeared to give positive results, but it is very difficult to assess this early work. The same applies to the clinical work of Herrman.

"Using definitely oestrus producing preparations Pratt and Allen claim to have produced enlargement of the human uterus in cases of both primary and operative amenorrhoea. Menstruation was not produced Zondek obtained more satisfactory results, While Bronha and Simonnet claim to have successfully treated ovaryectony atrophy, amenorrhea, sterility, dysmenorrhoea, infantile conditions of the genitalia, and menapause symptoms, Saqueur reports some success in treating amenorrhea.

" It is clear that in considering all such clinical work due attention must be paid to the difficulty of adequately controlling work on the human, and also to the psychological factor involved. Little can be deduced from the results at present, but it seems probable that oestrin will have some clinical use, especially in expediating labour".

There were some rather interesting clinical investigations carried out by Hoosen (I7) in I920. Although this was before the placental hormone was purified, she used a hormone supplied by Park Davis & Co. and desicated bovine placenta, with which she seemed to obtain definite results. Her work consisted in the administration of the hormone or desicated placenta to the puerperal women with in the first twelve hours. She found that by so doing, the babies began to gain weight earlier and a larger percent, regained their birth weight much more rapidly than without the extract. She concluded from these tests, that,

the secretion of milk is established on the first or second day, that the violent establishment of the mammary function is avoided, and, that the administration of the hormone will stimulate the mammary gland at any period during lactation.

A consideration of the hormone "Emmenin" will next be taken up. Since the preparation of "Emmenin", anterior-pituitary-like hormone, and theelin are more or less connected by the proceedure of Collip (IQ) they will be considered together here.

The preparation consists in the extraction of the human placenta with either alcohol or acetone. The extracts are then concentrated and fractioned. These fractions are divided into three portions eaah appearing to contain an active principle. The first fraction contains theelin. It is obtained by repeated extractions with ether of the acetic acid acidified aqueous concentrate of the original extract.

The second fraction is soluble in eighty five percent alcohol and is present in the original extract. Theelin is removed from this by repeated extraction with ether. However it is very difficult to remove and necessatates the extraction to be carried out ten times and the aqueous phase acidified with acetic acid after each extraction. This fractioncontains "Emmenin"

The third fraction consists in the precipitate obtained

from the original extracts by the use of alcohol up to eighty five percent concentration. It is then repeatedly extracted with water and ether and reprecipitated by alcohol to remove the other hormones. By this proceedure the anterior pituitary-like hormone is obtained.

Little is known concerning the chemical properities of "Emmenin" other than that which has been obtained during its isolation and, in carrying out experiments since no more of the hormone has been isolated than was necessary for experimental purposes. However, it is known that the hormone is soluble in eighty five percent alcohol, is non-protein in character and is unaffected by digestive enzymes, or boiling with dilute acetic acid. About one miligram is obtained from ond kilogram of placenta and one rat unit contains about .0015 miligrams of solids.

Since there has been no other work done, other than that of Collip's on the placental hormones, "Emmenin, and the anterior pituitary-like substance, his work will have to be used entirely and he will be quoted frequently.

The injection of "Emmenin" in immature rats produces oestrus in from three to seven days either by oral or subcutaneous administration. The amount used to produce oestrus is equivelant to .5 grams of placental tissue each day. Examination of the animals at this time shows gross and microscopic changes in both the uterus and vagina typical of oestrus. There is also an increase in the size of the ovaries as a result of the development of the follicles. Corpora lutea were not observed in the

ovaries at this time. These changes were also produced by "Emmenin which had been treated with pepsin and trypsin. There were however a number of immature rats which did not respond to the use of "emmenin"and consequently it is impossible to determine the rat unit.

There has been no demonstrable changes either in the immature or adult male or in senile female rats from the use of "Emmenin".

In female adult rats there were no changes in the oestrus cycle, impregnation, pregnancy or lactation with daily administration of a dose corresponding to one to ten grams of placental tissue (IO).

There are some very satisfactory results in the clinical cases treated with "Emmenin"(I8). One hundred and thirty five cases of deranged ovarian function were used, consisting of Amenorrhea, Dysmenorrhea, Polymenorrhea, Metrorrhagia, Menorrhagia and Menapausal symptoms. These cases had no gross or obvious pathology in the pelvis. They were observed for a period varying from three to nine months. A summary of the cases is as follows:

	No. of cases Treated	Improved
Amenorrhea		
Primary	8	I
Secondary		
Oligomenorrhoea	20	I8
Oligomenorrhoea (with lapses)	I4	I2
Regular (with lapses)	I9	II
Dysmenorrhoea	36	26
Polymenorrhoea	8	7
Menopausal symptoms	I8	I4

There was no result obtained either in metrorrhagia or Menorrhagia.

The patients were given a dose of "Emmenin" corresponding to seventy five grams of placenta daily. It was given in water or orange juice before each meal. Some of the patients were unable to tolerate this dose because of symptoms such as nausea and vertigo. In these cases the dose was reduced until there were no such symptoms produced. During the treatment of these cases there were no bad effects noticed on impregnation of gestation and four of the patients became pregnant while the treatment was being used.

Although this is a small group of cases from which to draw any conclusion, they do show promises of proving "Emmenin" a valueable therapeutic agent in many of the ovarian dysfunctions.

Little is known regarding the chemical properities of the anterior pituitary-like hormone of the placenta with the exception of the fact that it is not soluble in alcohol, is decomposed by enzymes and boiling. Considerable is known, however, about the chemical properities of the anterior pituitary hormone. In a recent article Collip (II) compares the physiological effect of the two hormones and there is such a difference in the results that he believes the two to be entirely different.

He bases his conclusion on the fact that the adminstration of the anterior pituitary-like hormone in doses equivalent to thirty two grams of placenta will not produce any increase in weight of

the ovaries as compared with the implants of the anterior pituitary. However this effect may be interpreted either as a matter of dosage or that there is an absence of some specific growth promoting substance in the anterior pituitary-like hormone. Facts supporting the latter interpretation are furnished by experiments on male rats. In male rats, especially the immature animals there is an increase in the weight of the seminal vesicles and prostate gland with both the implants of the anterior pituitary gland and use of the hormone. However the use of the hormone does not produce an incerase in the size of the testis while the implants of the gland do.

Although the above facts were obtained in a very scientific manner they are not conclusive enough to prove the two hormones to be different. While they suggest that there is a difference between the two hormones there are a good many factors which enter into the question and these factors will have to be decided by further investigation before a definite conclusion can be obtained.

The injection of the anterior pituitary-like hormone in immature female rats(IO) has resulted in the occurrence of oestrus on the fourth or fifth day. These animals at this time show the usual phenomena of oestrus in the uterus and vagina. In addition the ovaries show a marked increase in size and contain numerous corpora lutea. These conditions were not produced by the oral

administration, or by boiling the hormone.

In male rats (IO) there has been an increase in the rate of growth of the seminal vesicles and prostate gland with in ten days. However if the administration was carried on over a long period of time the normal animal tended to equal the size of the treated animal in regard to the size of the accessory sexual apparatus. Although there occured an hypertrophy in the accessory glands the gonads were unchanged.

Daily injections of the anterior pituitary-like hormone into adult females produced a slight irregularity in oestrus although the cycle remained the same. Normal pregnancy and lactation were not effected by its continuous use.

The clinical (I8) work on the anterior pituitary -like hormone is even more limited than that carried out with "Emmenin". It consists only in the use of the hormone in metrorrhagia, however the results were most encouraging. The extract was administered subcutaneously and contained approximately ten rat units per cc. There was no enlargement of the breasts or milk secretion occuring with the administration of this hormone.

There fore, from the work of Collip and Daisy, one may conclude: first, that the placenta contains three hormones, theelin, Emmenin and an anterior pituitary-like hormone. Second, that these hormones are alike in that they are oestrogenic in the immature female rat, but differ in chemical and other physiological properities. Third, that the isolation of these hormones has seemingly proven the placenta to be a gland of internal secretion,

Fourth that although the clinical experimentation has been limited, the results are very encouraging with some specific results. And fifth, that the future offers no limit for the possibility of treating ovarian dysfunction since one hormone may be used to stimulate the action of the ovaries while another may be used at the same time to carry on the function of the ovaries.

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