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BIOLOGIC EFFECTS OF PROGESTERONE AND PROGESTATIONAL AGENTS

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

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OUTLINE AND TABLE OF CONTENTS

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INTRODUCTION

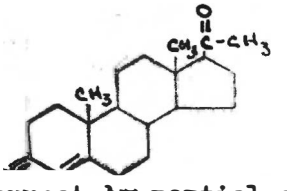
The middle of the twentieth century brought a new era in endocrine medium. Beginning with cortisone-like compounds, it was discovered that changing various molecular groups on the basic perhydrophenanthrene nucleus would, in certain cases, enhance the potency of the compound. Even more important, it was found that small chemical changes caused marked alterations specific activities. Thus, substances more potent than the naturally occurring cortisol were synthesized and additional modifications decreased the undesirable side effects (e.g. salt retention). After these modifications on the adrenal steroids, courageous investigators attached the other natural steroids, particularly progesterone, which has previously been considered unique in its actions. One group of these were the "progestins". Synthetic compounds were prepared which extended progestational activity in certain areas, and then progesterone itself became a hormone requiring re-investigation. Much of the earlier knowledge of this substance had been based on investigation of the "corpus luteum hormone", and so the effects of estrogen and relaxin had been incorporated into the results of experimental studies. However, progesterone had been used clinically for a number of years. The principle source was sow ovaries, and the pure compound was administered intramuscularly. Therefore the announcement of potent, orally active progestational substances was welcomed as a more convenient method of attaining progestational effects. But confusion has arisen as to whether

the synthetic progestational substances can completely duplicate the biological functions of the natural hormone. For instance, norethandrolone, an androgen derivative marketed in April 1956, for its anabolic effects, was soon discovered to be an active "progestin" as well, causing specific endometrial changes. However, its close similarity of structure and action to testosterone required specific evaluation. Thus, the object of this investigation is to gather additional basic information on the biologic activities of progesterone and compare it with various homologous substances.

I. HISTORY

Before 1900, Born (1), in Breslau, postulated the "important function of the corpus luteum during pregnancy." This theory was strengthened by the work of Fraenkel (2) who proved that pregnancy was impossible without a corpus luteum in rabbits. By 1907, Leo Loeb (3) had shown that the corpus luteum, through some unknown mechanism, made implantation possible. Bio-assays on extracts of corpus luteum were done by Bouin and Ancel (4) in 1910. Five years later, Hermann (5) found that lipoid extractions from the corpus luteum and placenta contained "an active substance, which, (in rabbits) produced uterine growth and congestion and endometrial cell changes." In 1922 Long and Evans (6) thought they had found this same substance i high levels in animals just prior to ovulation. Decidual formation was first related to corpus luteum extract administration by Corner (7) in 1928. In the 1930's the word "progestin" (impure progesterone) appeared in the literature. Mazer, Goldstein, Adler, Ehrhardt (8) and others confirmed its presence in the placenta as well as the corpus luteum. A variation of this was presented by Corner and Allen (9) in 1929, when they found that lipoid extracts of corpus luteum produced "progestational proliferation of the endometrium", while placental extracts produced "growth only". This was one of the first clues that more than one substance might exist in lipoid extracts of corpus luteum. Hisaw, Fevold, and Meyer (10) in 1930 showed other affects of lipoid

extracts of corpus luteum as producing relaxation of the symphsis pubis in guinea pigs, mucification of the vagina (later disproven), and sensitization of the endometrium so that decidua could form. The "proliferative substance" mentioned above later proved to be estrogen plus progesterone plus relaxin. In 1933 Allen and Meyer (11) separated estrogen from progesterone. Progesterone was isolated from the placenta (12) and human pregnancy plasma (12-A) by Salhanick et al. in 1954. Thus at the present time we enjoy the heritage of knowledge that a substance (called progesterone) is produced by both the corpus luteum and the placenta, that it is necessary to pregnancy and possibly to ovulation, and that it causes specific endometrial changes in the endometrium. From Allen's work, (13) progesterone was suggested as a substance which melted at 120° or 128° C. Slotta, Ruschig and Fels (14) found that the prism form of progesterone, M.P. 128°, separates on slow crystalization from aqueous alcohol. The needle form melts at 121° and can be crystalized from petroleum ether. The two forms yield identical derivatives and are interconvertible (15)(16). The early source of progesterone was sow ovaries, which contain several copora lutei each (17). In 1934 Slotta (18) proposed the formula:



which later proved to be correct by partial synthesis.

Several methods of bioassay of progesterone have been used.

The Corner-Allen technique (19) consists of: (A) castrating female rabbits at the proper stage of follicle ripening (as determined by mating); (B) Giving the animal intramuscular hormone; and (C) on the fifth day studying endometrium by histological examination for progestational proliferation. The Clauberg method (20) involves priming immature female rabbits with estrogen and then administering progestational agents. In 1947 Hooker and Forbes (21) injected .0006 ml. of progesterone into the uterus of 16-day castrate mouse, studying the endometrium for cell changes on the 4th or 5th day thereafter. High estrogen levels were later found to produce "false negatives" by this method, (21-A). The McGinty technique involves injecting the test material into the uterine horn of the estrogen-primed oophorectomized rabbit.

The official name, progesterone, was proposed in 1935 by Allen, Butenandt, Corner and Slotta. The League of Nations designated the International Unit in the same year as follows: "The specific progestational activity of 1.0 mg. of the international standard of B-Progesterone" (22).

II.(A)THE EFFECTS OF PROGESTERONE

The role of progesterone in cyclic changes in the reproductive cycle has long been known. Dempsey (23) demonstrated the superiority of progesterone in producing oestrous in ovariectomized guinea pigs in 1936. More recently, Robinson (24) stated that

progesterone was necessary for the production of heat in the ovariectomized ewe. Grant (25) had found previously, however, that the first ovulation by the ewe in mating season was not accompanied by heat. It was postulated, therefore, that the subsequent formation of the corpus luteum following the first ovulation released the progesterone necessary to produce estrus. The primates' cycle was described by Allen et al. (26) as follows, "A menstrual cycle in which ovulation occurs can be conveniently divided into a follicular and a luteal phase. The follicular phase extends from menstruation to ovulation and the luteal phase from ovulation to the following menstruation..... The endometrial modifications that take place during the follicular phase of the cycle can be duplicated in the castrated monkey by the injection of estrogen. Likewise, progestational condition characteristic of the luteal phase can be developed by giving progesterone. In fact, all the morphological and physiological features of anovulatory and ovulatory cycles can be reproduced in castrated monkeys by estrogen and progesterone." Allen's statements were supported primarily by the work of Hisaw et al. (27) in the early 1930's. Similarly, secretory and decidual changes were produced in the endometrium of oophorectomized women by Bradbury et al. (28) and others by the following procedure: 1 mg. of estrone was given daily for one week with the result that good proliferative changes were noted (as in follicular phase, above). Progesterone was given I.M. in doses of 5 mg. daily for seven days, along with the estrone, which was continued. Early secretory

changes were noted from this dosage (luteal phase, above). It was found that increasing the dose of progesterone to 50 mg. and estrone to 2 mg. brought about "complete secretory changes". Increasing the dose of hormones still further produced decidual changes, and bleeding followed cessation of the progesterone therapy even though the estrogen was continued. It is of interest to note here that Hisaw found that if progesterone treatment is prolonged from 25 to 30 days in monkeys, the endometrial glands enter a state called "secretory exhaustion" (29).

Presecretory, progestational endometria in castrated monkeys can be produced by progesterone, and experimentally will produce an implantation reaction if mechanically traumatized, (Hisaw)(30). It is described as follows: "The proliferated cells originate from the surface of the glandular epithelium and grow out into the surrounding stroma. Many attain the proportions of giant cells and fuse with their neighbors producing multi-nucleated masses. The reaction usually spreads from the point of injury and by the eighth day may involve the entire inner portion of the endometrium bordering the lumen."

The cyclic theory has been advanced as follows: (31) "The follicle-stimulating hormone, FSH, from the anterior lobe of the pituitary acts as the trophic principle in ovarian follicular growth. As the follicle matures, the level of its secretory product, estrogen, rises until it reaches that critical threshold level at which it inhibits further release of FSH and stimulates release of

the luteinizing hormone, LH.... LH then initiates, or triggers, rupture of the mature ollicle and formation of the corpus luteum. The corpus luteum, in turn, secretes progesterone which is required to prepare the endometrium for nidation. Progesterone, in turn, inhibits the premature release of LH so that supraovulation does not occur. Should conception fail to occur, the functional activity of the corpus luteum is short lived and with its disintegration, the progesterone level falls and menstruation (progesterone withdrawal bleeding) occurs. During this period of disintegration of the corpus luteum the beginning of the next cycle has already been initiated by the release of FSH and maturation of a new follicle." Theoretically, then, a defect of alteration in any one of these stages -- either pituitary or ovarian--- of this normally sequential series of interactions can result in menstrual irregularities, ovulatory failure, and/or amenorrhea." Certainly there are questions about this theory; not all phases have been proven even in animals, and much remains to be proven about the actual interaction of these hormones in the human. Species lines cannot be crossed with generalizations, as has been repeatedly proven. For instance, it has been postulated (as mentioned earlier) that pregnancy in animals is maintained by the corpus luteum hormones and that it is necessary in these species. However, removal of the corpus leteum in the human does not always terminate gestation. In 1928 Asdell (32) analyzed thirty-four cases in which bilateral oophorectomy had been performed during pregnancy, ranging from the first to the seventh

month. Only four instances of abortion occurred (32-A & 32-B). In 1938 Hartman (33) had stated that human beings, guinea pigs, horses and monkeys are exceptions to the otherwise general theory that the corpus luteum is necessary to maintenance of pregnancy. Furthermore, the necessity of ovulation and therefore progesterone to menstruation has long been disproven. Corner, in 1932, (34) in speaking of regularly menstruating monkeys stated: "To all outward observation the periods of these animals, the character of the bleeding and indeed all aspects of the menstrual phenomena were alike, and yet when they were killed, it was apparent from the examination of the ovaries that some of the monkeys had not ovulated or formed corpora lutea in the ovaries for months past, while others had ovulated with regularity during the cycles preceding autopsy." It remained to be found, however, whether bleeding of anovulatory cycles was due to progesterone withdrawal or some other phenomena. Corner, and later, Van Herwerden, stressed that menstruation which occurs during an anovulatory cycle involves bleeding from an interval type of epithelium of uterus; not the type now recognized as "progestational". Lass, Smelser and Kurzrock (35) chose 47 lactating women with regular menstrual periodicity from 4000 cases and found that 55% of 196 cycles were anovulatory, using the above criteria of endometrial changes. The above instances are examples of the discrepancies in the theory of hormone interaction necessary to cyclic changes. No doubt further exceptions and changes will

be made as the pure substances are isolated and tried experimentally.

Tulsky and Koff (32-A), in a series of 14 women who had been castrated during pregnancy, found that pregnancy continued normally in 12 of them. Pearlman & Thomas, Salhanick et al. (32-B) and others have suggested that the source in humans is probably placenta.

Whether or not progesterone precedes or is necessary to ovulation has been the subject of much controversy. Sawyer (36) presented evidence that progesterone initially facilitates the release of pituitary ovulating hormone in the rabbit; he claimed that it inhibited this same release later in the cycle. Tienhoven et al. (37) explained the progesterone mechanism behind ovulation in the hen as probably via some neural mechanism, since they were able to block the progesterone effect by a sympathicolytic agent. In this same year, Holmstrom (38) in reviewing a series of cases of functional uterine bleeding who had been treated with progesterone reported that 70% of patients under 35 ovulated within four months of the commencement of therapy. Endometrial biopsies were studied as evidence of anovulatory cycles prior to and following therapy.

Another well known effect of progesterone is its so called "thermal effect". This was postulated when Raoul Palmer (1938) commented on the simultaneous appearance of glycogen in the endometrium and rise in body temperature. There has been much evidence that the rise in body basal temperature which precedes ovulation is probably a "progestational effect" and that the relationship is quantitative (39-A). In 1838 Von Fricke studied the body temperature

in response to ovulation and menstruation. Many investigations followed, and finally Palmer and Devillers (1939) found that the "thermal effect" could be produced in ovariectomized women by the injection of 10 mg. of progesterone (39-B). In a complete review of cyclic basal temperature curves, Palmer (39-C) stated that the "shift of body temperature should be regarded as due to the luteinization of the mature Graafian follicle" and that real ovulation is usually followed by a temperature rise within 24 hours.

The effects of progesterone on the endometrium of the uterus can be conveniently summarized by studying four components: glands, epithelium, blood vessels and stroma. The glands first show elongation of the epithelial cells and the necks of the glands. When progestational influence continues, this effect progresses down the gland toward the base. The epithelium change follows this, consisting mainly of rearrangement of the nuclei, which retreat from the basement membrane leaving a conspicuous "clear zone", supposedly produced by intracellular deposits of glycogen. Actual secretion is thought to begin partly in response to estrogenic stimulation, appearing first in the necks of the glands. Submuclear vacuoles appear and migrate toward the cell membrane, where they will eventually rupture, enabling secretion to occur. The stroma becomes edematous in the early phase of progesterone influence, later becoming decidual.

Bradbury and Long (40) found that decidual changes, increased vascularity and very low pavement type of surface epithelium could

be produced on the endometrium by prolonged dosages of progesterone and estrogen. Estrogen alone did not produce such changes and progesterone alone often resulted in "breakthrough bleeding" before decidual changes could be identified. "Breakthrough bleeding" is defined as bleeding in the presence of the hormone. These authors reported a good "synergistic effect" and decidual changes by giving 25 mg. of progesterone (beginning in the luteal phase) and 2.5 mg. of estrogen daily, continuing this over three to six weeks.

Williams (40-A) reported the vascular effect of progesterone as being one of intensification of the estrogen effect, "particularly on the "baskets' of capillaries around the uterine glands and the subepithelial plexus of capillaries". Classically, the arterioles become more spiral following vasodilatation.

Cytologic changes are also seen in the vaginal mucosa, the mammary glands and the cervix under the influence of progesterone. The vaginal mucosa thickens up to the time of ovulation, and then during the luteal phase desquamates. Robertson, Maddux and Allen (41-A) reported that glycogen is deposited in the vaginal cells in the proliferative phase. Hisaw and Greep (41-B) found that progesterone alone has very little effect on the vaginal mucosa but does modify the action of estrogen (above) when both drugs are given simultaneously. The first indication of the inhibition of estrogen is the appearance of leukocytes in the vagina. The mucosa then thins, the leucocytes infiltrate and extend over the areas where the functionalis has disappeared. The Papanicolaou-smear shows the

desquamated cells to be nucleated and not completely cornified. According to Turner (1939) mammary glands are affected first by estrogen with marked alveolar growth and later by progesterone, with "rapid and extensive hyperplasia of the alveoli which occurs during the first half of pregnancy. During pregnancy or pseudopregnancy with the presence of active corpora lutea in the ovary, rapid lobule hyperplasia always follows" (42).

Turner and Frank (32) believed that estrogen must be present in order for progesterone to act upon the mammary gland (43). It has been shown that estrogens (Allen, 44) and progesterone (Hisaw, Greep and Fevold)(45) will restore the cervical epithelium in castrated monkeys.

The biochemical reactions in the body under the influence of progesterone are characteristic. Its role in metabolism and its excretion have also been studied. Probably the best known excretion product of progesterone is pregnanediol, established by Butenandt, 1930. While quantitative precision in extracting this substance from urine is extremely difficult, its qualitative presence has been widely used in tests to prove the presence of pregnancy and the pregnanediol test is of prognostic value in threatened abortions (46).

In general, progesterone is thought to be carried in the blood as a "protein bound substance". In the liver and in tissue pools it probably becomes glucuronide bound and from thence is excreted in the bile, or may reach urine via the bloodstream. More recent

information was acquired when M. Edward Davis et al. (47) administered radioactive tracer doses of progesterone to patients who were to receive therapeutic abortions. Repeating Bloch's experiment, they reaffirmed that progesterone may be synthesized from cholesterol in vivo. Further experimentation led them to believe that acetate→cholesterol→progesterone→pregnanolone→ pregnanediol represented a characteristic route of metabolism of progesterone in human pregnancy. They found evidence of local-synthesis of cholesterol in the corpus luteum, the fetal liver, adrenals, and the placenta. Intramuscular administration of C^{14}-progesterone resulted in surprisingly low concentrations of progesterone in the blood of pregnant women; possibly reflecting the rate of inactivation and disappearance from the blood of the secreted hormone.

Other biochemical reactions involved with progesterone have been utilized as measures of progestational activity. In 1955 Lutwak-Mann (48) established the hormonal control of carbonic anhydrase in the female reproductive tract with particular attention to progesterone influence of the levels found. She states that "Parallel assays were made of endometrial carbonic anhydrase activity and of progestational proliferation, both in intact and ovariectomized rabbits, following the administration of various amounts of progesterone, desoxycorticosterone acetate, and certain progesterone metabolites. In each instance where chemical estimation demonstrated an increase in endometrial carbonic anhydrase activity

this was associated with progestational proliferation." (49) Another recent paper (50) mentions that progesterone has been found to be a DOCA blocker in man. The fact that progesterone affects the deposition and metabolism of glycogen within cells has been mentioned earlier. In addition the hormone has a characteristic affect on the deposition of fat within the cell and in alkaline phosphatase.

In bovine-endometrium, Skjervøn (51-A) stated that alkaline phosphatase was distributed on four main uterine sites: the surface epithelium, the glandular epithelium, the stroma and the vascular system. He states that "the enzyme activity of the surface epithelium was limited to the distal cytoplasm as demonstrated by the formation of a black rim adjacent to the uterine lumen, whereas the basal cytoplasm and the nuclei were inactive. As a rule the superficial glands and especially the ducts showed the highest activity, whereas the deeper parts were scarcely active. The activity was the weakest during the estrus phase of the cycle... the strongest activity was observed 9 to 12 days after estrus... Biopsies taken in the luteal phase showed the same picture." McKay et al. (51-B) in a study of human endometrium stated that "alkaline phosphatase is still concentrated at the tips of the glandular epithelial cells early in the progestational phase... As this phase progresses two changes are noted in the activity of the enzyme. 1) It progressively diminishes in amount until at about day 22 it is no longer seen in the cytoplasm and 2) it shifts in location to the secretions of the gland lumina where

it is found in abundance during the middle portion of the progestational phase."

McKay (51-B) also noted that ribonucleoprotein diminishes in epithelial cytoplasm under progestational influence, acid phosphatase increases and glycoprotein "is deposited in increasing amounts during the progestational phase and is found almost exclusively in the secretions and at the luminal tips of the epithelial cells".

Lipid deposition in the uterus is influenced by hormonal changes essentially as follows: (51-A) "The most remarkable shifts of stainable fat globules were observed in the cytoplasm of the surface epithelium. The first fat drops appeared nine days after estrus, but the epithelium was already fairly deeply colored (by the stain) 6 days after estrus. Biopsies removed after day 9 in the cycle until one or two days before the next estrus nearly always contained fat globules of varying size and in varying numbers. They were usually not present throughout the epithelium, but fat-containing and fat free zones alternated. The drops were present sub-and supra-nuclearly, often forming radial stripes between nuclei. The larger drops were usually present supranuclearly. No definite cyclic variation in respect to the cytoplasmic arrangement of the crops were observed." The foregoing effects of the luteal phase and progesterone on the uterine endometrium have been part of the basis for measuring progestational effects, as will be seen later in the comparison of the newer progestational agents.

Other chemical and physiological changes produced by progesterone, to mention a few, include its ability to pause general protein catabolism and salt diuresis (51-C), and its androgenic effect (51-D). Landau et al (76) reported that: "The enhanced catabolism of protein appears, to be a direct or indirect effect on several protein tissues, not requiring the participation of other hormones or endocrine glands. The salt loss is probably explained by the competitive inhibition of adrenal salt retaining hormones at a renal level."

II.(B)TREATMENT USAGES OF PROGESTERONE

Based on the known effects of progesterone, the drug has been used therapeutically in many different entities, the only disadvantage being its intramuscular route of administration; It may be used orally in very high doses. Probably the earliest therapeutic use of progesterone was in attempts to prevent abortion. This idea has not been wholly accepted because of the concept that in many abortions the fetus is abnormal. However, in some instances it has been postulated that there may be a temporary lack of progesterone about the third month of gestation, when theoretically the corpus luteum is becoming less productive of progesterone while the placenta prepares to assume this role (52). The dosage regulation has been attempted by measurement of pregnanediol appearing in the urine, which should be in the neighborhood of 10-20 mg. daily. It has been claimed that at least 20 mg. per day should be given. If progesterone is used later in pregnancy, during premature labor, the dosage should be increased. Progesterone is an accepted means of treatment of functional uterine bleeding in young women. In 1942, Allen and Heckel (53) reported good results in treating this entity with intramuscular progesterone. The bleeding usually subsides during the administration of progesterone, but may be profuse on progesterone withdrawal, which essentially causes a "medical surrettage" and lasts for seven to eight days. Allen (54-A) claimed that in approximately one third of the cases treated the withdrawal bleeding

is followed by normal menstrual cycles. In another third, amenorrhea ensues for a long period of time, apparently until estrogenic hyperplasia recurs. In the remaining third (54-B) the withdrawal bleeding is followed soon by recurrent functional bleeding. Dosages necessary for the above were found to be from 5 to 10 mg. per day for six to ten days or 25 to 50 mg. as a single injection. The theory behind the use of progesterone for treating premenstrual tension has been known since before 1938, when Israel (55) proposed that the weight gain, edema, and irritability in this syndrome could possibly be due to a relative lack of progesterone, or, as some put it, "hyperestrogenism", in which there is too much "unopposed" estrogen. In seventy-eight cases of premenstrual syndrome treated with progesterone by Dr. Raymond Greene and Dr. Katherina Dalton between 1948 and 1952, 83% were relieved of symptoms (56). Because she felt there is a definite relationship between toxemia of pregnancy and "premenstrual syndrome", Dr. Dalton carried the idea one step further and treated all her cases of toxemia of pregnancy with progesterone if they had had previous symptoms of premenstrual tension. She states: "Progesterone therapy in toxemia was based on knowledge gained from its use in premenstrual syndrome Experience has shown that for successful use progesterone must be administered before premenstrual symptoms begin, usually from the 14th to 28th day, or throughout the cycle. ....It was found necessary (in pregnancy) to give progesterone in oil daily or on alternate days; larger doses did produce a longer

duration of effect. The scheme for progesterone therapy was formulated upon the recognition of the stage of minor symptoms preceding the development of toxemic signs, and the belief that the severity of minor symptoms is related to the degree of progesterone deficiency... Progesterone also brought speedy and complete relief to 34 cases of vomiting of early pregnancy. Ten cases of moderate and one case of severe toxemia have been treated on these principles, and all have gone successfully to full term without bed rest or surgical intervention and with the delivery of live babies." Dr. Dalton's recommendation, in toxemia and premenstrual syndrome are highly controversial.

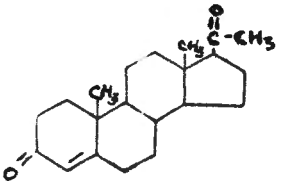
Progesterone has also been used in the treatment of endometriosis (Georgiana S. Jones)(58). Many investigators have also reported successfully using it in the treatment of secondary amenorrhea (59), dysmenorrhea (60), at menopause and in the aftermath of oophorectomy (61).

III. PROGESTATIONAL AGENTS: A REVIEW

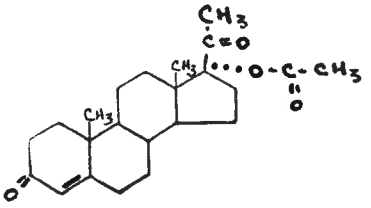
Within the past five years, many substances with "progestational activity" have been marketed. The idea was by no means new. While it has been shown that progesterone inhibits the menstrual cycle unless withdrawn, Hartman (62) found that a daily dose of testosterone also had a marked inhibitory effect on the menstrual cycle. Zuckerman, in 1937, (63) showed that the endometria of monkeys under such treatment became shallow, but bled after the testosterone was withdrawn. In the same year Klein and Parks (64) found that certain androgens would produce progesterone-like endometrial development in the uterus of rabbits. Green and Ivy and others found evidence that androgens would also postpone parturition in normal pregnancy, prevent abortion that follows castration, and interfere with the sexual differentiation of the newborn young. A selected number of representative progestational agents will be reviewed in this paper. Some of these, as will be shown, are actually testosterone derivatives. This is not surprising, since progesterone and testosterone have similar structures and effects. Freed (65), among others, reported the successful use of androgen for treatment of premenstrual distress and opened the way for the wider use of androgens and their derivatives.

Progesterone's effects, uses and implications have already been discussed and will be used as a basis for comparison of the progestational agents.

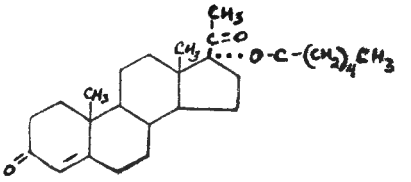
TABLE I.



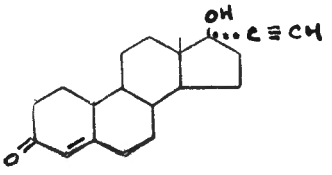
Progesterone



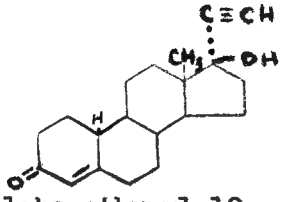
17-alpha-hydroxy-progesterone acetate
"acetoxyprogesterone"
(Prodox)



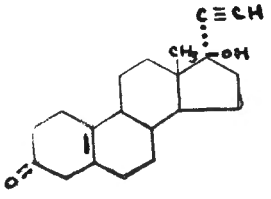
17-alpha-hydroxy-progesterone caproate
(Delalutin)



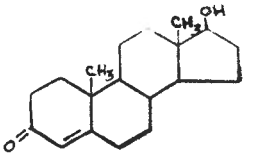
17-alpha-ethynyltestosterone
"anhydroxyprogesterone"
(Ethisterone)
(Pranone)



17-alpha-ethynyl-19-nor-testosterone
"norethindrone"
(Norlutin)

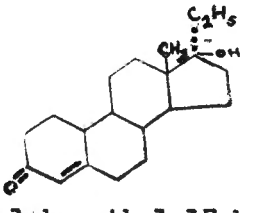


17-alpha-ethynyl-17-hydroxy-5-(10)-estren-3-one
"norethynodrel"
(Part of Enovid)



Testosterone

"



17-alpha-ethyl-17-beta-hydroxy-norandrosterone
"norethandrolone"
(Nilevar)

These willbe discussed in the following order:

(A) 17-alpha-hydroxyprogesterone acetate
(B) 17-alpha-hydroxyprogesterone caproate
(C) 17-alpha-ethynyl-testosterone
(D) 17-alpha-ethynyl-19-nor-testosterone
(E) 17-alpha-ethyl-17-beta-hydroxy-19-norandrostene-3-one
(F) 17-alpha-ethyl-beta-hydroxy-19-norandrosterone
(G) testosterone and other androgens

The very names of these agents suggest the ways in which they were structurally modified to become biologically intensified. Hydorxylation at the 17 position and subsequent esterification may increase the potency of progestational steroids. Other changes which are effective in increasing potency are removal of the angular 19-carbon methyl group or introduction of a double bond between the 11-12 carbon atoms.

Hydroxylation of progesterone in the 17 position was found to increase the biological activity of several compounds tested by Zarrow et al. in 1957 (67). In addition they showed that 17-alpha-hydroxy-progesterone is sixty times more potent than progesterone in the mouse. The study was extended to the human being by Salhanick et al. in 1957 and it was found that this same compound is inactive in the human. However, Junkmann (68) found that the free compound was also inactive in the rabbit. Junkmann discovered, and Davis and Weid (69) confirmed, however, that by using the esterified compound (17-alpha-hydroxyprogesterone caproate) it was active in the human and probably had "prolonged profestational activity." This esterified compound is also active in the rabbit (68).

These findings were confirmed by Salhanick et al. in 1957 (70) who also commented: "The biological difference between the free alcohol and the caproate ester is difficult to explain... A point of significance may be the different processes or rates of degradation which the two forms undergo. Thus, Langecker found in the male that the free alcohol of 17-alpha-hydroxyprogesterone was excreted as pregnane-3,17,20-triol and pregnane-3,17-diol-20-one but found no change in the excretion of pregnane-3,20-diol or 17-ketosteroids. On the other hand, the caproate ester administered to a man could not be recovered from the urine in amounts greater than 1 per cent. Administration of the caproate to a pregnant woman resulted in an increased excretion of pregnane-3,20-diol." It was stated by both David and Weid and by Salhanick et al. that the metabolism and mechanism of action o these substances remains obscure. The caproate ester of progesterone is produced by E. R. Squibb & Sons as 17-alpha-hydroxyprogesterone-17-n-caproate (Delalutin); The Upjohn Company produced the 17-alpha-hydroxyprogesterone acetate (Prodox) and a similar and as yet unpublished compound, U-8839 (See Table I). The latter is at present under clinical trial. Following their production these compounds were tested by many investigators. The acetate ester was tested by Davis and Wied (71). These workers concluded that "17-alpha-hydroxyprogesterone acetate is an orally effective progestational substance, since it a) induces secretory changes in the endometrium, b) induces luteal changes in exfoliated vaginal epithelial cells, c) reduces fern-like patterns

of the cervical mucus, d) causes a rise in basal body temperature, and e) induces withdrawal bleeding in castrates. The study was performed on three surgically castrated women." It may be noted by the reader that the size of this series was relatively small and did not include many of the type of subjects in which the clinician will desire to get an adequate progestational effect. These investigators also admitted that "endometrial biopsies showed minimal or mild secretory changes after daily administration of 25 or 50 mg. of "acetoxyprogesterone" acetate daily over a period of fifteen days." With larger doses more definite secretory changes were noted, but these apparently did not equal the effect of parenteral progesterone. In addition the authors stated that when compared with progesterone itself, "the activity per milligram is much less." This esterified progesterone derivative, however, was thought to be much more active than pregneninolone (anhydroxyprogesterone). In a series of 16 patients, Goldzieher (72) reported that 17-alpha-hydroxyprogesterone acetate had about the same potency on a weight basis as ethisterone and that it caused increased amounts of glycogen deposition in the endometrium. In the experience of several investigators, this substance is not truly progestational in that "breakthrough bleeding" occurs and the normal menstrual cycle is not actually prolonged.

Byrnes and Johnston (72-A) found that acetoxyprogesterone has more than twice the activity of ethinyltestosterone in the rabbit by the Corner-Allen method of bioassay. In addition they showed

evidence to prove that it has very little estrogenic (uterotrophic) activity on the uteri of ovariectomized female rats. In an assay designed to test the androgen effect of 17-alpha-hydroxyprogesterone they found that it had less tendency to increase the weight of seminal vesicles or prostate than did progesterone itself. (This experiment was performed on immature male rats.) In a three week study on toxicity in rats the authors conclude that no symptoms due to toxicity can be produced from high dosages of acetoxyprogesterone. They did note, however, that high dosages produced an increase in ovarian weight, thymus and adrenal glands.

A related compound, 17-alpha-hydroxyprogesterone caproate (known as Delalutin [E. R. Squibb & Sons]) has also been investigated (Table I). This drug is given by injection and dissolved in sesame oil with 30% benzyl benzoate. Its actions are much the same as the acetate ester previously discussed. Much of the material published about its activity has been composed of statements actually regarding its effect in rabbits or other animals. Kessler and Borman (73) reported that it was effective for about 21 days after administration to rabbits, whereas progesterone is effective only for about 8 days. They also stated that it shows no thymolytic, anti-thyroidal or glucocorticoid activity and that it is <u>not</u> anti-uterotrophic in immature female mice. It is apparently potentiated by estrogens. Velardo (74) reported that this esterified progesterone derivative is superior to progesterone in preventing abortion in rats. Smithberg, (75) on the other hand, reported that it is

"capable of inducing implantation but it is not capable of maintaining pregnancy in prepuberal mice." Landau (76) reported that no new progestational agent was able to "duplicate the metabolic effects of progesterone." He states that 17-alpha-hydroxyprogesterone caproate is catabolic, but has no apparent influence on sodium excretion as does progesterone. Whitelaw found that sixteen of seventeen patients treated for infertility problems showed excellent response with endometrium "varying from day 22 to late secretory." Follow-up showed that after therapy, five patients' cycles "were anovulatory and prolonged while two had irregular cycles." He also states that "basal body temperature failed to mirror the endometrial response. Administration of 17-alpha-hydroxyprogesterone caproate preovulatory causes a marked disturbance in the menstrual cycle, usually characterized by a long anovulatory phase." Anna L. Southam (77) reported a comparative trial of 17-alpha-hydroxyprogesterone caproate, 17-ethinyl-estraeneolone and 19-nor-ethinyl-testosterone. She stated thirty-two patients were given one of these drugs to produce artificial cycles in the face of "functional amenorrhea". One established cyclic menstruation following therapy. Only one other patient established continuing cyclic ovulatory menses. Four pregnancies occurred in 25 patients treated for infertility problems. This investigator did find that "bleeding was significantly decreased" in 24 patients with recurrent anovulatory bleeding when given the caproate ester. Gold and Cohen (78) reported the successful use of this compound in treating

amenorrhea, but that cyclical bleeding failed to follow discontinuance of its use. They went on to state that it caused satisfactory "medical surettage" in functional uterine bleeding and produced a decidual cast in three patients. It was found to be ineffective in suppressing postpartum breast engorgement and lactation. From the above discussion, one can conclude that much remains to be learned about the actual efficacy of this esterified hydroxy-progesterone substance.

In a review of the effects of 17-alpha-hydroxyprogesterone caproate, Reifenstein points out that the evidence "indicates that the duration of action of a progestational agent is influenced by the amount of estrogen that is present during the time that the progestational agent is exerting its action. Thus, Boschann, in 4 castrated women treated with the short acting estradiol benzoate induced menstruation-like bleeding between 7 and 11 days after the second of 2 single injections of 125 mg. of 17-alpha-hydroxy-progesterone caproate when the second injection of the progestational compound and the last injection of the estradiol benzoate were given simultaneously. In contrast, Davis and Wied found that when they employed the longer acting estradiol valerate as preliminary and simultaneous therapy, the progestational effect of 17-alpha-hydroxyprogesterone caproate was prolonged to between 14 and 19 days. Even the duration of action of free progesterone is affected by the duration of the estrogenic activity that is administered with it." Note that with the simultaneous injection of both estrogen and

progestational substances, the bleeding may have been due to estrogen withdrawal. This possibility also exists in other data frequently presented as evidence of progestational withdrawal bleeding. It has already been mentioned that testosterone itself has long been known to have certain progestational effects. By changing the structure slightly it becomes 17-alpha-ethynyl-testosterone, also called "anhydroxyprogesterone." It has been marketed as "ethisterone". (See Table I). Feiser and Feiser (80) make the following statement about it: "One of the most interesting related compounds [to progesterone] is the 17-alpha derivative of testosterone; the name 17-isopregneninolone is sometimes used for this substance in order to emphasize the fact that it possesses significant luteoid, but not testoid, activity... The ethynyl group in the chief product of addition has the alpha-orientation, which is the opposite to that of the two-carbon chain of the cortical hormones. Although alpha-ethynyl-testosterone has only about one-third the activity of progesterone by subcutaneous injection, it is more active than progesterone on oral administration (4 mg. active; progesterone inactive at 60 mg. orally). The substance was introduced into clinical medicine under the name Proluton C in 1941. Reduction of the ethynyl group to the vinyl decreases the activity somewhat."

Ethisterone will produce withdrawal bleeding in five days following the cessation of multiple dose administration, and has the advantage of being orally administered. This compound was also

found to have androgenic effects by Emmens and Parkes in 1939 (81). Greene and Dalton in 1953 found ethisterone effective in only 47% of cases treated for premenstrual syndrome, whereas progesterone was 80% effective. These investigators used deep intramuscular injections of progesterone, usually 25 mg. every other day. Ethisterone was administered orally, beginning with doses of 30-75 mg. daily and increasing the dosage until symptoms began to disappear. Therapy was started the day before the anticipated onset of symptoms. Side effects of ethisterone were noted to be nausea and vertigo. Effects on the endometrium, as might be expected, are not entirely "progestational" and increased stroma with little glandular secretory activity have been noted following ethisterone therapy. According to Zarrow et al. (81-A) 17-alpha-ethynyl-testosterone gives a "++" effect as compared to a reaction of "++++" for progesterone (in relatively large doses).

Ferin (83) in France studied 17-methyl-19-nor-testosterone in women and found it to have progestational effect. The effect of making the preceding compound 19-Nor was to increase all the previously known potency of action. 17-alpha-ethynyl-19-nor-testosterone (norethindrone) has been marketed under the name of Norlutin by Parke-Davis & Co. It has been found to be five times as effective as ethisterone (84) as a progestational agent. Its side effects include spotting before the anticipated onset of menstruation, lethargy, and nausea. Ten to twenty mg. is given starting on the fifth day of the cycle and ending on the twenty-third

in order for five-day withdrawal bleeding to occur.

Pincus, Zarrow et al. (85) in a comparative study of several 19-Nor compounds reported that this compound (using the Clauberg test) gives a four-plus effectiveness at dosages of 0.2 mg. to 5.0 mg. as compared to progesterone which gives a one-plus effect at 0.2 mg. and a four-plus effect at 2.0 mg. Norethindrone was also found to be ineffective as compared to progesterone in maintaining implantation in ovariectomized rabbits carrying fertilized eggs; the authors conclude that its efficiency does not parallel the apparent results of the Clauberg test. Later, they brought out that this compound was also ineffective in decidual activity on the rat. Two samples of this drug were tested for estrogenic activity, one being "highly purified," and this showed less estrogenic effect. Both samples were found to be "considerably less active than the estrone standard". Relatively small doses of this drug were found to be potent inhibitors of ovulation in the rabbit, both orally and intramuscularly. The authors conclude by stating that 17-alpha-ethinyl-19-nortestosterone has "previously been reported as an active progestin by conventional assay in rabbits and in women. On closer scrutiny of the array of tests which we have employed, characterization becomes rather difficult... Thus, this compound, which is 10 times as active as progesterone by the Clauberg assay is clearly much less active as a deciduomagenic agent."

Another 19-Nor progestational steroid is included in a product

marketed under the name of Enovid (Searle). Its active progestational agent, norethynodrel, is combined with 17-ethynylestradiol-3-methyl ether, an estrogenic agent. This fact must be born in mind in evaluating the effectiveness of this compound as a progestational substance, for most clinical data are evaluated in the light of incomplete effects. Norethynodrel is 17-alpha-ethynyl-17-hydroxy-5-(10)-estren-3-one. It is partially soluble in water, freely soluble in alcohol and readily absorbed from the intestinal tract. It has previously been mentioned that estrogen will enhance the effect of progestational agents and so the estrogenic compound has been purposefully retained in this agent. Drill and Saunders (86) state that "Norethynodrel has three main properties: First, it has a progestational action when administered orally; second, it has inherent estrogenic effects and third, it inhibits the pituitary gland." These authors found that by the McGinty bioassay technique (injecting the test material into the uterine horn of the estrogen-primed ovariectomized rabbit) there was an almost complete lack of response to 100 ug. of norethynodrel as compared to the excellent response in the control rabbit which received 0.2 ug. of progesterone. Photomicrographs reveal many branched glands in the latter case, while in the former, the glandular appearance is much like that of the estrogen control alone. Using the Clauberg technique, the authors state that "an excellent result was obtained." However, close scrutiny reveals that while at one mg. dosages, progesterone gave a 1 + response and norethynodrel gave a 3.3+ response, when

the dosage was increased to 10 mg., progesterone gave a 2.5+ response and norethynodrel was only slightly higher, giving a 3.0+ response. The above responses followed oral administration. By subcutaneous injection, the Clauberg technique revealed that norethynodrel produced "only minimal" progestational effects.

Pincus, Rock and Garcia reported that Enovid (in humans) inhibited ovulation at daily doses of from 5 to 20 mg. At the 5 mg. level, however, there was "breakthrough bleeding" and with this dosage, although menses was typical, in terms of duration and quantity, it was unsatisfactory as to the time of its occurrence. These disorders were rare at daily doses of 10 to 20 mg. and the menstruation closely simulated the accepted normal patterns. In these patients the incidence of ovulation was indirectly observed with the aid of basal body temperature graph recordings, endometrial biopsies and vaginal smears and pregnanediol excretion. Later, although the authors apparently found it necessary to use dosages of 10 to 20 mg., they state that "The duration of flow was about the same but with dosages of 10 and 20 mg. the patients complained of scantiness of the amount of flow." The authors found Enovid very effective in preventing ovulation and in Puerto Rico, this characteristic was put to use as an oral contraceptive agent (88). Kupperman and Epstein (89) found that dosages of 5 mg. of Enovid produced increased size of seminal vesicle, prostate and levator ani (anabolic) in parabiotic rats. Roland (90), in using Enovid to

treat patients with anovulatory cycles and amenorrhea, observed nausea and breast engorgment in the patients treated. One patient out of ten with anovulatory cycles conceived after therapy with Enovid. Three patients with amenorrhea had withdrawal bleeding following cessation of medication. The basal temperature charts showed biphasic curves with Enovid therapy. The thermal shift occurred within 24 hours of the initiation of therapy when the dosage was 20 mg. daily; it occurred 10 days later when the dosage was 10 mg. The author states that "Four different components of the endometrium were observed individually. This was done because it was often difficult to say whether or not a particular endometrium was in secretory or a proliferative phase. Of all the specimens of endometria [sic] which were studied there was rarely one that could be interpreted as secretory, that is, involving all four components, namely: the glands, epithelium, blood vessels and stroma. The over-all impression was that the glands and blood vessels were arrested in the proliferative phase while the epithelial cells and the stroma showed progestational activity following Enovid. The duration of therapy did not matter as much as the amount of daily dosage." Later he states, "The endometrium which is produced as a result of Enovid therapy cannot be called secretory by conventional standards since not all the components show progestational changes. Whether or not it is absolutely necessary for nidation that all components show good progestational activity

requires further investigation." Kupperman, in discussing the above presentation, made the observation that he had "noted that when Enovid alone was administered to patients with ovarian failure, in other words to those patients lacking endogenous estrogen activity, we were never successful in inducing withdrawal bleeding." This is an interesting contrast to the statement later made by Dr. Gold (91) that one patient on her first course of therapy for secondary amenorrhea showed "excellent" estrogenic effect on a vaginal smear taken five days after the initiation of 30 mg. of Enovid daily. Nelson (92) observed that there is "lack of evidence that Enovid will maintain pregnancy. Apparently this point has not been examined in women, although Drill proved that it failed to do so in ovariectomized animals. However it is apparent that Enovid does not produce the same effect in humans as in animals."

Pincus (93) tried Enovid on a series of 16 psychotic men. He observed: lower outputs of 17-ketosteroids, inhibition of adrenal cortical secretion (presumably by the inhibition of pituitary ACTH) and absent spermatogenesis, also severe maturation acrests, soft, small testis and flat atrophic prostate gland development. He concluded that "the sterilizing" effect on men is "clearly indicated" by data collected.

Norethandrolone (Nilevar, Searle) is still another agent with reported progestational effects. Its chemical name is 17-alpha-ethyl-17-beta-hydroxy-19-norandrosten-3-one. It is known for its

anabolic activity in promoting nitrogen retention. However, it causes notable progestational effects. In 1957 Epstein and Kupperman (89) showed that two out of six patients treated with this substance developed a few secretory changes in the endometrium after treatment for primary amenorrhea. Ten out of seventeen patients with secondary amenorrhea developed "secretory" endometrium and three had a biphasic basal temperature curve. Seven out of seventeen patients with anovulatory cycles responded "well", three again showing definite biphasic thermal shifts. Heller found that this substance is "not a potent gonadotrophin inhibiting agent in the human being". Despite the biphasic temperature rise, this substance has shown itself uniquely different from progesterone in two respects: A) Therapy is associated with no increase in pregnanediol excretion, and B). The endometrium examined on endometrial biopsies shows a predominance of stromal hyperplasia with decidua-like changes without a commensurate glandular response.

In addition to the effects of testosterone already mentioned, it has been shown that shrinkage in weight of the rat uterus which usually accompanies ovariectomy is prevented by daily doses of testosterone propionate. The mucosa becomes "greatly thickened and develops lace-like foldings resembling the endometrium of pregnancy or that seen after treatment with estrogen plus progesterone" (94). Mazer and Mazer (95) state that the daily administration of 2 mg. to spayed rats after estrogen priming produces well marked progestational changes within two weeks.

It has been pointed out that testosterone differs from progesterone in that larger doses are required and true deciduomata is not produced in the laboratory animal. It has also been shown that concomitant administration with estrogen produces "cystic hyperplasia" of the endometrial glands (96).

Similar results have been shown in the primate, Senile women, with proper "estrogen priming" have been made to have cyclic uterine bleeding with intermittent doses of testosterone (97). Endometrial biopsies reveal slight secretory endometrial changes.

In their book on Androgens (94), Dorfman and Shipley state that "Androgen has been shown to affect uterine motility. When testosterone is administered to spayed rabbits primed with estrogen the normal contractile response of the myometrium to oxytocin is inhibited. This, once again, is a progesterone-like action."

Perhaps the undesirable side effects of androgens, other than masculinization, should be mentioned in passing. These include a tendency to cause sodium chloride retention and thus precipitate edema. Jaundice has also been reported as a side effect.

DISCUSSION

It is apparent from the above discussion that several problems will be encountered by those interested in new steroid compounds. Perhaps most important at this time is a re-evaluation of the semantics involved. A progestational agent need not be one which necessarily reflects <u>all</u> the activity of progesterone. Testosterone

itself, as has been shown, exhibits many of the effects of progesterone, yet has heretofore be undefined as a progestational substance. It would seem that the term "progestational" may be redefined in the future as applying to a substance which exhibits some of the desirable effects of progesterone. In so defining the substance, however, one must not forget its possible concommitant androgenic effects, and in several instances, because the new progestins are not pure, their estrogenic activity must also be taken into consideration.

Thus clinically, the effects of one of the new progestins may be more useful and/or practical than either progesterone or estrogen in some instances, and contraindicated in others. For example, long-term therapy in high dosages by some of the new agents could result in permanent androgenic side effects. Since the definition of progestational substance "apparently extends all the way to testosterone itself, the physician must consider these new progestins as compounds with "certain progesterone-like effects." A careful analysis as to which effects are dominant must ensue. It is hoped that some of the newer progestins may obliviate the inconvenience of parenteral administration and produce useful progesterone-like activity in vivo without exhibiting androgenic and other undesirable concommitant effects.

TABLE II. EFFECTS OF PROGESTATIONAL AGENTS COMPARED

	Progesterone	Acetoxy-Progesterone U-8839	Delalutin	Ethisterone	Norlutin	Enovid	Nilevar	Methyl Testosterone
I. Uterotrophic	slight	slight	slight	+ -	?	+	+	?
II. Endometrium								
A. Histological								
1) glands	+	modified	modified	-	+ -	Arrested	Arrested	Arrested
2) stroma	+	+ -	+ -	+	+	+	+ weak	thin
3) decidua	+	+ -	+ -	-	+	+	+ weak	+ -
4) epithelium	+	+ -	+ -	+ -	+ -	+ -	+ -	+ -
II. Systemic								
A. Thermogenic	+	+	+	-	+	+	+	+ -
B. Electrolyte (salt losing)	+	-	-	-	-	-	-	-
IV. Pituitary Inhib.	+ $\bar{c}$ estrogen	Possib.	+ $\bar{c}$ estrogen	?	+ $\bar{c}$ estrogen	+ $\bar{c}$ estrogen	+ $\bar{c}$ estrogen	+
V. Ovulation	- $\bar{c}$ estrogen	- $\bar{c}$ estrogen	inhibits $\bar{c}$ estrogen	-	inhibits $\bar{c}$ estrogen	inhibits	+ $\bar{c}$ estrogen	+ inhibits
VI. Maintenance of Pregnancy	+	-	-	-	-	-	+	+
VII. Withdrawal bleeding	+	+ 3 days	+ 2 weeks	5 + days	+	+	+	+
VIII. Maintenance of menstrual cycle	+	+ -	+ -	-	+ inhibits	+	+	+
IX. Breakthrough bleeding	-	+ -	+ -	+ -	+ -	+ -	+ -	+ -
X. Vagina	+	+	+	+	+ -	estrogenic	-	+
XI. Breasts	+	+ -	+ -	-	-	+ -	-	-
XII. Androgenic								
A. Myotrophic	+ -	+ -	+ -	+	+ -	+ -	+	+
B. Hirsutism	+ -	-	+ -	+ -	+ -	-	+ -	+
C. Prostate	+	+ -	+	+	+	+	+	+
D. Seminal vesicles	+ -	-	?	+	?	+	+	+
XIII. Estrogenic	± -	-	-	± -	± -	+	± -	-

IV. RESULTS OF CLINICAL TRIAL OF PROGESTERONE AND PROGESTATIONAL AGENTS

A) Selection of Patients:

Group No. 1:

These patients were those who presented themselves with complaints of secondary amenorrhea. They were thoroughly examined and discovered to have no other contributing pathology. Pelvic examinations and Papanicolaou-smears were negative. These patients were all in the reproductive age group, between the ages of 17 and 40. Certain of them had developed amenorrhea secondary to pregnancy.

This group of patients was treated with various doses of 17-alpha-hydroxy-progesterone acetate (Prodox) or U-8839 (an experimental progestational agent) and/or progesterone intramuscularly for control purposes.

In certain cases endometrial biopsies were performed to demonstrate adequate estrogen effect and that endogenous progesterone levels were not manifestly produced prior to treatment. Patients who were pregnant did not receive biopsies, but it was possible to observe whether or not pregnancy or miscarriage resulted from the therapy instituted.

Group No. 2:

All female patients in the reproductive age group and female patients under the age of 60 years who were hospitalized

at Hastings State Hospital, Hastings, Nebraska filled out forms giving information as to age, parity, menstrual cycles and cooperation. The medical histories of these patients were also reviewed and previous surgery, treatment and pelvic examination reports were noted. Patients were excluded from the clinical trial group on the basis of pelvic pathology or other severe systemic pathology, severe psychiatric problems, inability to cooperate, intact hymenal rings and age group (i.e. patients over 60 were not included because of the probability of uterine and endometrial atrophy). On examination, some patients were found to have stenotic cervices, adhesions within the vagina, or other conditions which made endometrial biopsies painful, impossible or undesirable. These were also excluded from clinical trial. Papanicolaou-smears were proven negative on all patients.

The premenopausal patients were given progesterone or progestational agents in various doses and modes in an attempt to prolong the luteal phase of their cycles.

The post-menopausal patients were first "estrogen primed" and after microscopic evidence of adequate endometrial proliferation was observed, they were given various forms of progesterone and progestational agents by various route, along with the estrogen. These patients were followed by Papanicolaou-smears and indometrial biopsies, and treatment was stopped whenever contra-indications arose.

Group No. 3:

These patients presented themselves to the University of Nebraska College of Medicine Clinics or to various associated doctors with complaints supposedly amenable to treatment with progestins. These were treated in various ways, as shown in Table VI. All patients were carefully screened for other contributing pelvic pathology. Four patients (N-1, N-2, N-3, N-4) complained of "pre-menstrual tension" in association with regular normal periods. They were given two therapeutic drugs, numbered 81-C and 81-B. One of these was Prodox (17-alpha-hydroxy-progesterone acetate) and one was a placebo tablet, which looked and tasted exactly the same as Prodox itself. They were instructed to take one tablet daily of 81-C beginning 5 days prior to expected menses and to cease medications wherever bleeding occurred. The next month they were instructed to do the same with 81-B, and so on, alternating placebo tablets and Prodox from month to month without knowing which tablet actually contained medication. Other patients in group No. 3 are still on trial therapy and biopsies of endometrial tissue are to be taken and evaluated at a later date.

B) Therapeutic trial drugs and methods of treatment:

The following pharmaceutical agents were employed:

1) Progesterone

   a) solution for intramuscular injection

   b) suppository form for vaginal application

      (AY-5303) Ayerst Laboratories Inc.

c) tablets for oral administration

Progesterone 5 mg. (compressed tablets) Upjohn & Co.

2) Estrogen

a) Diethylstilbestrol perles for oral administration

Upjohn Company

3) 17-alpha-hydroxy-progesterone acetate (Prodox)

a) tablets for oral administration

4) 17-alpha-hydroxy-progesterone caproate (Delalutin)

a) solution for intramuscular injection

5) norethynodrel plus estrogen (Enovid)

6) norethindrone (Norlutin)

Patients and/or nurses in charge of treating patients were carefully instructed as to methods of administration of the drugs. For instance, the progesterone for intramuscular administration was injected deeply into the muscle. The vaginal suppositories were supposedly inserted deep into the vagina with a vaginal applicator upon retiring. A careful record was kept of administration of oral compounds, injections and suppository administration.

C) Method of Biopsy:

The patients were placed in lithotomy position. After the cervix was visualized (using a speculum), a biopsy was taken of endometrial tissue using a Novak or a Randall suction curette.

The tissues were immediately fixed in solutions of formalin neutralized with calcium carbonate or in Elfman's fluid solution for phospholipid stain. All solutions were freshly prepared at the time of biopsy.

Tissue sections were prepared by routine histological techniques. They were stained with hematoxylin and eosin or with histochemical stains for phospholipids.

D. Results and Conclusions:

The patients in Group No. 1 (See table III) were given various dosages of Acetoxyprogesterone (Prodox-Table I) in an attempt to treat amenorrhea. It was noted that 50 mg. of oral therapy produced spotting only instead of true withdrawal bleeding, reflecting the low dosage level. A 75 mg. dose, on the other hand produced what appeared to be adequate withdrawal bleeding in four out of five instances. This may reflect the fact that these patients were producing hormones of their own and estrogen levels involved were not therefore known. Three cases of proven pregnancy received dosages of 100 mg. Prodox each, and in no case did withdrawal bleeding occurr. This probably indicates the high level of endogenous progesterone existing during pregnancy. It is also notable in that no untoward effects on the pregnancy were observed. This lends evidence that Prodox probably will not precipitate or instigate bleeding in the presence of adequate amounts of progesterone.

Doses of 100 mg. of Prodox produced withdrawal bleeding within 48 hours except in the case where ovarian failure had occurred; this is probably due to the absence of adequate "estrogen priming". This confirms the idea that acetoxyprogesterone as well as progesterone and others of the new progestins probably require prior estrogenic stimulation to be effective. No ill effects of acetoxyprogesterone were noted.

It would seen from these data that acetoxyprogesterone could be a useful agent in producing "medical curretage" simply and

TABLE III

Group No. 1

Test for Withdrawal Bleeding with Prodox

Patient	Diagnosis	Dose	Route	Result	Explanation
1	Secondary Amenorrhea	50 mg.	'O'	Spotting 48 hours	Partial Withdrawal bleeding (incomplete)
2	Anovulatory Bleeding	50 mg.	'O'	Spotting 48 hours	" "
3	Secondary Amennorrhea	75 mg.	'O'	Withdrawal bleeding 48 hours	
4	Secondary Amenorrhea	75 mg.	'O'	Spotting 48 hours	Incomplete Withdrawal bleeding
5	Anovulatory Bleeding	75 mg.	'O'	Withdrawal bleeding 48 hours	
6	" "	75 mg.	'O'	Withdrawal bleeding 48 hours	
7	Secondary Amenorrhea	75 mg.	'O'	Withdrawal bleeding 48 hours	
8	Pregnancy	100 mg.	'O'	No apparent effect.	Pregnancy - Withdrawal, endogensis progesterone
9	" "	100 mg.	'O'	" "	" "
10	" "	"	"	" "	" "
11	Postpartum Amenorrhea	"	IM	Withdrawal bleeding 48 hours	
12	Ovarian Failure	100 mg.	'O'	No withdrawal bleeding	Endometrium not estrogen primed (Ovarian failure)
13	Secondary Amenorrhea	"	"	Withdrawal bleeding 48 hours	

without difficulty. It can also be used to secure regular cyclic bleeding in "controlled cycles".

The patients in Group No. 2 received a variety of therapeutic agents and dosages with progesterone administered as a control (see tables IV and V).

(Table IV) In the premenopausal age group, an attempt was made to prolong the luteal phase of normal menstrual cycle by beginning progestational therapy on the 21st day. Progesterone itself prolonged the cycle beyond the 28th day in several instances, but breakthrough bleeding occurred in all cases with dosages lower than 100 mg. per day except one. From this it would seem that somewhere between 50 and 100 mg. progesterone per day is necessary to prolong the luteal phase. In one case (VS) where menses ceased for 2 months, a decidua-like stroma was noted. This confirms and extends the data of Bradbury.(40)

Vaginal progesterone was uniformly unsuccessful in prolonging the luteal phase for very long. This may reflect the difficult technique of administration. In order to be properly absorbed, the suppository must be placed far into the vagina, behind the cervical os. This was probably not attained in most instances. One case received Delalutin and has been prolonged past the 30th day (to date).

Attempts to prolong the luteal phase with acetoxyprogesterone were questionably successful. Breakthrough bleeding occurred in 2 out of 3 cases, although the cycle was somewhat prolonged in one case. One patient is receiving 300 mg. daily and to date

(28th day) no "breakthrough bleeding" has occurred. It will be of interest to note the final result in this case, as in laboratory animals it has been found that increasing the dosage beyond lower limits does not increase the potency of the drug.

U-8839 is an experimental progestational steroid, at present under clinical trial. It's structure is related to acetoxyprogesterone. Two patients received 10 mg. per day of this new steroid, beginning treatment on the twenty-first day of their cycles. Two patients received 30 mg. daily under the same program and in both no breakthrough bleeding has occurred to date, one being prolonged to the 46th day.

The patients in this premenopausal group are still under investigation. Control biopsies before treatment revealed normal endometrial changes in all cases. Since the luteal phase has not been prolonged in most cases, it has been impossible to get worthwhile biopsies for slide evaluation. Biopsy results were somewhat disappointing. One patient (CB) whose cycle was prolonged for two months on IM Progesterone grossly seemed to have heavy endometrial tissue, but biopsy sections revealed only blood clots. The other patient (VS) whose menses ceased on IM progesterone (100 mg. per day) developed good decidual tissue on microscopic study.

Since it is known that progesterone in large doses not only will prolong the luteal phase, but will go on to cause decidual formation, it is apparent that at the dosage levels used in this experiment, the new steroids do not necessarily imitate progesterone's luteal effect.

TABLE IV

PREMENOPAUSAL AGE GROUP

ATTEMPT TO PROLONG LUTEAL PHASE OF CYCLE
BEGINNING 21st DAY OF CYCLE

Substance:	Route:	Dose:	Patient	Age	Result:
Progesterone	IM	25 mg.	V.M.	39	"Breakthrough bleeding" 33rd day
	IM	50 mg.	C.B.	26	Menses ceased for two months.
	IM	50 mg.	V.G.	35	"Breakthrough bleeding" 28th day
	IM	50 mg.	L.G.	38	"Breakthrough bleeding" (scant) 44th day
	IM	100 mg.	V.S.	45	Menses ceased - two months
Progesterone	Vaginal	50 mg.	M.P.	34	"Breakthrough bleeding" 28th day
	Vaginal	250 mg.	C.Z.	27	"Breakthrough bleeding" 34th day (?)
	Vaginal	250 mg.	D.H.	29	"Breakthrough bleeding" 30th day
Delalutin	IM	2 cc. day	D.H.	29	Prolonged past 30th day
Prodox	"O"	50 mg.	C.Z.	27	Prolonged cycle to 44th day
		100 mg.	M.E.	37	"Breakthrough bleeding" 28th day
		300 mg.	V.M.	39	Prolonged past 28th day
U-8839	"O"	10 mg.	L.G.	38	"Breakthrough bleeding" 28th day
		10 mg.	M.E.	37	"Breakthrough bleeding" 28th day
		30 mg.	E.L.	38	Prolonged cycle to date(26th da
		30 mg.	M.P.	34	Prolonged to 46th day to present date

The patients in the postmenopausal age group (See table V) presented different problems. These patients were carefully selected in the age group where, although menses had ceased, the endometrium would still respond readily to hormonal stimulation. All of these patients received preliminary priming doses of 1 mg. of estrogen daily for at least 4 weeks before progestational agents were added to the regimen. In all except six cases (which will be discussed) the estrogen was continued concurrently with the progestin chosen. Control biopsies were taken prior to estrogen administration and after 4 weeks of estrogen therapy in order to determine whether adequate proliferative endometrium had developed. In 14 out of 15 cases, this dosage was sufficient to induce a proliferative change, as substantiated by microscopic studies.

Four patients were used as "estrogen controls". All of these developed proliferative changes in the first four weeks. Two received 1 mg. estrogen daily; two received 5 mg. per day. No progestational agent was used. At the end of 8 weeks none of these patients had shown any tendency toward "breakthrough bleeding". All had developed dense proliferative changes; one (on 1 mg. estrogen daily) showed "hyperplastic dense stroma" and one (on 5 mg. estrogen daily) developed atypical glandular changes.

By accident, six patients were discontinued from estrogen therapy on the same date that the progestin therapy was started. (See asterisks on table V)

Several of these patients showed "breakthrough bleeding" at about the time one would expect withdrawal bleeding from the cessation of the estrogen therapy. It is known, however, that progesterone itself in adequate doses (above 50 mg. daily) when administered immediately following discontinuance estrogen therapy, will prevent estrogen withdrawal bleeding. Other observations resulting from this accidental withdrawal of estrogen therapy are as follows: Patients receiving Progesterone (25 mg.) Intramuscularly, 180 mg. orally, and 250 mg. vaginally developed breakthrough bleeding approximately two weeks after progesterone therapy was begun. By this time estrogen had been restarted and they had received it for approximately 10 days along with the progestational agent. The patient receiving Prodox (50 mg. per day) showed "spotting" on the 7th day, about the same time one would have expected estrogen withdrawal bleeding. The patients receiving 50 mg. Progesterone IM daily and 100 mg. Prodox daily did not breakthrough even though estrogen was discontinued. By repeating the above experiment and using different progestins, possibly one could establish doses of the new progestational agents more nearly parallel to their progesterone equivalent than has been done previously.

Two patients taking intramuscular progesterone developed cervical polyps which had not been noted on previous examination. One of these had definite progestational changes on endometrial biopsy. One patient on Prodox and three patients on various doses of U-8839 have shown no breakthrough bleeding to date, biopsies will be taken later. These patients have received therapy for 21 days or more.

*The estrogen priming dose was accidentally discontinued for five days at the time that the progestin was begun.

TABLE V

POST-MENOPAUSAL AGE GROUP

A COMPARISON OF THE EFFECTS OF PROGESTINS ON THE ESTROGEN-PRIMED UTERUS.

Progestin:	Route:	Dose:	Estrogen Priming Dose:	Patient:	Age	Endometrial Effect:
None			1 mg. 8 weeks	B.H.	51	Proliferative Endometrium.
None			1 mg. 8 weeks	B.E.	53	Hyperplastic dense stroma.
None			5 mg. 8 weeks	J.D.	53	Atypical proliferative Endometrium
None			5 mg. 8 weeks	V.H.	53	Proliferative Endometrium.
Progesterone*	I.M.	25 mg.	1 mg.	M.Z.*	48	Breakthrough bleeding and polyp formation (cervical polyp).
Progesterone*	I.M.	50 mg.	1 mg.	E.H.*	52	Proliferative Endometrium with progestational shape of glands and polyp formation (cervical).
Progesterone*	Vaginal	250 mg.	1 mg.	D.A.*	41	Breakthrough bleeding after 14 days.
Progesterone*	Oral	180 mg.	1 mg.	F.M.*	50	Breakthrough bleeding on 13th day.
Prodox	Oral	25 mg.	1 mg. throughout	A.B.	56	No bleeding. Results to be determined.
Prodox*	Oral	50 mg.	1 mg.	V.P.*	46	Slight spotting on 7th day.
Prodox*	Oral	100 mg.	1 mg.	O.K.*	47	Atypical endometrium: Did not bleed through.
U-8839	Oral	5 mg.	1 mg. throughout	M.B.	57	No breakthrough bleeding to date. Results to be determined.
U-8839	Oral	10 mg.	1 mg. throughout	M.L.	58	No breakthrough bleeding to date. Results to be determined.
U-8839	Oral	30 mg.	1 mg. throughout	A.W.	47	No breakthrough bleeding to date. Results to be determined.

FIGURE I

Legend to Figure I:

(A) Patient V. S. (Table IV).

Endometrium on 8th day of normal cycle showing straight glands, normal stromal proliferative changes and non-secretory epithelium. (100x).

(B) Patient B. H. (Table V).

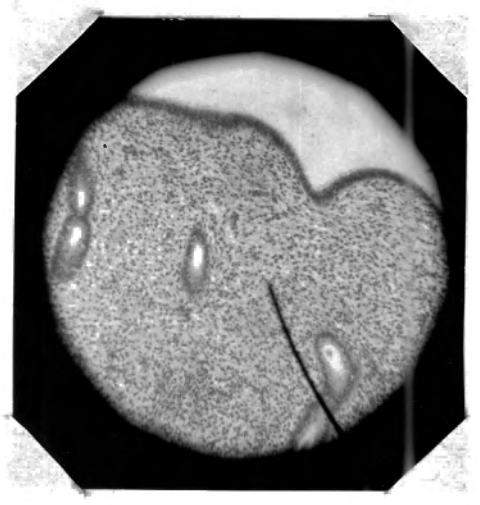
Postmenopausal patient's endometrium after 1 mg. of diethyl-stilbestrol therapy for 21 days. Note similarity to (A). (100x).

(C) and (D) Patient C. Z. (Table IV).

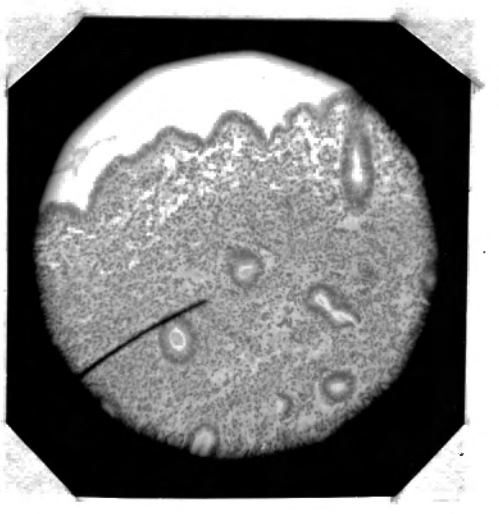
Atypical glandular change after 6 days of 50 mg. acetoxy-progesterone daily, started on the 20th day of cycle, which was prolonged to the 44th day. Note convuluted gland with minimal epithelial vacuolization and secretion. (430x).

FIGURE I

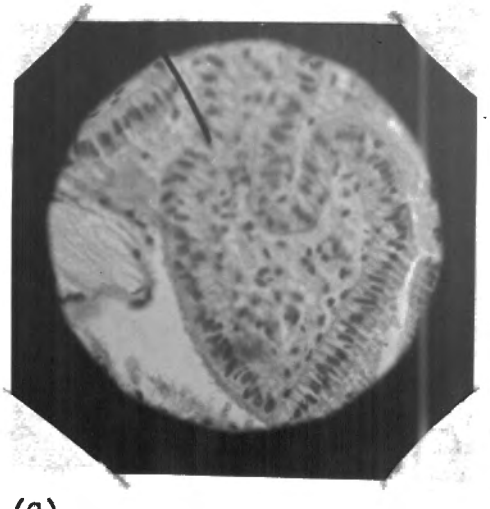
Endometrial Changes After Estrogen and Acetoxy-Progesterone



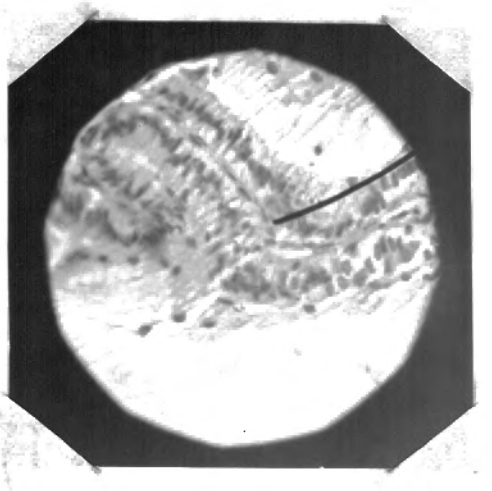
(A)



(B)



(C)



(D)

The patients in Group No. 3 are of even more diverse background, diagnostically speaking (Table VI).

Patient A, after 5 successive trials on 50 mg. IM progesterone, was given a trial on Prodox 50 mg. Withdrawal bleeding occurred in 4 days following progesterone therapy and within 48 hours following Prodox. Neither Prodox nor progesterone (50 mg. dosages) were effective in producing withdrawal bleeding when the patient had ovulatory cycles.

In two out of three trials, 10 mg. of U-8839 produced 4 day withdrawal bleeding, in effect more similar to progesterone than Prodox.

Enovid therapy in doses of 10 mg. (day 5 to day 23) resulted in normal menses, but regular cycles did not ensue.

Vaginal progesterone (250 mg.) resulted in satisfactory withdrawal bleeding.

One case of threatened abortion was treated with Norlutin and then Prodox; (Table VI). No data can be derived from this case.

Four patients with premenstrual tension were treated in a control study (Sub-Group No. 3, table VII). They received 25 mg. per day Prodox for five days prior to one period and a placebo tablet (17-alpha-ethynyl-testosterone, 2 mg.) for five days preceding the next period. The girls reported about the same amount of relief of symptoms with either of these medications. In no case was the regularly expected onset of menses nullified by the therapy. No conclusions were noted as regards premenstrual tension.

From the above data one can conclude that U-8839, as well as Prodox produce adequate withdrawal bleeding when administered properly. Small doses (25 mg.) of acetoxyprogesterone or of 17-alpha-ethynyl-testosterone premenstrually do not interfere with cyclic menstrual bleeding.

TABLE NO. VI
Group No. 3

University of Nebraska College of Medicine Patients

Progestin	Dose	Route	Patient	Diagnosis	Result
IM Progesterone	50 mg.	IM	A-Trial 1	Anovulatory Amenorrhea	Withdrawal bleeding 4 days
"	"	"	A-Trial 2	"	" "
"	"	"	A-Trial 3	"	" "
"	"	"	A-Trial 4	"	" "
"	"	"	A-Trial 5	"	No bleeding. Ovulatory cycle confirmed by basal temperature record
Prodox	50 mg.	'O'	A-Trial 6	Anovulatory Amenorrhea	Withdrawal bleeding 48 hours
"	"	'O'	A-Trial 7	"	No effect. Ovulated according to Temp. graph
U-8839	10 mg.	'O'	B-Trial 1	Amenorrhea, estrogen primed	Withdrawal bleeding 4 days
"	"	"	C-Trial 1	Postpartum Amenorrhea	" "
"	"	"	D-Trial 1	Secondary Amenorrhea	No withdrawal bleeding.
Enovid	10 mg. day 5 to 23	'O'	E-Trial 1	Irregular shedding	Normal menses. day 27 and 28, following periods-Irregular.
Norlutin	10 mg.	'O'	F-Trial 1	Threatened Abortion - spotting	Profuse bleeding
Prodox + thyroid - gr i + stilbestrol-gr i	12.5 mg.	'O'	F-Trial II	Threatened Abortion - Profuse bleeding	Bleeding ceased. Delivered 5 lb. 6 oz. baby by cesarean.
Vaginal Progesterone	200 mg.	IM	G-Trial 1	Myomata - Irreg. bleeding	Withdrawal bleeding - 24 hours

TABLE VII

Progestin	Patient	Dose	Route	Diagnosis	Result
Prodox	N-1 N-2 N-3 N-4	25 mg./ day 5 days prior to period	'O'	Premenstrual Tension	Bled at regularly expected onset of menses. Some relief of symptoms.
Placebo capsule (17-alpha-ethynl-testosterone)	N-1 N-2 N-3 N-4	"	'O'	"	"

DISCUSSION

E. Discussion. A review of our data and the results of others emphasize that the following, at least, should be considered in the evaluation of the new progestational steroids.

1) Side-effects. Particularly the "androgenic component" of the derivatives.

2) The need for and effects of estrogen in conjunction with their use.

3) The fact that species lines cannot always be crossed in testing these drugs; this applies especially to the primate.

For example, progesterone itself has certain androgenic effects, such as causing an increased incidence of pseudo-hermaphroditism in utero when administered in high dosage levels to pregnant mothers certain of the other substances (e.g. Nilevar) have potent nitrogen-retaining properties.

At least two of the new progestins are admittedly and purposefully contaminated with estrogenic substances. This is not undesirable, for estrogen may enhance the effects of progesterone. But the investigator must clearly distinguish between estrogenic effects (such as estrogen withdrawal bleeding and hyperplasia) and progestational effects when studying the new compounds.

In reviewing the literature on these new compounds, it becomes apparent that much of the proven effects of therapeutic trials on laboratory animals has not been repeated in the human. The fact that these compounds may give entirely different effects in the rat as compared to the rabbit has been mentioned. Widespread

clinical application should not be made on the basis of animal testing alone. Clinical trial is necessary before the true place of these compounds has been proven.

Certainly these new progestins have a place. With judicious use, as has been shown, they are safe and effective in producing medical curretage and aiding in establishing cyclic uterine bleeding. Whether their use extends further, to the prevention of abortion and maintenance of pregnancy has not yet been proved. The expected incidence of future abortion and of spontaneous reconciliation of the threatened abortion must not be forgotten, as the following table shows:

TABLE VIII

Recurrent Abortion: Effect of Treatment

On Incidence of Abortion in Percent.

Author:	Treatment:	Incidence Percent:	No. Patients:
Randall (98)	None		15
Speert (99)	None	21%	Not recorded.
Smith & Smith (100)	Hormone therapy	26%	81

It is of interest also to note that Guterman (101) found low pregnandiol levels in only 17% of patients with abortive problems. Other authors have found progesterone to be as low as 10% effective on incidence of recurrent abortion. Therefore, since progesterone itself has been disappointing as a treatment for abortion, it might be pertinent to question the effectiveness of progesterone substitutes.

In addition there has been much confusion as to interpretation of biologic effects on the endometrium. Many scientists feel that the effect of the new progestins is to produce a rather atypical glandular effect instead of the true secretion effect desired (Figure I)

According to Crosson (102), the most gratifying results obtained with the new progestins is in the treatment of menometrorrrhagia. Bleeding will decrease significantly twelve to twenty-four hours after the oral administration of an adequate dose of the progestin. Withdrawal bleeding usually occurs 2 to 5 days after the oral administration of the drug is stopped. These drugs (in larger dosages) may prove useful in prolonging the luteal phase and stimulating the "rebound phenumenon".

Already, new progestins not mentioned in this review are under clinical trial. Several of these, such as 17-alpha-1-methylallyl-19-nor-testosterone promise to be effective in 1/5 to 1/10 of the necessary dosages of nonethynodrel and norethindrone. Much remains to be ascertained particularly the metabolic pathways and mode of action.

SUMMARY:

1) Since the synthesis of orally effective progestational agents, a re-evaluation of their effects as well as the effect of progesterone itself seems necessary; terms such as "progestational" need to be re-defined.

2) Certain of the new progestins were tested in at least three areas.
   A) Prolongation of the luteal phase of the normal menstrual cycle was effected by some but not all of the substances.
   B) Withdrawal bleeding using the newer agents was proved practical and safe in at least 15 trial cases.
   C) The ability of the new progestins to produce tru and complete progestational effect on the estrogen primed endometrium is very doubtful thus far in this experiment.

3) Conclusion:

As well as re-defining the semantics involved in describing "progestational effects", the progestins themselves need still further investigation. Because small chemical alterations produce such specific changes in biologic effect, it is entirely possible that many different progestins will be in use for widely variant purposes. No one new oral progestin, however, is capable of completely reproducing the effects of the naturally occurring hormone, progesterone.

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