THE CHEMICAL AND PHYSIOLOGICAL PROPERTIES, AND CLINICAL USES OF THE CORPUS LUTEUM HORMONE, PROGESTERONE*

WILLARD M. ALLEN

Professor of Obstetrics and Gynecology,
Washington University School of Medicine, St. Louis

THE corpus luteum hormone, progesterone, owes its discovery to two events: First, a considerable amount of physiological experimentation indicated the production of a special hormone by the corpus luteum; second, after the preparation of active estrogenic extracts it was found that these preparations did not produce the effects characteristic of the corpus luteum.

The first actual experiments to establish the corpus luteum as an endocrine gland were made by Fraenkel[1] in 1903. He found that removal of the corpora lutea in the first few days of pregnancy prevented the continuation of pregnancy. Loeb[2] in 1907 made the next contribution by showing that the endometrium of the guinea pig is sensitized while under the influence of the corpus luteum so that simple, mechanical trauma at the time when the embryos would be implanting, results in the formation of a decidual tumor at the site of injury. This experiment showed that the endometrium requires the presence of the corpus luteum before it can respond normally to the presence of the embryo. A little later, in 1910, Bouin and Ancel[3] showed in the rabbit that the endometrium undergoes marked proliferative changes as the corpus luteum develops, and further, that removal of the corpora lutea prevents this proliferation. Here then was the first evidence that actually the embryos in Fraenkel's experiments arrived in a uterus which was not prepared by the action of the corpus luteum to receive them. Coincident with the growth of the corpora the endometrium proliferates rapidly, as we now know because of the action of progesterone, and the embryos survive. If the corpora are removed, this proliferation does not take place and growth of the embryo stops, as was shown by Corner[4] in 1928, as soon

* Read January 2, 1941 at the Annual Meeting of The New York Academy of Medicine.

as it reaches the uterus. This proliferation is essential for the implantation of the embryo, and hence has been called progestational proliferation.

Alterations of the endometrium of similar significance are found in association with the growth of the newly formed corpus luteum in all species that have been investigated, and in the woman we now know that the premenstrual changes are due in part at least to the progesterone produced by the corpus luteum. Such alterations, no matter how dissimilar they may appear to be in different species, are essential for the safe implantation of the embryo.

The corpus luteum hormone, of course, has many properties other than those specifically pertaining to the uterus but they are presumably all related to the problem of preparing the body for pregnancy and the safe continuation of pregnancy.

None of the specific effects of the corpus luteum can be satisfactorily and completely produced by estrogens. Pregnancy cannot be maintained in a castrated rabbit with estrogen. The endometrium of the guinea pig cannot be sensitized by estrogens. Premenstrual changes cannot be produced in primates with estrogens. For these reasons, George Corner and I began our work on extracts of the corpus luteum.

To study the effect of a corpus luteum extract, it is necessary only to castrate the rabbit about eighteen hours after mating, and then to give the preparation to be tested for five days to see what effect it will have on the endometrium and on the embryos. If the extract contains no corpus luteum hormone, the endometrium shows no progestational proliferation and the embryos do not develop; if on the other hand the extract contains corpus luteum hormone, the uterus undergoes typical progestational changes and the embryos develop exactly as in normal pregnancy, even though the rabbit has been deprived of her own corpora lutea almost from the time of ovulation.

This method of testing the extracts enabled Corner and myself to prepare active crude extracts of the corpus luteum. These crude extracts produced progestational changes in the endometrium, maintained pregnancy to term in castrated rabbits and sensitized the guinea pig's endometrium so that deciduomata could be produced experimentally. In short, these crude extracts contained a hormone (or hormones) which did all the things that the corpus luteum was supposed to do, at least in so far as the rabbit and guinea pig are concerned. Because of its

obvious relation to pregnancy, it was named progestin, i.e., a substance necessary for pregnancy.

The active principle was independently isolated in 1934 by Butenandt;[5] Slotta, Ruschig and Fels;[6] Allen and Wintersteiner;[7] and Hartmann and Wettstein.[8] It was found to be a neutral compound, a di-ketone with the empirical formula $C_{21}H_{30}O_2$. This immediately suggested that it was closely related to pregnandiol, a dialcohol previously isolated by Marrian[9] in 1929 from the urine of pregnant women, with the formula $C_{21}H_{36}O_2$, the detailed structure of which had already been established by Butenandt.[10] The supposed relationship was proved soon by Butenandt by the preparation of progesterone from pregnandiol and from stigmasterol. It has since been prepared from cholesterol and sarsapogenin and possibly other sterols.

Progesterone is soluble in most organic solvents, and in vegetable oils, such as sesame oil, olive oil and peanut oil, but it is insoluble in water. It is ordinarily dissolved in a bland vegetable oil and is given by intramuscular injection. Despite its insolubility in water, absorption from the oily solutions is quite rapid. The effect is not prolonged and anyone who has had much experience with the use of progesterone in animals gives little credence to reported remarkable clinical results where small doses were given once weekly. For example, in certain experiments in rabbits the omitting of a single daily dose may be sufficient to disturb a pregnancy being maintained artificially by progesterone. In women, too, progesterone deprivation frequently is followed by menstrual bleeding within forty-eight hours. If one is attempting therapy with progesterone, injections should be given each day. In this respect it is quite different from the esters of estradiol and testosterone, the absorption of which extends over a period of several days.

The conditions for which progesterone may be used in women are probably rather few but before discussing them it may perhaps be profitable to mention some of the physiological effects in animals. The endometrium of the rabbit responds to progesterone the same as it does to action of the rabbit's own corpora. Pregnancy can be maintained to full term in the absence of the ovaries, and delivery can be prevented by continued administration of adequate amounts. It sensitizes the endometrium of the rat and the guinea pig so that deciduomata can be produced experimentally. In short it substitutes completely for the corpus luteum, except that for some types of response estrogen must be given

at the same time, i.e., some physiological responses attributed to action of the corpus luteum are in reality due to combined action of estrogen and progesterone.

Progesterone also has a specific action on the uterine muscle. It is well known from the experiments of Knaus[11] that the reactivity of the rabbit's uterus to pituitrin is materially changed during pseudopregnancy or pregnancy. If a segment of the uterus is removed from an animal in heat and suspended in a suitable saline bath, spontaneous contractions occur and can be recorded by means of a kymograph. If now a small amount of pituitrin is added, there is an immediate and prompt response, shown by a typical tetanic contraction. A similar response is, of course, obtained in the human uterus at the time of delivery when pituitrin is given. This sensitiveness to pituitrin in the sexually mature rabbit is always present when no corpora lutea are present in the ovaries. If functional corpora are present, as in pregnancy or pseudopregnancy, the uterus similarly studied in vitro, is not responsive to pituitrin. The uterus is refractory. This pituitrin-inhibition, as it is called, is due to the action of progesterone and can be produced experimentally by giving rabbits 1.0 mgm. of progesterone daily for five days (Makepeace, Corner and Allen).[12]

In vivo studies carried out by recording the uterine motility in anesthetized rabbits by means of an intrauterine balloon show too that progesterone has a quieting effect on the uterus. It has been shown that estrogen induces strong, rhythmical contractions and that progesterone brings about quiescence (Allen and Reynolds).[13] The effects are noticed rather quickly, indicating prompt absorption, even though the hormone is dissolved in an inert vegetable oil. It is this quieting effect on the myometrium which has led to the use of progesterone in dysmenorrhea, threatened abortion and premature labor.

The effects on the endometrium of the primates are of even greater interest because it has now been amply demonstrated that the cyclic changes which are known to occur in the corpus luteum phase of the cycle can be produced experimentally by the use of progesterone. The exact details of dose are not as well worked out as in the rabbit but several observers have produced premenstrual changes in both monkeys and women with progesterone.

The menstrual cycle of the primate is inseparably associated with the corpus luteum, whenever the cycle is ovulatory, but to complicate

matters, many cycles in monkeys are anovulatory and we have fairly good evidence that many types of abnormality in the human cycle are accompanied by and probably due to disturbances in the mechanism of ovulation. The types of cycles can be best explained by discussing the methods of inducing artificial menstruation.

Menstrual bleeding can be produced experimentally in both women and the lower primates by either of two mechanisms. If a castrated individual is given estrogen for a short period of time, say two or three weeks, the endometrium responds by moderate growth. If the hormone is then discontinued, vaginal bleeding similar to menstruation occurs, beginning about seven to ten days after the last injection. This bleeding is called estrogen-deprivation bleeding. The amount of estrogen necessary to produce this depends on the particular preparation used. With estradiol benzoate, for example, it requires 1.5 to 2.0 mgm. per week.

Before passing on to the second mechanism of producing bleeding, it may be well to dwell on the significance of this observation. Since bleeding can be produced with this one hormone alone, it becomes obvious that some menstrual cycles might actually occur because of this same mechanism. Follicles may grow, produce estrogens and then, without ovulation having occurred, undergo atresia. The estrogen level falls and bleeding occurs because of estrogen deprivation. This is obviously not a complete cycle because there has been no ovulation, hence, no corpus luteum, and therefore the endometrium does not progress to the premenstrual or secretory type. This type of cycle, called an anovulatory cycle was described first in the monkey by Corner. This was considered by many gynecologists, both in this country and in Europe, an interesting peculiarity of the monkey, but of no significance as far as the woman was concerned. Such is certainly no longer the case and many cycles in women are undoubtedly anovulatory. One of the first evidences of ovarian dysfunction is failure of ovulation.

Estrogen-deprivation is not the only method of producing menstruation experimentally. It was soon found that administration of progesterone would prevent estrogen deprivation bleeding. If a monkey is given estrogen, and then if progesterone is given, beginning as soon as the estrogen is stopped, no bleeding occurs. However, when the progesterone is discontinued typical bleeding occurs. Progesterone, therefore, prevents estrogen-deprivation bleeding, but its withdrawal leads to pro-

gesterone-deprivation bleeding. This means that bleeding can be due to two different types of endocrine change. Externally the bleeding is the same, but the type of endometrium from which the bleeding occurs is quite different in the two cases. In the estrin stimulated endometrium, bleeding occurs from an interval type of endometrium; whereas in the estrin and progesterone stimulated endometrium, bleeding occurs from a premenstrual endometrium.

A final way of producing bleeding probably nearly approximates the mechanism present in the normal ovulatory cycle. If a monkey be given an adequate amount of estrogen continuously, it is possible to produce menstrual bleeding simply by the administration of progesterone for a few days. Bleeding occurs two to three days after the last injection. This means that bleeding will occur whenever the progesterone level falls, regardless of the amount of estrogen present, within certain rather wide limits (Corner).[14]

An interesting variant of this experiment has been recently carried out in women by Zondek.[15] He has shown that administration of progesterone to a normal woman in the first half of the cycle leads to bleeding 48 to 72 hours after the last injection without at the same time necessarily preventing the appearance of the next normal menstrual period at the expected time. This only serves to emphasize the point that progesterone-deprivation is followed by bleeding provided the stimulation has been sufficient.

By way of confusing the issue it might be appropriate to point out what is already probably obvious to you, namely, that bleeding can occur from almost any sort of endometrium. The degree of development of the endometrium depends on the amount and duration of action of the two ovarian hormones but the bleeding occurs from hormonal deprivation (except possibly in hyperplasia), the stage of development of the endometrium being incidental and in no direct way related to the cause of the bleeding.

The final question is, what clinical uses can be made of the various physiological properties of progesterone?

Progesterone may be used in dysmenorrhea, the theory being that the pain is due to ischemia resulting from strong contractions of the uterus. Progesterone does relax the uterine muscle in both animals and humans and hence, should be beneficial in dysmenorrhea. The general opinion, I think, is that about one-half of the cases of dysmenorrhea are

benefited. My own experience has not been extensive, but in several patients there can be no doubt but that marked improvement was noted. The progesterone has to be given before the period has begun and I have had the best success when 1.0 to 2.0 mgm. was given intramuscularly on the last three days immediately preceding the flow. I have got the impression also that it is the progesterone given in the last 24 hours before the onset which produces the effect. Unfortunately the treatment is not curative in any sense of the word since the next period is usually as bad as ever.

Threatened abortion is, of course, theoretically the indication par excellence for progesterone. Excision of the corpus luteum of pregnancy results in abortion in most mammals (including the human in early pregnancy) and, experimentally, progesterone will maintain pregnancy normally following excision of the corpora. Transfer of this information to the human is not so justifiable, however, because there is very little evidence that abortion is necessarily due to corpus luteum deficiency. In many instances it may be and probably is due to corpus luteum deficiency but this is frequently due to the presence of a defective embryo in utero. The administration of progesterone to such a case, of course, might defeat the natural process of expelling the defective embryo. I have seen one such case where the abortion was prevented on two different occasions only to have a defective embryo expelled at the fourth month in which the placenta and membranes were intact but without a fetus. In case of a threatened abortion in which the bleeding is slight and the uterus of normal size and consistency for the period of amenorrhea, progesterone is, of course, indicated. It can do no harm and it may be beneficial.

The question of dose is paramount. In normal pregnancy at the third month 10 to 20 mgm. of pregnandiol are excreted daily (Venning).[16] If this be taken to indicate the amount of progesterone metabolized, it is obvious that 5 to 10 mgm. as a minimum should be given daily until the symptoms have subsided, i.e., for a week or so. It seems probable that some abortions may occur because the placenta does not begin elaborating sufficient progesterone quite soon enough, and if this be so, then progesterone given for a short time might be expected to give good results.

Habitual abortion is also considered an indication for progesterone. This is a rare condition and some of these cases will occasionally carry

a fetus to term after many previous miscarriages with no treatment. Many patients will have two or three spontaneous miscarriages only to have the following pregnancies progress normally. It is very difficult, therefore, to evaluate the beneficial results reported in the literature, especially since the doses used have usually not been large. In habitual abortion progesterone, if used at all, should be started almost as soon as pregnancy can be diagnosed and it should be continued probably to the fourth or fifth month, the reason for beginning early being that the fetus may fail to develop normally if the progesterone level is inadequate. In the rabbit progesterone deficiency in early pregnancy leads to defective development and early death of the embryos.

Functional bleeding is one condition worth special consideration. Amenorrhea or bimonthly bleeding usually do not impair the health, but continuous bleeding leads to considerable ill health, and rarely death has occurred from blood loss due to functional bleeding. The exact cause of the bleeding is not known. It is reasonably certain, however, that ovulation is not occurring and hence that progesterone is not acting. The use of pituitary hormones for this condition is based on the assumption that ovulation may be induced, and a normal cycle established. This can rarely be accomplished by this means. There is one other method of approaching the problem that is often successful. This involves the use of progesterone. As I mentioned earlier, progesterone has the property of preventing estrogen-deprivation bleeding and also of preventing the onset of normal menstrual bleeding, if given in adequate amount. It is logical, therefore, to try it, especially since continuous functional bleeding and hyperplasia are presumably associated with progesterone deficiency. There is an added reason. Some investigators believe that hyperplasia and the associated bleeding are due to hyperestrinism. If so, progesterone is the natural hormone to use because there is ample evidence indicating that progesterone reduces or at least alters the effect of estrogens.

We (Allen and Heckel)[17] have given progesterone to several adolescent girls with more or less continuous bleeding with rather uniform results. The general plan has been to give progesterone intramuscularly, 2 to 10 mgm. daily, for from five to ten days. Ordinarily the bleeding decreases markedly after the second and third dose but a small amount of brownish discharge may persist. When the injections are discontinued, the bleeding may recur within 48 to 72 hours and persist

for four or five days, resembling in every respect a slightly profuse menstrual flow. Bleeding does not invariably decrease when progesterone is given. Occasionally the spotting or even moderate bleeding may continue during the course of injections but usually cessation of injections is followed by increased bleeding. In every instance where this result has been obtained the flow has stopped completely after a few days. This bleeding is probably a true progesterone-deprivation bleeding. Bleeding may occasionally increase during the course of injections. The reason for this is not clear at present but there are so many peculiarities about bleeding that one can only record the observation and await the explanation. For example, I have seen bleeding occur in a castrated woman receiving estrogen daily, during a course of injections of progesterone, hence with no deprivation of either hormone. In other cases, usually when the smaller doses were given, the bleeding has stopped but there has been no recurrence as a result of the withdrawal of progesterone.

These results, by themselves, are of considerable interest but the subsequent course has been even more interesting. In most instances several fairly normal periods have followed the progesterone therapy. The first patient treated by this scheme was given crystalline progesterone in 1935. She was fifteen years old and had had continuous bleeding for two months. She was given 2.0 mgm. daily for five days and the bleeding stopped. Menstrual periods began about a month later and have been quite regular ever since. Another patient of seventeen years who had been bleeding daily for two years, was given 5 mgm. daily for six days. The bleeding stopped after the third injection. A copious menstrual period began two days after the last injection and lasted five days. During the next six months the periods were somewhat irregular but there were no prolonged periods and there was no episode of prolonged bleeding. I cite these two only as examples of what has occurred. In some cases there has been a recurrence of functional bleeding after several months of normal cycles, but the same beneficial results were obtained by a second course of progesterone.

The beneficial effects in functional bleeding from progesterone should not be permitted to obscure the fact that functional bleeding is a manifestation of ovarian dysfunction. As such it may indicate hypothyroidism and many patients with functional bleeding have been permanently relieved by the use of thyroid.

Amenorrhea, one of the most troublesome types of endocrine dysfunction when it is not amenable to thyroid extract and improved nutrition, provides another type of disturbance in which progesterone may be used. In view of the observation that the vicious cycle of continuous bleeding may frequently be broken by progesterone, I have tried it in several cases of amenorrhea in which there was no marked atrophy of the reproductive organs. In several cases the administration of 5 to 10 mgm. daily for six days was followed by menstrual bleeding, the onset being 48 to 72 hours after the last injection. One of these cases treated recently had had no menstrual periods for two years and had failed to menstruate following the use of equine chorionic gonadotropin, and human chorionic gonadotropin and had proved intolerant to thyroid extract. Two menstrual periods were induced by the use of progesterone, following which a normal period occurred. Another patient who had not menstruated for one and one-half years bled for four days after only three injections of progesterone. I cite these observations only to show that bleeding can be induced in amenorrheic individuals as a result of progesterone deprivation. Estrogen deprivation also is followed by bleeding in amenorrheic patients but it is practically never followed by normal cycles. It seems too much to hope that cycles produced by progesterone deprivation would be followed by normal periods but at least it can be no more of a failure than most of the other hormones that have been used.

There is one other compound closely related to progesterone which I shall mention. This compound is ethinyl testosterone (anhydro-hydroxy-progesterone, pregneninolone). It was prepared originally by Inhoffen and Hohlweg[18] in 1938 and was shown to be a compound with progesterone-like properties when given by mouth. This compound is far less active by mouth than is progesterone intramuscularly but it is much more active by mouth than is progesterone by mouth in the rabbit. In the guinea pig, however, Soderwall[19] (1940) has recently shown that progesterone and pregneninolone have about the same activity, the oral dose of either being about 20 times the injected dose of progesterone. This compound is also mildly estrogenic and mildly androgenic.

The indications for use of pregneninolone are the same as those for progesterone. It produces nausea occasionally but it is probably nontoxic in doses of even 100 to 200 mgm. per day for a period of two weeks or so. A recent report by Cohen and Stein[20] (1940) indicates that

it may be toxic in rats, hence caution must be observed in its use.

In conclusion it is proper to emphasize that the corpus luteum hormone has a firm place in the physiology of reproduction, and our appreciation of its many properties helps us to approach disturbances of ovarian function more rationally. It does not serve as a "cure-all" for any of the dysfunctions of the ovary but it is another tool which can be used in attempting to ascertain the cause of such disturbances. If used with that in mind the results are bound to be instructive.

REFERENCES

1. Fraenkel, L. Die Function des Corpus luteum, *Arch. f. Gynäk.*, 1903, *68:*438.
2. Loeb, L. Ueber die experimentelle Erzeugung von Knoten von Deciduagewebe in dem Uterus des Meerschweinchens nach stattgefundener Copulation, *Zentralbl. f. allg. Path.*, 1907, *18:*563.
3. Bouin, P. and Ancel, P. Recherches sur les fonctions du corps jaune gestatif. sur le determinisme de la préparation de l'utérus à la fixation de l'oeuf, *J. physiol. et path. gen.*, 1910, *12:*1.
4. Corner, G. W. Physiology of the corpus luteum; the effect of very early ablation of the corpus luteum upon embryos and uterus, *Am. J. Physiol.*, 1928, *86:*74.
5. Butenandt, A. Neuere Ergebnisse auf dem Gebiet der Sexualhormone, *Wien. klin. Wchnschr.*, 1934, *47:*934.
6. Slotta, K. H., Ruschig, H. and Fels, E. Reindarstellung der Hormone aus dem Corpus luteum, *Ber. d. deutsch. chem. Gesellsch.*, 1934, *67:*1270.
7. Allen, W. M. and Wintersteiner, O. Crystalline progestin, *Science*, 1934, *80:*190.
8. Hartmann, M. and Wettstein, A. Ein krystallisiertes Hormon aus Corpus luteum, *Helvet. chim. acta*, 1934, *17:*878.
9. Marrian, G. F. The chemistry of oestrin; preparation from urine and the separation frcm an unidentified solid alcohol, *Biochem. J.*, 1929, *23:*1090.
10. Butenandt, A. Über das Pregnandiol, einen neuen Sterin-Abkömmling aus Schwangeren-Harn, *Ber. d. deutsch. chem. Gesellsch.*, 1930, *63:*659.
11. Knaus, H. Zur Physiologie des Corpusluteum, *Arch. f. Gynäk.*, 1930, *140:*181; *141:*374; 395.
12. Makepeace, A. W., Corner, G. W. and Allen, W. M. The effect of progestin on the in vitro response of the rabbit's uterus to pituitrin, *Am. J. Physiol.*, 1936, *115:*376.
13. Allen, W. M. and Reynolds, S. R. M. Physiology of the corpus luteum; the comparative actions of crystalline progestin and crude progestin on uterine motility in unanesthetized rabbits, *Am. J. Obst. & Gynec.*, 1935, *30:*309.
14. Corner, G. W. The ovarian hormones and experimental menstruation, *Am. J. Obst. & Gynec.*, 1939, *38:*862.
15. Zondek, B. and Rozin, S. Production of uterine haemorrhage in the normal cycle and in amenorrhoea through progesterone, *J. Obst. & Gynec. Brit. Emp.*, 1938, *45:*918.
16. Venning, E. H. Further studies on the estimation of small amounts of sodium pregnanediol glucuronidate in the urine, *J. Biol. Chem.*, 1938, *126:*595.
17. Allen, W. M. and Heckel, G. P. *Unpublished observations.*
18. Inhoffen, H. H. and Hohlweg, W. Neue per os-wirksame weibliche Keimdrüsenhormon-Derivate: 17 Aethinyl-oestradiol und Pregnen-in-on-3-ol-17, *Naturwissenschaften*, 1938, *26:*96.
19. Soderwall, A. L. Induction of sexual receptivity in estrogen conditioned spayed female guinea pigs by orally administered progesterone and pregneninolone, *Endocrinology*, 1940, *27:*840.
20. Cohen, M. R. and Stein, I. F. Laboratory and clinical experience with oral pregneninolone, *Am. J. Obst. & Gynec.*, 1940, *40:*713.