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REVIEW ARTICLE

Oral Contraceptives: Therapeutics *versus* Adverse Reactions, with an Outlook for the Future I*

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Keyphrases ☐ Oral contraceptives—mechanisms of action, adverse effects, review ☐ Contraceptives, oral—mechanisms of action, adverse effects, review ☐ Thromboembolic disorders—relationship to oral contraceptives, review ☐ Cancer and hyperplasia—relationship to oral contraceptives, review ☐ Hypertension—relationship to oral contraceptives, review ☐ Carbohydrate metabolism—effect of oral contraceptives, review ☐ Diabetes—relationship to oral contraceptives, review ☐ Lipid metabolism—effect of oral contraceptives, review ☐ Protein metabolism—effect of oral contraceptives, review ☐ Liver dysfunction and jaundice—relationship to oral contraceptives, review ☐ Amenorrhea and galactorrhea—relationship to oral contraceptives, review

Oral contraceptives have been in general use for only slightly more than a decade, but during this time a large volume of literature has accumulated concerning primarily the mechanisms of action and the adverse reactions of the estrogenic and progestagenic components of these drugs. In addition, numerous reports have been published concerning clinical studies in which these synthetic hormones have been employed for the purpose of contraception, but in dosage forms and on dosage schedules differing, sometimes markedly, from those of the commercially available oral contraceptives. The purposes of this review are to discuss and to summarize the more recent developments concerning these various aspects of hormonal contraception. However, since the mechanisms of action of the oral contraceptives involve modifications of the hormonal and hormone-related events occurring during the normal ovulatory menstrual cycle, such a review would be incomplete unless prefaced by a summary of these events.

GENERAL CONSIDERATIONS

Menstrual Cycle—The recently increased accumulation of knowledge concerning hypothalamic–pituitary–ovarian interrelationships in the human have been made possible as a result of the discovery of the hypothalamic-releasing hormones (factors) (1) and the development of competitive binding techniques (2) that permit the accurate measurement of the minute amounts of gonadotropins and gonadal steroid hormones present in as little as 1 ml. of serum.

A number of recent reviews (1–15) summarized the observations made in numerous studies since the late 1960's. Briefly, gonadotropin [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)] secretion by the anterior pituitary is under the control of the hypothalamus which secretes small polypeptides into the portal system connecting the median eminence of the hypothalamus with the anterior pituitary. These hormone-specific releasing factors (FSH-RF and LH-RF) [designated by some investigators as releasing hormones (FSH-RH and LH-RH) (1)] act on the pituitary to stimulate the release and, perhaps, also the synthesis of FSH and LH. Furthermore, evidence indicates that two hypothalamic centers may be responsible for LH release: a "tonic" center concerned with the regulation of basal LH secretion and a "clonic" ("cyclic") center responsible for triggering the ovulatory surge in LH secretion. The hypothalamus, in turn, is influenced by the estrogen and progesterone secreted by the ovary

* *Editor's note:* Part II of this article will appear in the March 1973 issue of the *Journal of Pharmaceutical Sciences*.

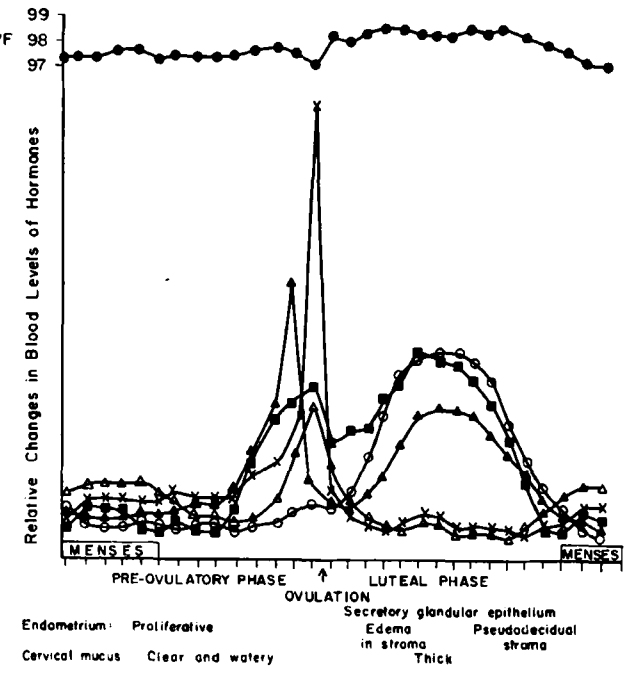


Figure 1—*An idealized composite of the hormonal and hormone-related events occurring during the normal menstrual cycle (2–15). Key: basal body temperature (●—●), FSH (△—△), LH (×—×), estrogen (▲—▲), 17α-hydroxyprogesterone (■—■), and progesterone (○—○).*

in response to gonadotropic hormone stimulation. Depending on conditions, their effects may be either inhibitory or facilitative; that is, they may exert a negative or positive feedback on the hypothalamus. In addition, the tropic hormones themselves may exert a negative feedback on the hypothalamus (3). Finally, emotional and external environmental factors, mediated by various areas of the CNS, can also influence the hypothalamic control of the menstrual cycle (induction of psychogenic amenorrhea, for example) (4).

The various hormonal and hormone-related events occurring during the normal menstrual cycle are depicted in Fig. 1. Since the units of measure vary for the individual hormones and since absolute concentrations vary among individual women, only relative, qualitative changes during the cycle have been indicated. Indeed, the possibility was suggested (5) that an occasional woman may be sufficiently stressed by daily blood sampling procedures that an adverse effect on hormonal events during the cycle may be produced; hence, the differences (*vide infra*) observed among various studies may or may not represent true physiological variability.

As shown in Fig. 1, toward the end of the luteal phase of the preceding cycle, concentrations of estrogen and progesterone decline; in response, the previously suppressed FSH and LH levels begin to increase, initiating another cycle of follicular maturation (6). Estrogen secretion increases markedly, culminating in a sharp peak followed by a drop prior to ovulation; then there is a subsequent smaller rise during the luteal phase.

The preovulatory rise in estrogen secretion exerts a negative feedback on the secretion of FSH which, therefore, declines. LH levels continue to rise, however, culminating in a sharp preovulatory surge of LH; following ovulation, LH levels decrease to values comparable to or less than those seen during the preovulatory phase. The cyclic (clonic, ovulatory) release of LH is thought to be due to a positive feedback effect of rising estrogen levels (7) and/or a negative feedback effect due to the dip in estrogen secretion that may precede the peak LH level (3).

The possible role that may be played by preovulatory progesterone secretion by the follicle and/or the adrenal (8) in the triggering of LH release in the human is a matter of controversy (9). 17α-Hydroxyprogesterone has been shown to increase prior to the LH peak, but this compound is not believed to be of physiological significance (10). The small periovulatory increase in progesterone secretion, on the other hand, appears to be a consequence of LH secretion rather than a stimulus for it, although the possibility that it may act centrally in a facilitatory manner to maintain and prolong the surge of LH has not been ruled out (7).

The low postovulatory secretion of LH appears to be responsible for stimulating the newly formed corpus luteum to continue secreting increased amounts of progesterone and estrogen. Nevertheless, despite continuous exogenous administration of LH, it has been shown that the secretory capacity of the corpus luteum declines (spontaneously or perhaps due to some unknown luteolytic factor) at 9–11 days after ovulation (7).

Most investigators also find a midcycle peak in FSH secretion, usually coincident with the LH peak (2, 3, 6, 7), although sometimes preceding (3) or following it (9). Its physiological significance is unknown, and the physiological stimulus responsible for it is merely speculative (7).

Concurrent with the preovulatory peak in estrogen secretion, a drop in basal body temperature is usually seen (3, 11); following ovulation, there is a rise in basal body temperature which parallels the rise in progesterone levels. Sometimes the rise in temperature is abrupt, as shown in Fig. 1, but at other times it is slow, requiring several days before preovulatory levels are exceeded. Results of a study (12) comparing these two types of temperature rise suggest that ovulation may not bear a similar temporal relationship to the low temperature point in these two conditions, *i.e.*, ovulation may occur later when there is a slow ("staircase") rise.

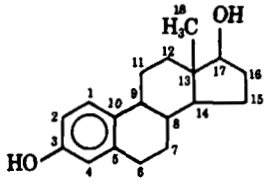
In addition to the negative and positive feedback effects that ovarian steroids have on the hypothalamus, they also produce cyclic changes in the endometrium and the cervical mucus. Specifically, prior to ovulation the endometrium is of a proliferative type due to the action of estrogen alone; following ovulation, progesterone and estrogen combine to produce secretory changes in the glandular epithelium and edema in the stromal tissue. Toward the latter portion of the cycle, pseudodecidual changes occur in the endometrial stroma (13). Very late in the cycle, as a result of the decline in estrogen and progesterone production, menstruation occurs; the endometrium responds to the withdrawal of the hormonal support by desquamating (14).

During the preovulatory phase and especially just prior to ovulation, the cervical mucus becomes increasingly more favorable, in physicochemical char-

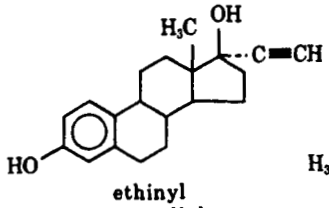
acteristics, to the migration of sperm (15). It forms a thin, clear, watery liquid which can be stretched into threads (spinnbarkeit). It is rich in sodium chloride, a decisive factor in determining consistency, and when dried on a slide, crystals of the salt form fern-like patterns (arborization); viscosity is minimal and cellular debris virtually absent. Finally, the micelles comprising the mucus are thought to be present in long chains arranged approximately in parallel order. In contrast, the mucus becomes thick under the influence of progesterone in the luteal phase. There is coiling of the mucoid molecules, and the micelles form a dense network containing much cellular debris; spinnbarkeit and arborization (ferning) disappear.

Chemistry—The chemical structures of the two estrogens and six progestagens present in the commercially available oral contraceptives are shown here. The structures of naturally occurring estradiol and testosterone, to which the synthetic compounds, respectively, are chemically related, are included for comparison.

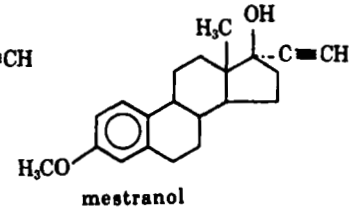
estrogens



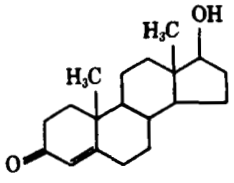
estradiol



ethinyl estradiol

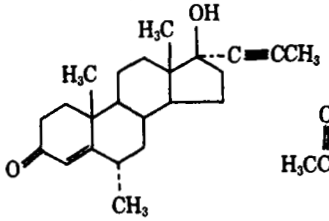


mestranol (ethinyl estradiol 3-methyl ether)

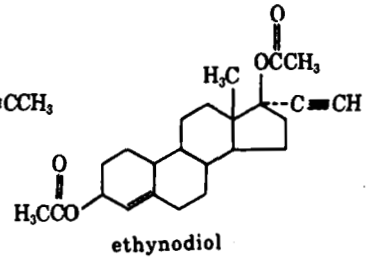


testosterone

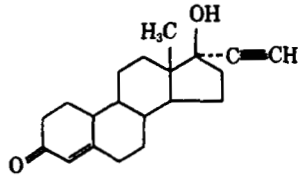
progestagens



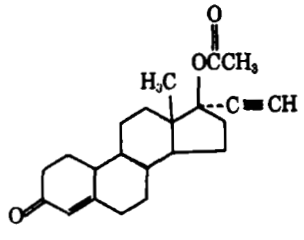
dimethisterone



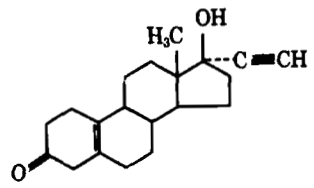
ethynodiol diacetate



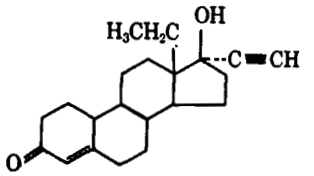
norethindrone (norethisterone)



norethindrone acetate



norethynodrel



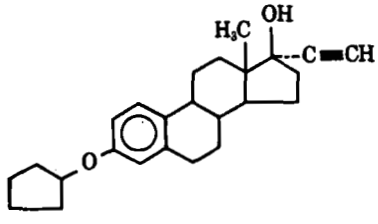
norgestrel

Removal of the 19-methyl group from testosterone, yielding 19-nortestosterone, markedly reduces the androgenicity of the molecule (16). It is actually the latter compound to which the progestagens, excluding dimethisterone, are more closely related. Addition of the 17α-ethinyl group confers progestational activity on 19-nortestosterone (17), and further modifications have resulted in compounds which, on the basis of data obtained from studies in lower animals (16), appear to differ slightly among their other biological properties (18).

Dimethisterone, a potent antiandrogen, for example, is without estrogenic or androgenic properties in contrast to ethynodiol diacetate, which has some estrogenicity and slight androgenicity. Norethindrone, norethindrone acetate, and norgestrel all possess some androgenicity, while norethynodrel has estrogenic activity thought to be due to the double-bond configuration between carbon positions 5 and 10 in the latter compound (19).

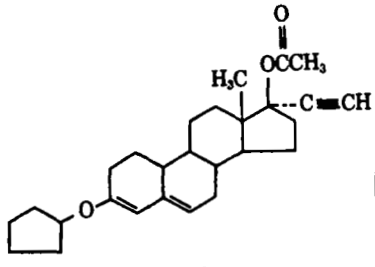
The chemical structures of a number of other steroids with contraceptive properties are also shown here.

estrogen

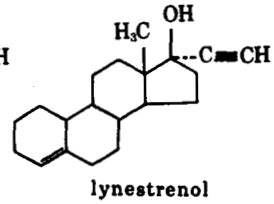


quinestrol

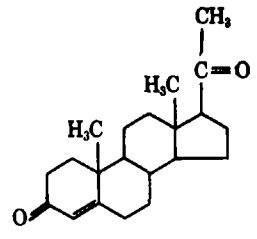
progestagens



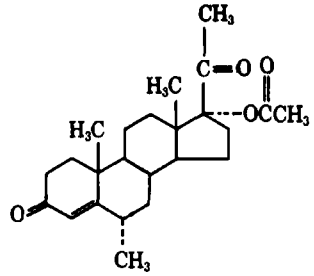
quingestanol acetate



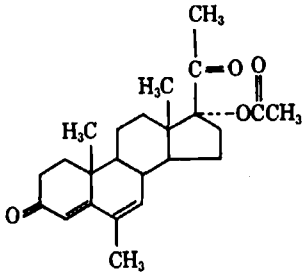
lynestrenol



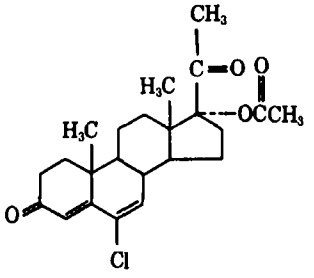
progesterone



medroxyprogesterone acetate



megestrol acetate



chlormadinone acetate

These compounds have been and/or are being used alone or in combined estrogen–progestagen preparations, largely in investigational studies, in the United States and elsewhere (*vide infra*).

Estradiol is not active orally; addition of the 17α-ethinyl radical, however, as in ethinyl estradiol and its 3-methyl ether, mestranol, renders the molecule orally active (17). The 3-cyclopentyl ether derivative quinestrol is not only active orally but has an extremely prolonged duration of action due to its slow release from fat stores (20). In contrast, quingestanol acetate, the 3-cyclopentyl enol ether derivative of norethindrone acetate, is not stored in body fat and does not have prolonged activity (21). Results of a study (22) of other 19-nortestosterone 3-cyclopentyl enol ethers suggest that the nature of the parent ketone, rather than enol etherification *per se*, governs the biological behavior of such compounds.

Lynestrenol is another 19-nortestosterone derivative present in oral contraceptive preparations that are commercially available outside the United States. Like norethindrone acetate and its derivative, quingestanol acetate, lynestrenol is a progestagen which has some androgenicity (18).

Finally, there are three progestagenic steroids shown that are structurally related to progesterone, the structure of which is given also. Neither progesterone nor 17α-hydroxyprogesterone has activity when administered orally; the progesterone molecule, furthermore, does not lend itself to useful derivatives (17). Esterification of the 17α-hydroxyl group of the second compound, however, gives this molecule progestational properties, and further modifications enhance its contraceptive effects (23). Specifically, a major pathway of 17α-hydroxyprogesterone catabolism involves hydroxylation at C-6. Addition in this position of substituents such as chlorine, as in chlormadinone acetate, or a methyl group, as in medroxyprogesterone acetate and megestrol acetate, hinders the catabolism of the compound, thereby enhancing its biological effects. Finally, medroxyprogesterone acetate has slight androgenicity while chlormadinone acetate and megestrol acetate are potent antiandrogens (18).

Contraceptive Preparations—The commercially available oral contraceptives are of two types, the combined products and the sequential preparations. The various doses and combinations available in the United States are listed in Table I. It can be seen that doses vary not only among the different progestagens but also for the same progestagen used either with the same or a different dose of estrogen. The reasons for the variability are multiple and are related to the factors of potency, dosage regimens and therapeutic uses other than contraception (see footnotes to Table I), and side effects.

Although much valuable preliminary information can be obtained by studying the effects of oral contraceptive components in experimental animals, the potency of these compounds, as assessed by animal studies, may not always be relevant to their effectiveness in the human. Consequently, Swyer and Little (24) compared a number of progestagens, with and without estrogen, with respect to their ability to postpone menstruation in normal women given 20-day courses of progestagen from Day 20 of the cycle. The respective doses (and variations available for given progestagens) listed in Table I for norethindrone, norethindrone acetate, norethynodrel, ethynodiol diacetate, and norgestrel are consistent with their respective potencies as determined by the menstruation postponement assay; dimethisterone was not studied in this manner.

The combined preparations containing the highest doses of norethindrone and norethynodrel have been used for the treatment of endometriosis as well as for contraception. The rationale behind this use is that the higher the dose of progestagen the greater are the degree and probability of occurrence of endometrial suppression (25).

The occurrence of side effects, such as lack of withdrawal bleeding on the one hand and breakthrough

Table I—Oral Contraceptive Preparations

Estrogen	Dose, mcg.	Progestagen	Dose, mg.
Ethinyl estradiol	100	Dimethisterone	25.0[a]
	50	Ethynodiol diacetate	1.0[b]
	50	Norethindrone acetate	1.0[b]
	50	Norethindrone acetate	2.5[b]
	50	Norgestrel	0.5[b]
Mestranol	100	Ethynodiol diacetate	1.0[c]
	50	Norethindrone	1.0[c]
	60	Norethindrone	10.0[d,e]
	80	Norethindrone	1.0[b]
	80	Norethindrone	2.0[f]
	100	Norethindrone	2.0[d,g]
	75	Norethynodrel	5.0[d,e]
	100	Norethynodrel	2.5[d]

[a] Sequential preparation: 16 tablets of ethinyl estradiol followed by six tablets of ethinyl estradiol plus dimethisterone. [b] Combined preparation: 21-day dosage schedule. [c] Combined preparation: 20- and 21-day dosage schedules. [d] Twenty-day dosage schedule. [e] Used also for treatment of hypermenorrhea and endometriosis. [f] Sequential preparation: 14 tablets of mestranol followed by six tablets of mestranol plus norethindrone. [g] Used also for treatment of hypermenorrhea.

bleeding on the other, may also be dose related (26); higher doses of progestagen may be associated with scanty withdrawal bleeding, while lower doses may be associated with breakthough bleeding. It has also been suggested that a higher dose of estrogen (*i.e.*, 80 mcg. mestranol instead of 50 mcg.) may be needed by some women to avoid spotting and breakthrough bleeding. Nevertheless, it was shown that by the sixth cycle the differences between the two doses were negligible with respect to the occurrence of breakthrough bleeding (27). In view of this observation and of the possible association between more serious adverse reactions and estrogen dosage (*vide infra*), it would seem wiser to rely on higher progestagen dosage preparations to control breakthrough bleeding in patients for whom this effect is a significant problem.

The variations in estrogen dosage are also of interest from the point of view of contraceptive effectiveness. A number of investigators (28–36) studied the effectiveness (pregnancy rate) and/or the ovulation-blocking effects (pregnanediol measurements and/or endometrial biopsy) of various doses of both ethinyl estradiol and mestranol, alone as in the initial portion of a sequential regimen or in combination with a progestagen. The results of one study (28) seem to imply that a dose of 80 mcg. mestranol is necessary to produce the same (and acceptable, although less than complete) degree of ovulation blockade as 50 mcg. ethinyl estradiol; mestranol, however, apparently was not studied at a dose of 50 mcg. A subsequent study (29) showed that a daily dose of 100 mcg. ethinyl estradiol was necessary to inhibit ovulation consistently. Furthermore, six of eight patients ovulated during treatment with 80 mcg. mestranol following a nontreatment cycle. The results of a more recent study (30) support the data of Jackson *et al.* (29) concerning ethinyl estradiol; three pregnancies, two of which were attributed to method failure, occurred among 80 women treated for 695 cycles with 70-mcg. doses of ethinyl estradiol, yielding a pregnancy rate of 3.5/100 woman-years of use.

In larger scale studies (31–33) in which the contraceptive effectiveness of 80 mcg. mestranol was investigated, pregnancy rates were low. In one report (33) the pregnancy rates for the 27 centers in which the study was carried out showed a distribution comparable to that for published data concerning various combination products; the data from the 27 centers averaged out to a rate of 1.12/100 woman-years. The authors pointed out, however, that factors such as the reluctance of women to admit not having taken the preparation correctly until they were assured that they were not pregnant made it difficult to determine the true effectiveness of any contraceptive.

Finally, two investigations were carried out to compare the effectiveness of various contraceptive preparations. Results of a 4-year study (34) yielded pregnancy rates of 0.1/100 woman-years for combined preparations and of 0.5/100 for the sequential preparations; these values increased to 0.7 and 1.4, respectively, when patient failures were included. Unfortunately, the doses of estrogen and progestagen used were not given. The other study (35) compared nine preparations, four of which were sequentials. All preparations contained 80 mcg. mestranol except for two combined preparations that contained 75 and 125 mcg. mestranol, respectively. The preparations were used for total numbers of cycles ranging from 71 to 6721. The seven pregnancies attributed to method failure occurred in patients using three of the four sequentials; however, since the directions given were to wait until the 5th day of withdrawal bleeding before beginning a new 20- or 21-day course of medication, 11 days of no medication elapsed for three of the patients in whom withdrawal bleeding had been delayed. Resumption of medication on the 6th or 7th day after the last tablet of the previous cycle, regardless of whether or not withdrawal bleeding had occurred, became the retrospective and, probably, safer recommendation.

There have been reports of women omitting tablets and becoming pregnant, of women omitting tablets and not becoming pregnant, of women ovulating during treatment but not becoming pregnant, and of women not omitting tablets but becoming pregnant (30–36). Some data also suggest a lesser effectiveness with use of the sequentials (34, 35). These phenomena can be explained at least partly on the basis of the mechanisms of action of the contraceptive preparations (*vide infra*). However, very few studies have taken into consideration the absolute frequency of coitus. Since both ova and sperm have a finite viability, pregnancy would not be expected to occur if coitus had not taken place close to the day(s) of missed medication and/or breakthrough ovulation. As will be shown later, the combined preparations provide secondary, backup mechanisms in the event a breakthrough ovulation occurs. However, data are not available to indicate whether coincidental lack of coital exposure took place at the time that tablets of either type were missed by patients who did not subsequently become pregnant. At best, an occasional study (30) reported the average coital exposure per week or that patients were required to have frequent (no numerical value given) intercourse as a prerequisite for receiving the test medication (35).

Mechanisms of Action—Both the combined and sequential preparations are believed to prevent ovulation by inhibiting the release of the gonadotropin-releasing factor(s) from the hypothalamus (1, 16); that is, the synthetic hormone(s) exerts a negative feedback on the hypothalamus in a manner similar to that by which the naturally occurring hormones act. The major difference, however, is that the oral contraceptives provide a constant (inhibitory) dosage, a steady-state level, of synthetic ovarian hormone(s) in contrast to the changing (and, hence, sometimes stimulatory) levels of naturally occurring steroids provided during the normal, untreated menstrual cycle (*vide supra*).

As a result of hypothalamic suppression by an oral contraceptive, gonadotropin output from the pituitary is decreased. However, results of studies (37–41) of serum and urine samples obtained from women taking various oral contraceptives have revealed both qualitative and quantitative differences between the effects of the combined and sequential preparations. Such differences are to be expected in view of the normal hormonal interrelationships already discussed. Specifically, the sharp midcycle peak in LH secretion was

almost always abolished by both the combined (37–41) and sequential preparations (37, 38), as was the midcycle peak of FSH (37, 38, 41). In addition, basal, tonic secretion of FSH was suppressed to about 70% of control values by both types of preparation. In contrast, whereas the combined preparations decreased tonic LH secretion to about 20–30% of the control value, sequential preparations decreased LH values to only about 50% of the control (37). Furthermore, large, multiple fluctuations in LH levels, "inappropriately timed LH peaks," were found in serum samples obtained from several women during the estrogen phase of their sequential medication (38). An additional peak in LH secretion occurred in five of eight patients following addition of the progestagen on the 16th day of medication.

In summary, if FSH is sufficiently suppressed by the estrogenic compound of both the combined and sequential preparations, follicular growth will be minimal (16). Therefore, the occurrence of ovulation would be unlikely even in the presence of the higher LH levels found during sequential therapy since there would be no ripe follicle mature enough to ovulate. Nevertheless, modest fluctuations in FSH secretion have been observed, even in women receiving combined preparations (37, 41), suggesting that follicular growth might proceed further in some women than in others.

The possibility of ovulation occurring is increased when a tablet of either type of preparation is missed, although one preparation containing 0.5 mg. norgestrel and 50 mcg. ethinyl estradiol was shown to be effective when administered on alternate days (42). The possibility of a pregnancy occurring is increased, especially when a tablet of the sequential type is missed. These possibilities are further increased when more than one tablet is omitted. Finally, although taking a missed tablet as soon as it is remembered or doubling the dosage when more than one is missed will serve to end the cycle of medication at the appropriate time, such measures are not likely to provide adequate contraception in the case of the sequentials nor even in the case of the combined preparations if more than one tablet of the latter type is omitted. Consequently, use of an additional method of contraception for the remainder of the cycle is advisable whenever oral contraceptive tablets have been omitted. Explanations for taking this precaution lie partly in the results of studies (43–45) in which various doses of estrogens and/or progestagens were administered daily to women.

Briefly, FSH concentrations in the urine were found to increase with daily subtherapeutic doses of ethinyl estradiol (20 mcg.) (43) and mestranol (10 mcg.) (44), whereas therapeutic doses [70 and 100 mcg. (43) and 50 and 100 mcg. (44), respectively] usually depressed FSH excretion. Furthermore, high daily doses (2 mg.) of one progestagen, ethynodiol diacetate, suppressed FSH (44) and LH (45) secretion while lower doses [0.25 and 0.50 mg. (44) and 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg. (45)] often resulted in high levels of FSH and LH, respectively.

Although the progestagen–estrogen ratio in combination products apparently influences the degree of FSH and LH suppression (44), the important point to be realized from these studies is that low doses of these contraceptive steroids do not suppress, and actually may stimulate, FSH and/or LH secretion. Hence, when tablets are missed, blood levels of the steroids decrease, resulting in an increased output of FSH and/or LH by the pituitary. Follicular growth or, possibly, ovulation may then occur if sufficient FSH secretion has been occurring in spite of the medication. Assuming the lowering of steroid levels has merely resulted in a subsequently increased secretion of gonadotropin, which has in turn merely resulted in follicular maturation, doubling the dosage upon remembering the omission would increase blood steroid levels and possibly trigger an ovulatory surge of LH, just as rising steroid levels appear to do during the normal, untreated cycle. Indeed, changing the dosage of estrogen during the combined portion of a sequential regimen (75 mcg. ethinyl estradiol for 16 days followed by 50 mcg. ethinyl estradiol plus a progestagen for 5 days) was associated with a pregnancy rate of 15.4/100 woman-years in comparison with the rate of 6.3 obtained when the dosage of ethinyl estradiol remained 75 mcg. throughout the 21-day sequential cycle (46). Furthermore, low doses of conjugated estrogen (0.1 mg. daily on Days 9–14 of the cycle) were used to induce ovulation at a predictable time (97% of 336 ovulations occurred no later than Day 16) in patients wishing to use the rhythm method of birth control (47).

Pituitary suppression tends to be more complete with the combined preparations, as indicated by a marked decrease usually in both FSH and LH secretion, with the probable consequence being a more marked lack of follicular development. However, these preparations also provide secondary contraceptive mechanisms that can be of importance in the event of a breakthrough ovulation. One such mechanism involves the effect of the progestagen on the cervical mucus. In contrast to the watery mucus facilitatory to sperm penetration (15) present during the preovulatory phase of the untreated cycle and throughout the estrogenic phase of the sequential regimen, the mucus present during treatment with the combined preparations is similar to that found during the normal luteal phase; that is, the cervical mucus is thick, scanty, and cellular, providing a physical barrier to penetration by sperm (13). The physical barrier hypothesis, moreover, is supported by electron microscopic evidence (48) which indicates a close approximation of, and significant cross-linkages between, the main fibers comprising the cervical mucus obtained from women taking combined preparations. This mechanism, furthermore, is thought to be the one primarily responsible for the contraceptive effectiveness of low-dose progestagen-only preparations (*vide infra*).

Endometrial changes occurring under the influence of combined preparations differ from those observed during the normal, untreated cycle. As a result of the presence of progestagen along with estrogen early in the cycle, glandular secretion begins promptly and reaches a peak within 4 days of the start of medication (13). The glands then involute and may acquire an atrophic appearance. The stroma, after passing through a phase of edema, reaches a pseudodecidual pattern earlier and of greater intensity than in the untreated

cycle. It has been suggested that such an endometrium may be unfavorable in some manner for the implantation of a fertilized ovum (23, 49).

Investigational Steroid Contraceptives—A number of orally administered progestagens have been or are being investigated for their ability to control fertility in the absence of exogenous estrogen. Most studies involved chlormadinone (50–55) and, more recently, norgestrel (55–60), although results obtained with continuous administration of low doses of norethindrone (51, 61, 62), norethindrone acetate (55), and megestrol acetate (55) were reported as well. A discussion of the observations made in women taking chlormadinone acetate is virtually of academic interest only, since this drug was withdrawn from the market due to the finding of mammary gland nodules in dogs treated with it (63). Nevertheless, the results of clinical studies with this drug provide much valuable information concerning the mechanism(s) by which low dose progestagen-only preparations may exert their contraceptive effects.

It was indicated above that the primary antifertility effect of low dose progestagen-only preparations was thought to be the result of their production of a cervical mucus that was "hostile" to sperm. Indeed, studies (50) with doses lower than 0.25 mg. chlormadinone acetate revealed a failure to produce inhibitory changes consistently in the cervical mucus; these doses also did not afford completely effective contraception. Higher doses produced greater inhibitory effects on the cervical mucus and greater contraceptive effectiveness. When doses higher than 0.3 mg. were used, suppressive effects on the endometrium were obtained; these effects presumably contributed to the contraceptive effectiveness of the drug.

Subsequent studies of volunteers taking daily doses of 0.5 mg. chlormadinone acetate (51–54) or 0.5 mg. norethindrone (61) revealed discrepancies among factors normally associated with the occurrence of ovulation and, hence, with one another. These factors include the midcycle LH (and FSH) peak(s), biphasic basal body temperature, and increased serum progesterone and increased urinary pregnanediol levels during the second half of the cycle. The fact that sometimes one, although not always all, of these factors might be negative in patients taking chlormadinone acetate suggested the possibility of a central effect (52) of the dose to which some patients might be more sensitive than others (53). In addition, these observations raised the question as to whether there might be some minimal levels of LH and pregnanediol compatible with normal ovulation and corpus luteum function (51). In other words, would the gonadotropin levels found during chlormadinone acetate therapy be sufficient merely to cause luteinization of one or more follicles but not ovulation (53) or, if ovulation did occur, would it be followed by an "inadequate luteal phase" (54, 61)?

Similar observations were made in studies employing low doses of norgestrel. Specifically, the results suggested that in addition to producing inhibitory effects on the cervical mucus and on the endometrium, norgestrel in doses of 0.03 (56), 0.05 (57), and 0.075 (58) mg. appeared also to interfere with the formation and/or functioning of the corpus luteum. The progestagens, norethindrone, norgestrel, chlormadinone acetate, and medroxyprogesterone acetate, administered orally for several days following ovulation, have, in fact, been shown (64) to decrease progesterone production by the corpus luteum. However, the doses used for norethindrone (5–100 mg.), norgestrel (2–5 mg.), and chlormadinone acetate (50 mg.) were higher than those used clinically for progestagen-only contraception. The mechanism by which hormone production was decreased was not determined, but it was not likely to have involved luteolysis since the administration of human chorionic gonadotropin (HCG) resulted in increased production of progesterone despite continued administration of the synthetic progestagens.

It seems apparent that the mechanism(s) by which daily administered oral preparations of low dose progestagens exert their contraceptive effects is (are) complex and still somewhat obscure. By means of culdoscopic examinations (51) and ovarian biopsies (52), for example, it was shown that ovulation does occur in some, but not all, women taking these preparations. Moreover, inconsistencies among parameters normally indicative of ovulation have been found in some women. Furthermore, although it is generally agreed that the production of a cervical mucus that prevents the entrance of sperm into the uterine cavity is the primary mechanism by which these preparations act, postcoital tests, although usually poor, are not always so, in women using these contraceptives (55, 60). Finally, the "hostility" may not be entirely due to a physical barrier but may also be biochemical in nature (65). Trypsin, for example, is thought to affect sperm penetration of the zona pellucida of the ovum, and it has been postulated that one mechanism might involve antitrypsin in the cervical mucus (60).

Whether due to a physical and/or a biochemical effect, the production of cervical mucus that is nonpermeable to sperm formed the basis on which clinical trials of precoital progestagen-only contraceptives were undertaken (66). Megestrol acetate, at a single oral dose of 0.5 mg., was shown to be effective when coitus was limited to the period between 4 and 14 hr. after dosing; 17 women were studied for a total of 187 cycles, and coital frequency was estimated to be once every 3 days on the average. A previous group of 26 women studied for a total of 468 cycles also achieved contraceptive protection with this preparation, although there was one patient failure. In these patients, coital activity had been restricted to between 5 and 10 hr. following dosing. Protection was not achieved, however, in a third group of patients in whom coitus took place largely between 20 and 21 hr. after dosing. This preparation appears to have advantages for women exposed only occasionally to intercourse; however, frequent coitus requires frequent medication, with the result that cycle irregularity may occur, similar to that observed with continuous progestagen administration (*vide infra*).

It should be obvious that women using oral contraceptives of the combined type have an advantage over those using sequentials since additional contraceptive mechanisms are provided by the progestagen. Unfortunately, in the absence of exogenous estrogen (*i.e.*, in patients using the continuously administered progesta-

gen-only oral preparations), high pregnancy rates were reported in some studies. Rates for 0.05 mg. norgestrel vary from 0.8 (59) to 6/100 woman-years (62); for 0.5 mg. chlormadinone acetate, the rates vary from 0.2 to 9/100 woman-years (62). In his own study using 0.35 mg. norethindrone, Board (62) obtained a rate of 0.6/100 woman-years based on 154 women observed for a total of 1888 months. For norethindrone acetate, on the other hand, a rate of 4/100 was obtained by Mears *et al.* (55); however, the group in this study consisted merely of 41 women, only 19 of whom completed an entire year's treatment. In retrospect, the influence that factors such as ethnic group (67), especially if a marked difference in body size is involved, and coital frequency might have had on these results cannot be determined. In view of the high pregnancy rates reported in some of these studies, it would seem unwise for women to use progestagen-only oral contraceptives unless they wish merely to space their families rather than to eliminate pregnancy completely.

In addition to their drawback of having a variable degree of effectiveness, progestagen-only preparations also produce a high incidence of menstrual irregularity manifested by breakthrough bleeding and prolonged cycles (15, 26, 50, 59, 62). Better cycle control can be obtained by administering estrogen in addition from Day 19 or 21 to Day 25 (68), the so-called reverse sequential regimen (26). Although contraceptive effectiveness was reported for this regimen (68), the advisability of giving estrogen to women in whom ovulation but not necessarily follicular development may have been delayed is questionable in view of the stimulatory effect that estrogen may have on the hypothalamus (*vide supra*).

Progestagen-only contraception has been achieved by administering the compounds by other than the oral route. A depot form of medroxyprogesterone acetate, for example, has been shown to be effective when injected intramuscularly at a dose of 150 mg. every 3 months (15). Inhibition of LH release, increased viscosity of the cervical mucus, and thinning of the endometrium are all believed to contribute to the contraceptive effect. Unlike the oral progestagens, the injectables appear to be as effective as the commercially available combined oral preparations (26). Like the oral progestagens, however, the injectables have erratic bleeding as a major side effect. Nevertheless, amenorrhea eventually develops in many patients and may persist for up to 12 months after the last injection.

Other means of delivering contraceptive doses of progestagens in a continuous manner that have been tried recently include: (*a*) the subcutaneous implantation of silastic capsules containing progestagens such as medroxyprogesterone acetate and megestrol acetate (26), (*b*) the use of a silicone rubber ring impregnated with medroxyprogesterone acetate and placed around the cervix (26), and (*c*) the intrauterine administration of progesterone contained in a silastic capsule attached to an intrauterine contraceptive device (IUD) (15). However, insufficient studies have been published concerning these newer methods to permit their critical evaluation.

Clinical studies have been carried out also with long-acting, intramuscularly injected preparations containing an estrogen as well as a progestagen. These preparations share with the progestagen-only injectables the advantage that effective hormonal contraception can be achieved without requiring the patient to remember to take daily medication. In addition, it was suggested (69) that cycle control may be slightly better with the combined injectables; the latter, however, have usually been administered once a month in comparison to once every 3 months for the progestagens.

Examples of combinations that have been shown to be clinically effective include estradiol undecylate plus norethindrone enantnate [5 and 50 mg., respectively, or 10 and 70 mg., respectively (69)], 5 mg. estradiol cypionate plus 25 mg. medroxyprogesterone acetate (70), and 10 mg. estradiol enanthate plus 150 mg. 16α,17α-dihydroxyprogesterone acetophenide (71). The last preparation, studied in 385 patients during 4512 cycles, produced continued or irregular bleeding in 7.5% of the patients.

The depot concept was also applied to orally administered formulations in which the long-acting estrogen, quinestrol, was combined with one of several short-acting progestagens (15). In one study (20), for example, a dose of 2 mg. quinestrol alone was given on Day 1 of the first cycle; on Day 22 and then subsequently at 4-week intervals, doses of 2 mg. quinestrol plus 5 mg. quingestanol acetate were given. The effect of the progestagen was primarily to induce withdrawal bleeding, although the latter began at variable times following dosing: 8–14 days after administration in 65% of 7441 cycles observed in 719 patients and 5–20 days later in 92% of the cycles. The effect of the estrogen, on the other hand, was to prevent ovulation during the subsequent 4 weeks. However, the progestagen may have contributed to the contraceptive effect as well; six patients, four of whom did receive the quinestrol later than Day 1, became pregnant during the first cycle in which they received the estrogen alone, yielding a pregnancy rate of 4.0/100 woman-years. When the first cycle and subsequent patient failures were eliminated from the total data, the pregnancy rate was calculated to be 2.1/100.

Estrogens alone have been used postcoitally to prevent implantation in the event that fertilization may have taken place, as in rape cases (26) and condom failures (72) for example. Several possible mechanisms of action have been suggested (73), including disturbances in endometrial histology and histochemistry, tubal locking that would prevent the ovum from reaching the uterus, and rapid expulsion of the ovum from the tube and uterus. Various dosage regimens also have been suggested: 1 mg. ethinyl estradiol b.i.d. for 5 days following coitus (15), 0.5 mg. ethinyl estradiol or 5–50 mg. diethylstilbestrol daily for 4–6 days following coitus (74), 5 mg. diethylstilbestrol five times per day for 5 days beginning within 48–72 hr. after coitus (72), 75 mg. diethylstilbestrol for 3 days following coitus (75), and 25 mg. diethylstilbestrol b.i.d. for 5 days beginning immediately after coitus (73). The larger doses especially are likely to produce nausea and vomiting; consequently, one investigator (72) recommended administering an antiemetic along with the estrogen. Because of its

tendency to produce these symptoms, this method of contraception is not likely to be used by patients on a regular basis. Furthermore, its effectiveness is difficult to evaluate since a large number of patients in whom the method has been tested have been rape cases, and the certainty of pregnancy following rape is not thought to be very great (74).

ADVERSE REACTIONS: THROMBOEMBOLISM

The manifestations of adverse reactions (side effects) to oral contraceptives are not limited to abnormalities produced in the reproductive system, but rather they involve almost all systems of the body. Oral contraceptive therapy is rather unique since an abnormal, nonphysiological response in the normal reproductive system of the female is the therapeutic result. In addition to or perhaps as a consequence of this effect, other abnormalities, both mild and severe, may occur. In the following discussion, the term "side effect" will be used in its broadest form and will represent any change from normal in systems other than those specifically involved in the production of contraception.

Side effects may be produced in three ways: (*a*) an abnormality may be produced directly by an effect of the drug on the tissue, (*b*) the effect may occur indirectly as a result of a change in endogenous hormonal balance produced by the drug, and (*c*) the abnormality may be the consequence of a combination of (*a*) and (*b*). Unfortunately, little concern has been given to determining which possibilities may be correct.

Problems in Assessment of Side Effects—One major problem encountered in the assessment of the side effects of oral contraceptives is that the literature concerning this subject is controversial (76, 77). This enigma seemingly has resulted from controversial data that have been published, and the problem has been compounded by biases from several sources.

There are biases that are inherent in a particular type of investigation or reporting system. Such biases as are seen in retrospective epidemiologic studies, wherein patient records are searched to obtain data, have been identified in studies (78) concerned with the evaluation of side effects of oral contraceptives. They are usually considered normal and expected, and allowances can be made for them.

However, certain other biases have had a marked effect on the investigations and on the conclusions drawn from them. These biases are not usual, measurable, or accountable for, and they arise from various sources. Some seem to have originated from personal prejudices on the part of certain investigators who allowed their prejudices concerning the "pill" to affect the design of their experiments, the reporting of their results, and the conclusions derived. Such prejudices are not surprising when one considers the polemic attitudes prompted by religious and social influences (79, 80).

Other biases, often termed diagnostic biases, are more insidious and may have contributed to the problem of assessing the side effects of oral contraceptives. Seigel (81) pointed out that the evaluation of data from case-control studies may be biased because the proportion of women hospitalized who take oral contraceptives may be larger than a corresponding group who do not. He ascribes this observation to the fact that oral contraceptive users probably see their physicians more often. In addition, since both the physician and patient are likely to be aware of some of the undesirable effects of these agents, the women in this group may be diagnosed and hospitalized more readily than those in the general population. Therefore, the cases that come under scrutiny in these studies may yield a disproportionate number of incidences of adverse effects in oral contraceptive users. Seigel concluded that when analyzing studies of conditions such as thromboembolic disorders, particularly when they are not severe, the potential bias must be carefully assessed.

The controversies and biases have been polarized further by influences from political arenas, various antipill factions, representatives of government agencies, and people of the news media seeking sensationalism (79, 82). The influence of these factors on the reported side effects of the pill and on the use of the pill itself was discussed by Kistner (79). Jeffcoate (83) stated: "At times, there has appeared to be almost a campaign to discredit the pill, and the views of prejudiced if well-meaning individuals have been given disproportionate emphasis and allowed to outweigh scientific evidence."

Another major problem particularly pertinent to the assessment of the common, minor side effects has been the paucity of double-blind clinical trails. Such testing procedures are essential to determine whether an observed effect is causally related to the drug or due to other factors such as psychological effects. In this type of procedure, the tablets containing drug and those containing an inert material, as well as the packages in which they are contained, are identical in appearance; the packages are identified by a code, the key to which is kept under seal. The obvious problem with this type of study is finding women who will agree to subject themselves to this randomized dosing, the consequence of which might be pregnancy. It is also difficult to find investigators who are willing to charge themselves with the responsibliity of conducting such a study. However, it is possible to find populations of women who are not at risk of pregnancy, such as prison inmates (84); alternatively, other contraceptive devices such as intrauterine devices (85) or condoms can be provided. Obviously, with the danger of pregnancy obviated, investigators are less reluctant to conduct the experiment, and subjects can be found more easily. These double-blind clinical trials should be expanded to correlate the incidence of side effects with various dosages of the estrogens and progestagens, as well as with dosages of various combinations thereof.

Another difficulty encountered in evaluating the occurrence of side effects, which has made comparisons among data from different studies unreliable (86), is the variability in the protocols and reporting systems of these studies. No unified system or protocol exists; as a result, different variables are present in the various studies and often they are not fully described. These variables include: (*a*) a wide range of populations in terms of ethnic, cultural, and socioeconomic background; (*b*) variations in patient management; and (*c*)

variations in the methods used for collecting, recording, and evaluating data (84). For example, in clinical trials with oral contraceptives, the "open-ended" approach is often utilized to obtain information from the patient, wherein the patient is requested to volunteer any information concerning changes in her health status that she might have noticed. On the other hand, specific questioning about certain symptoms can be used; this probing type of question yields a higher incidence of side effects (85).

In the area of rare, major side effects such as thromboembolism, retrospective epidemiologic studies have been somewhat productive but few in number. Their reliability is open to question because of a lack of information concerning the incidence of many of the reported side effects in the general population (87), the small number of patients studied, and the lack of appropriate controls. Any disease that occurs in a normal population may also occur in a population that uses oral contraceptives. The important question is whether or not it occurs more frequently in women using oral contraceptives (84). The above-mentioned problems were described at some length by Tietze (84).

Although often used, the division of side effects of oral contraceptives into major and minor categories is arbitrary. Since the status of many side effects may change rapidly as new information is uncovered, no further designations of this type will be used.

Thromboembolic Disorders—The first association between oral contraceptives and thromboembolism was reported in 1961 when Jordan (88) described the development of bilateral pulmonary embolism in a woman following the use of norethynodrel (10 mg.) with mestranol for the treatment of endometriosis. Symptoms developed in the right lung 10 days following the termination of treatment and in the left lung at 17 days posttreatment. The patient also had been taking cyclizine and perphenazine to control the nausea produced by the steroid drugs. Jordan stated that the norethynodrel with mestranol might have provoked the pulmonary embolism and infarction. He made no reference to the possible effects of the other drugs. Later in the same year, two cases of fatal pulmonary embolism occurred in two young women taking oral contraceptives (89).

Subsequently, many cases such as these were reported in the literature, as well as to various government agencies both here and abroad. These thromboembolic episodes were reported most frequently in the lungs and the large leg veins, as well as in the coronary, cerebral, and mesenteric vessels. In 1963, Tyler (89) commented on 347 instances of thrombophlebitis that had been reported since 1961 in women using norethynodrel with mestranol for contraception. These isolated cases had been reported to the manufacturer of the contraceptive product. Thirty-five instances of fatal pulmonary embolism occurred during the same period. In his discussion of the latter, Tyler pointed out that other etiologic factors might have been involved. He recognized the fact that thrombophlebitis and pulmonary embolism could occur spontaneously in the general population but that no control figures for this spontaneity were available.

Since thromboembolic disorders are known to occur in young women who do not use oral contraceptives (90), these isolated case reports cannot serve to establish a relationship between oral contraceptives and thromboembolism.

One of the first organized efforts to determine whether a relationship might exist between thromboembolism and the use of oral contraceptives was a conference sponsored in 1962 by G. D. Searle and Co. (90). The participants' comparisons of the incidence of thromboembolism in oral contraceptive users and in the general population yielded no evidence to suggest a causal relationship between oral contraceptive therapy and thromboembolism.

In 1963, a committee of nine medical experts, assembled by the Food and Drug Administration (FDA), reported a study of 350 cases of thromboembolism in women using norethynodrel with mestranol. These cases included those reported both to FDA and to the manufacturer. The members of the committee reviewed the pertinent literature and conducted experiments in their own laboratories pertaining to hypercoagulability. They concluded that there was no evidence that this contraceptive preparation produced hypercoagulability and that there was no significant increase of thromboembolic deaths in the oral contraceptive users (91).

In 1965, Cahal (92, 93) reported that in the 12 months ending August 31, 1965, 16 thromboembolic deaths in oral contraceptive users had been reported to the Committee on Safety of Drugs in England; only 13 would have been expected, based both on the estimated occurrence in the general population and also on a round, theoretical figure of 400,000 oral contraceptive users. Cahal admitted that the accuracy of the latter figure was open to question and that the incidence of deaths might have been underreported. Cahal went on to point out that when the deaths due to thromboembolism were further subdivided according to site of the embolism, eight were found to have occurred in the lung in contrast to the two that would have been expected on the basis of mortality statistics found in the Registrar General's Statistical Review. Cahal himself concluded that the numbers involved were too few to state that the differences were meaningful.

Weatherall (94) was critical of Cahal's estimate of two expected instances of death due to pulmonary embolism in the general population, pointing out that pulmonary embolism commonly was not reported as an underlying cause of death when it occurred during some other disease. She stated that because of the method of reporting, only approximately 13% of the instances in which pulmonary embolism was the underlying cause of death were being reported as such; therefore, the estimate of death due to pulmonary embolism given by Cahal was seven times too low. However, in 1969, Vessey (90) quoted without qualification the fourfold difference in pulmonary embolism occurrence between users and nonusers that had been reported by Cahal.

In 1966, the Advisory Committee on Obstetrics and Gynecology of FDA issued its first report (95). Using similar basic data and procedures as the earlier *ad hoc*

committee (91), this group calculated that on the basis of the estimate of users in 1962, 1,187,000, the expected mortality rate from pulmonary embolism would be 12. They adjusted the latter figure to 10 since they felt the estimated number of users might have been inflated. The actual number of such deaths reported to FDA was 13; this difference was not statistically significant. On the basis of these figures, 13 reported and 10 expected, a relationship very similar to that found by the earlier *ad hoc* committee was evident.

This group also pointed out that since the number of oral contraceptive users had increased from 1,187,000 in 1962 to 5,000,000 in 1966, the number of deaths from pulmonary embolism should have increased to 50. However, the reported number was 13, exactly the same as in 1962. This discrepancy was thought to be due to gross underreporting, which they attributed to physicians becoming increasingly fearful of reporting adverse reactions and deaths because of possible legal reprisals. It was further pointed out that mortality statistics probably were unreliable, since the reporting of the underlying causes of death was left to the judgment and conscientiousness of the certifying physician, and these might vary with the physician. The discrepancy also could be due partly to a greater awareness of adverse reactions leading to more rapid diagnosis and treatment.

The Advisory Committee concluded that the data derived from mortality statistics were inadequate to determine an association between oral contraceptives and thromboembolic disease. However, they indicated that if the oral contraceptives were involved in the pathogenesis of thromboembolic disease, the incidences were very infrequent. The group suggested a need for well-controlled epidemiologic studies.

All of the foregoing studies were of the retrospective type. Vessey (90) pointed out that such studies suffer from an absence of comparable control data and also depend upon reported episodes of thromboembolism. He agreed with the Advisory Committee (95) that gross underreporting was a problem.

In 1961, the Records Unit and Research Advisory Service of the Royal College of General Practitioners in England (96) organized the recording of diseases by a number of general practitioners. Each of 29 practitioners made note of every woman in his practice, age 15–49, who had suffered a new episode of various thromboembolic disorders. The practitioner also categorized the thromboembolic disorder into one of 14 groups. Some of these categories took into account the pregnant and puerperal states.

Two control patients were selected for each thromboembolic patient. The controls were matched for age (within 5 years), parity, and marital status. At the interviews, the patients were questioned as to the type of contraceptive used prior to and at the time of the episode, as well as to the length of time that the form(s) of contraception had been used. There were 147 "affected" patients as well as 294 controls. It was found that a statistically significant excess of oral contraceptive users existed in the affected group as compared with the control group. The excess was present for superficial thrombophlebitis and generally for conditions on the venous side of the circulation. Data for the arterial side of the circulation were insufficient to give significant results. The data also indicated an increase in thromboembolic disorders for women in the pregnant and puerperal states. The investigators concluded that the latter conditions were associated with a fourfold increase in thromboembolic disorders and that oral contraceptive use was associated with a twofold increase. In addition, the study pointed out that the incidence of venous thromboembolic disease increased with age and parity. The authors acknowledged that the number of patients in their study was small and pointed out that prospective studies might provide more reliable data.

In 1968, Inman and Vessey (97) reported on their studies of thromboembolic deaths occurring during 1966. Four hundred ninety-nine death certificates of 20–44-year-old women were obtained in which thrombosis or embolism of the pulmonary, cerebral, or coronary vessels or peripheral venous thrombosis was mentioned. If thromboembolism was a terminal event in the course of another fatal disease, the case was not used. Many other cases were omitted for a variety of reasons, such as: (*a*) the attending physicians were not available, (*b*) some patients had not been registered with a doctor or hospital, and (*c*) some individuals were not at any risk of pregnancy. The number remaining after all exclusions, which formed the basis of the report, was 334. These were subdivided as follows: 95 attributed to pulmonary thrombosis or embolism, 209 to coronary thrombosis or myocardial infarction, and 30 to cerebral thrombosis or embolism.

Each death was investigated by a medical officer who completed a questionnaire with the assistance of the physician who had attended the patient during her terminal illness. The information obtained in the questionnaire was supplemented by hospital case notes and postmortem reports. In addition to data concerning the fatal case, the physician also supplied information concerning four to six controls who were of similar age and parity. In all, 998 controls were used in the analysis.

Since many conditions were believed to be predisposing toward thromboembolic disorders, the questionnaires were assessed and divided into three categories: Class A, patients with no predisposing conditions; Class B, patients with known predisposing conditions who were neither pregnant nor puerperal; and Class C, patients who were pregnant or who had been delivered during the month before the onset of the episode; this last class was not discussed in this paper. An important fallacy of this paper was that no information was obtained about the health of the control women.

The results showed that 77 deaths occurred from pulmonary embolism. Of these, 26 were in Class A, 16 of whom had been taking oral contraceptives; 4.2 would have been expected from the control group data. The difference between the two was stated to be highly significant ($p < 0.001$). Fifty-one deaths were noted in Class B; however, since there was uncertainty concerning the use of oral contraceptives by two of the women, only 49 were used for tabulation. Of the 49, nine had been using oral contraceptives whereas 6.8 would have been expected. This difference was not significant.

Coronary thrombosis was implicated in 205 deaths, 89 of which were in Class A. The authors reduced the number to 84 since the history of oral contraceptive use was doubtful in five patients. Of the 84, 18 deaths occurred in users while 11.4 would have been expected. This difference was not statistically significant. The number of 116 patients in Class B was reduced to 110 because of uncertainty of oral contraceptive use. Only five of the 110 had been using oral contraceptives whereas 12.6 would have been expected.

Twenty-seven deaths were attributed to cerebral thrombosis, 10 in Class A and 17 in Class B. Five of the 10 patients in Class A had been using oral contraceptives whereas 1.5 would have been expected; this difference was statistically significant. Of the 17 patients in Class B, one was excluded because of a poor history of oral contraceptive usage. None of the other 16 had used oral contraceptives.

In summary, the authors stated that a strong relationship existed between the use of oral contraceptives and death from pulmonary embolism or cerebral thrombosis, but not from coronary thrombosis, in the absence of predisposing conditions (Class A). In the presence of predisposing conditions (Class B), no significant relationship was found to indicate an increased number of deaths from any type of thromboembolism, although one might theoretically have been expected. In fact, for coronary thrombosis a statistically significant difference was found that indicated a decrease in the number of deaths associated with this type. As a possible explanation, the authors suggested that physicians might have been reluctant to prescribe oral contraceptives for women suffering from serious chronic diseases. However, since the health status of the controls was not known, this group was not divided into classes as was the thromboembolic group. Therefore, a more legitimate summary statement would involve a comparison of Class A plus Class B *versus* the controls. When this comparison was made, a statistically significant difference between oral contraceptive use and thromboembolism was seen only where pulmonary embolism was involved.

In the same issue of the *British Medical Journal*, Vessey and Doll (98) reported the results of another study concerning women admitted to 19 general hospitals for deep vein thrombosis and pulmonary embolism. The nosological index of each hospital was searched and case notes were reviewed relating to women who: (*a*) had been hospitalized in 1964–1966, (*b*) were 16 to 40 years old, and (*c*) had been diagnosed as having suffered from thromboembolic diseases in any vein except the cerebral, coronary, hepatic, or mesenteric. Patients having had pulmonary embolism or infarction were included. Women were excluded if they: (*a*) were single or widowed, (*b*) had a predisposing condition, (*c*) were pregnant, postmenopausal, or sterilized, (*d*) had suffered only superficial thrombophlebitis, or (*e*) had died. Two case controls were obtained for each affected patient. Those selected were suffering from an acute medical or surgical ailment and matched the affected patient with regard to hospital, date of admission, age, parity, and absence of attributes that had been used to exclude patients from the affected group. Patients were interviewed in their homes by a medical social worker.

For the 3-year period, 399 patients were found who had suffered pulmonary embolism or venous thrombosis. After exclusions were made for the reasons described, only 61 patients remained in the study; 122 controls were selected. Three sets of patients were lost for various reasons, so the study group contained 58 affected patients and 116 controls.

The results showed that 26 of 58 (45%) patients admitted for deep vein thrombosis or pulmonary embolism had been taking oral contraceptives during the month before the start of the episode; in the control group admitted for other conditions, 10 of 116 (9%) had been taking them. The difference was highly significant ($p < 0.001$). Further comparison of the data suggested that in the presence of a history of previous thromboembolic disease, use of oral contraceptives did not lead to further thromboembolic episodes.

The type of preparation used was known for 23 affected patients and for eight controls; none had used the sequential type. Without giving specific data, the authors stated that there was no indication that any one preparation was more likely to produce thromboembolism than another.

Finally, based on hospital admission records, Vessey and Doll (98) estimated the incidence of venous thromboembolic disease to be approximately 5/100,000 in nonusers of oral contraceptives and 47/100,000 in users; in other words, the calculated risk of hospital admission for this disease was nine times greater in oral contraceptive users.

The investigation was amplified further to study the incidence of cerebral thrombosis and coronary thrombosis, using the same criteria and protocols already described. Only nine patients were available who had had a confirmed cerebral thrombosis. Five of the nine had been using oral contraceptives for periods of from 1 week to 4 years immediately prior to becoming ill. The expected number from the control group data was one. Despite these few patients, the authors stated that the difference between the two groups was statistically significant.

Thirteen women were available who had suffered a coronary thrombosis. None of these had taken oral contraceptives during the month before the onset of the disease, a figure not statistically different from that of 0.7 obtained from the control data.

In a later article, Vessey (90) stated that these three British studies (96–98) were subject to the usual problems of case-control studies, but that their statistical inadequacies were less than those of earlier studies.

A "leading article" (99) published simultaneously in the same journal also reviewed the three British studies (96–98). Three conclusions were presented that had been drawn from the results of those studies as well as from data from studies (100, 101) in which estrogens had been used for purposes other than contraception: (*a*) sequential preparations might be more dangerous since they contained more estrogen than the combined type, (*b*) the continuous low dose progestagen technique might afford a means of avoiding the thromboembolic effects, and (*c*) the administration of estrogens for any

purpose, particularly when given in high doses or for long periods, carried a definite risk.

Strong objection to these conclusions was voiced by Reid (102) who claimed that this article led to a minor panic in Australia. In particular, he objected to the conclusion that the estrogenic component primarily was responsible for thrombosis, since Vessey and Doll (98) had not been able to show such a difference. In addition, the other evidence referred to was concerned with ethinyl estradiol given to men in doses higher than those used in women for contraception (100) and with an estrogenic substance other than those used in oral contraceptives and which had been used in higher than equivalent contraceptive doses (101).

Vessey and Weatherall (103) estimated the mortality rates due to venous thromboembolism for the years 1963–1967 based on known mortality rates per million per annum for the years 1953–1962. Separate data for males and for females were further subdivided for four age groups. The actual mortality rates were higher than predicted in three groups: 35–44-year-old males, 20–34-year-old females, and 35–44-year-old females. The authors acknowledged that the slopes of the regression lines they fitted to the data were subject to considerable standard errors, especially at the lower ages, although they did not include their values. They also suggested that similar factors could have been operating in all three groups to increase their mortality rates. Nevertheless, they felt that the increased mortality in the two groups of females was sufficiently high to be accounted for at least partly by the use of oral contraceptives.

In 1968, Drill and Calhoun (104) summarized data from a number of sources in an effort to determine the incidence of thromboembolic disease (thrombophlebitis and thromboembolism) during pregnancy, the puerperium, and oral contraceptive use, as well as in nonpregnant nonusers of oral contraceptives. They found that in nonpregnant women, 20–44 years of age, the natural incidence of thrombophlebitis, based on hospital admissions, was 0.91 (range 0.65–1.08)/1000 women/year. This mean was derived from averages of data for 1947–1962 obtained from three independent sources.

Drill and Calhoun were critical of the figure given by Vessey and Doll (98). The latter investigators had derived a figure of 0.05 case/1000 women/year in nonpregnant women, 16–40 years old. Drill and Calhoun stated that this rate was not comparable with results obtained in other studies. They concluded that the most satisfactory estimate was approximately 1/1000/year based on data from hospital admissions. When the incidence was based on patients' visits to the physician, the mean of the averages from five independent sources for 1955–1966 was 2.2 (range 1.2–3.0) cases/1000 women/year.

The incidence of thromboembolism in the puerperium reported for European studies was 10.4 (range 4.0–21.5) cases/1000 deliveries, while in the United States the incidence was 3.1 (2.6–6.4). Drill and Calhoun (104) pointed out that if these figures were extrapolated to a yearly basis, they would be even higher than the figure for nonpregnant women.

During the antepartum period, the incidence was found to be considerably below that in the postpartum period. The incidence of thrombophlebitis in the antepartum period, based on 379,766 pregnancies reported in 15 studies, was 0.56 ± 0.15 case. When this figure was extrapolated from the 9-month period to 1 year, the rate became 0.74/1000 woman-pregnancies/year. The authors pointed out that the incidence in the antepartum period was approximately one-half that found in nonpregnant females of childbearing age when based on data from hospital admissions and approximately one-fourth that found in similar women when based on data from visits to physicians.

Drill and Calhoun pointed out that in early reports published between 1956–1959, norethynodrel with mestranol was used in doses of 20–30 mg. (norethynodrel)/day for various menstrual disorders but that no thrombophlebitis was reported. A compilation of 29 of the early (1959–1966) major studies with norethynodrel and mestranol revealed one case of thrombophlebitis in 4271 woman-years of use, yielding a calculated incidence of 0.23 case/1000 women/year. In these studies the doses of norethynodrel ranged from 2.5 to 10.0 mg. In 16 studies in which norethindrone (2.0–10.0 mg.) with mestranol was used, three instances of thrombophlebitis were found during 4427 woman-years of use, yielding a calculated figure of 0.68 case/1000 women/year.

Drill and Calhoun also compiled the data from six major studies, reported between 1962 and 1965, which employed eight different contraceptive preparations and encompassed a total of 50,781 woman-years of use. A total of 28 cases of thrombophlebitis occurred in the entire group, providing an incidence rate of 0.55 ± 0.37 (range 0–3.7) case/1000 women/year. They stated that taken singly or totally the data from these studies did not provide any evidence of a relationship between oral contraceptives and thrombophlebitis.

The authors were critical of the 1967 study conducted by the Royal College of General Practitioners (96) because the matching of affected patients with controls had not been exact. Also, if the two affected patients who had stopped taking their oral contraceptives more than a month prior to the episode were dropped from the list, and if a figure of 10% were estimated for use of oral contraceptives in the general population, an incidence rate of 2.4 would be obtained. This overall rate would be well within the normal range.

Drill and Calhoun (104) also pointed out that the final figure arrived at by Vessey and Doll (98) for the estimate of risk of thromboembolic disease in oral contraceptive users, as judged from data on hospital admissions, was 0.5 case/1000 women/year in comparison with their figure of 0.55.

Studies involving the use of oral contraceptives for the treatment of endometriosis, in doses greater than those used for contraception, also were described by Drill and Calhoun (104); 456 patients were reported to have been treated on a continuous basis without a single report of thrombophlebitis.

Mortality statistics were presented for deaths due to thromboembolic disease and to pulmonary embolism in 20–44-year-old men and women in England and Wales for 1956–1966. The apparently slight increase over the 10-year period existed for both men and

women; as a result, there was no significant change in the ratio of deaths in women to those in men.

Data concerning the incidence of pulmonary embolism, both idiopathic and with other factors present, in young adults at a London Hospital for 1954–1964 also were presented by Drill and Calhoun. There was a significant increase over that time period. However, they pointed out that similar numbers of males and females had been involved, and that none of the females had been taking oral contraceptives.

Seigel and Markush (105) reviewed U. S. mortality statistics in which various thromboembolic diseases were involved. They stated that the mortality trends in this country were consistent with those shown by the British studies, which indicated an association between oral contraceptives and venous thrombosis. As in the British studies, an association with coronary thrombosis was not shown. However, the U. S. trends, unlike the British ones, did not suggest a relationship with cerebral embolism and thrombosis either. The relative risk of death from venous and pulmonary embolism estimated by Seigel and Markush was of the same order of magnitude as indicated in the British studies.

In 1969, Vessey and Doll (106) published a subsequent report of their findings which was a continuation of the study described previously (98); the same procedures were used throughout and similar results were obtained. Data obtained on 26 additional patients were added in this study to those obtained from the original 58 patients; in addition, several other factors were examined. For example, a comparison was made of the number of patients affected with thromboembolism and the number that would have been expected to be affected with regard to the type of oral contraceptive combination used. Seven combinations of two estrogens and seven progestagens had been used by the group. None had used sequential products, and no doses for the combined products were given. The authors concluded that the data did not provide any evidence that the risk of thromboembolism might be greater with preparations that they believed to be more inherently estrogenic. The data were reviewed in an attempt to determine whether the duration of use of the oral contraceptives at the time of onset of the attack might be a determining factor. The data did not suggest to them that either short-term or long-term use might carry a greater risk of producing thromboembolism.

In their earlier report, Vessey and Doll (98) noted that the affected patients, on the average, were heavier smokers than were the controls. In this study, they found no evidence to verify the earlier findings. Frederiksen and Ravenholt (107) retabulated the data of Vessey and Doll (98, 106) and concluded that these data could not be used as evidence against the possibility that cigarette smoking could potentiate the role that oral contraceptives might play in the pathogenesis of thromboembolism.

Crombie and Cross (108) were critical of Vessey and Doll's report (106). They suggested that most of the difference between the higher estimate of risk of thromboembolism in oral contraceptive users supplied by Vessey and Doll and their own lower estimate could be attributable to an increased hospital admission rate, on the order of 2.5 :1, for users as opposed to nonusers.

Hougie (109) also was critical of the estimated ninefold increase in thromboembolic disease in oral contraceptive users given by Vessey and Doll. He quoted a study in which a methodical examination of one lung resulted in the finding of pulmonary emboli in 52% of 263 autopsy cases while routine autopsy procedures on the other lung revealed pulmonary emboli in only 12%. Since the possibility that oral contraceptives might be implicated in thromboembolism had been recognized by the medical profession since 1962, Hougie suggested that more extensive physical examinations and autopsy procedures might have been carried out on users as opposed to nonusers in an effort to find evidence of thromboembolism.

Sartwell *et al.* (110) reported in 1969 on an epidemiologic, retrospective, case-control study conducted in five large eastern cities in the United States. This study involved 15–44-year-old women who had been discharged from 43 hospitals within the previous 3 years following a thromboembolic episode of any type. From their hospital histories, cases were excluded on the following grounds: a chronic condition thought to be predisposing to thromboembolism, recent trauma or surgery, pregnancy, sterility, and conditions that might be considered as contraindications to oral contraceptives. Some cases were excluded later because of uncertainty of diagnosis, prior attack of thrombophlebitis, or evidence of some predisposing condition. Of 2648 records of cases of thromboembolism abstracted at the hospitals, 2387 were rejected because of the reasons given above; only 175 cases with an unequivocal diagnosis of idiopathic thromboembolism were finally accepted.

Married control patients were individually matched with the cases for age within 5 years, residence, race, parity, hospital room pay status (private, semiprivate, or ward), and discharge from the same hospital within the same 6-month period. The same exclusions, on the basis of infertility or chronic disease, were also applied to the controls. During the study, however, in three of the cities, unmarried cases and controls were included because: "it had become evident that idiopathic thromboembolism was relatively frequent among younger, unmarried women, and it seemed unwise to disregard this source of case material."

The most common thromboembolic problem was thrombophlebitis of the lower extremity (115 cases) followed by thrombophlebitis with pulmonary embolism (19 cases), pulmonary embolism (18 cases), intracranial or carotid vascular lesion (13 cases), retinal vascular lesion (six cases), and thrombophlebitis at other sites (four cases). Coronary artery disease was not represented.

The relative risk of thromboembolism was determined by dividing the number of instances, 57, in which only the case had used oral contraceptives by the number of instances, 13, in which only the control had used them. By using this formula, a relative risk of 4.4 was determined. Sartwell *et al.* (110) pointed out that 26 of the cases were student nurses, all of whom had thrombophlebitis of the lower extremity and only two of whom had used oral contraceptives. They suggested that some

hospital jobs that involved standing on one's feet for considerable periods might be conducive to thrombophlebitis of the lower extremities.

A comparison of the period of use of the oral contraceptive preparations ranged from between less than 1 week to 2 years before admission. There was no evidence to indicate an increased risk with use up to 2 years.

The oral contraceptive preparations used in this study included three sequential products and nine combination products. The doses of estrogen ranged from 50 to 150 mcg. (mestranol) or 50 to 100 mcg. (ethinyl estradiol), and doses of seven different progestagens ranged from 1.0 to 25.0 mg. To determine whether either the combined or sequential products might show a greater propensity toward producing thromboembolism, a comparison of these two types of products was made. Fifteen cases and no controls had used the sequential type; the corresponding numbers for the combination products were 48 and 23, respectively. It was concluded that these ratios displayed a statistically significant difference. Furthermore, in the group of sequential users, seven of 15 developed pulmonary embolism. However, the doses of mestranol were higher in some of the combined preparations than in the two sequential products containing this estrogen. No additional information was given to indicate an association, or a lack thereof, between any of the other disorders and any of the preparations.

Inman *et al.* (111) analyzed reports of thromboembolism, following the use of oral contraceptives, received by drug safety committees in the United Kingdom, Sweden, and Denmark. The purpose was to determine whether the risk of thromboembolism might be related to the nature and/or doses of the steroids in the various preparations. The numbers of reports attributed to each product were compared with the distribution that would have been expected based on market research estimates of sales, assuming that all products carried the same risk.

In comparing sequential *versus* combined preparations containing corresponding doses of ethinyl estradiol or mestranol, Inman *et al.* (111) found no evidence to indicate that either type of preparation might have had a greater propensity to produce venous or arterial thromboembolism. In contrast, a comparison of the number of reports of thromboembolism with various doses of the two estrogens showed a consistently higher number of reports in which preparations containing higher doses of the estrogens were used. This trend was observed for cases of pulmonary embolism, cerebral thrombosis, and coronary thrombosis.

Observed and expected reports of thromboembolic disorders in patients using 12 different combinations of estrogen and progestagen were presented. These involved doses of mestranol of 50, 75, 100, and 150 mcg. and doses of ethinyl estradiol of 50 and 100 mcg., in combination with one of six different progestagens. No two preparations contained the same dose of any progestagen and, therefore, no direct comparisons could be made. Furthermore, the total number of patients using the preparations varied from less than 15 up to 76; as a result, when thromboembolism was categorized into four different types, there were often less than six affected patients per preparation. Nevertheless, the authors pointed out a "significant" deficit of reports in all categories of thromboembolism for the combination of mestranol (100 mcg.) and norethynodrel (2.5 mg.), in comparison with other combinations of progestagens with this dose of mestranol. Also, a "significantly higher" number of reports of venous thromboembolism was found for the combination of ethinyl estradiol (50 mcg.) and megestrol acetate (4 mg.) when the latter was compared with other combinations of progestagens with the same dose of ethinyl estradiol. The authors suggested that such inconsistencies might represent an influence of the progestagenic components.

It was concluded in this study that there was a positive correlation between the risk of thromboembolism and the dose of estrogen but that there was no difference between the two estrogens, nor between the combined and sequential products when these products contained the same type and dose of estrogen.

Nanni (112) criticized this study because it had been based on voluntary reports from physicians and because no patient control data had been used. He also objected to the use of a market research estimate of sales as a substitute for control data.

Anderson (113) compared mortality rates for women, 15–44 years old, in Ontario, Canada, for 1959–1961, when oral contraceptives had not been used, and for 1966–1968, the most recent period of contraceptive use for which mortality statistics were available. He estimated that 20–30% of the women in the age group studied during the second time period had used oral contraceptive preparations.

According to Anderson, the death rate from venous thromboembolic disease had increased from 0.32/100,000 women/year in 1959–1961 to 0.97 in 1966–1968 (+200%); the corresponding increase for men was from 0.36 to 0.45/100,000 men/year (+25%). The increase in mortality attributed to cerebral thrombosis was from 0.24 to 0.38 (+58%) for women and from 0.29 to 0.34 (+17%) for men. Increased mortality due to thromboembolic disease in other vascular beds was not evident.

This retrospective study did not utilize case controls of any type. Furthermore, the author stated that it was well recognized that the validity of diagnostic information on death certificates often was doubtful. However, he felt that if a causal relationship did exist between oral contraceptives and thromboembolism, "it would be surprising if the new treatment did not eventually result in a change in the death rate."

Anderson pointed out that although the oral contraceptives seemingly had caused a large percentage increase in venous thromboembolism and cerebral thrombosis, these causes of death represented a very small fraction of the total mortality for that age group. Furthermore, the decrease in mortality rate associated with pregnancy and childbirth was much greater than the increase in mortality supposedly produced by oral contraceptives.

In contrast to previous studies that had merely determined the incidence of thromboembolic disease, Irey *et al.* (114) conducted a study concerning the type of

pathological lesion found in the blood vessels of women who had suffered from thromboembolism. Blood vessels from 20 women who had succumbed to thromboembolism while taking oral contraceptives were studied histologically and compared with similar preparations from 22 women in the same age category who had not been taking oral contraceptives but who had died from diseases associated with thrombosis and thromboembolism. The oral contraceptive users ranged in age from 18 to 41 years. Fifteen were white and five were black, and none had conditions thought to be predisposing to thromboembolism. The duration of use of the oral contraceptives ranged from 5 weeks to 13 months; two patients had been taking sequential preparations, and 15 others had been taking the combined type. Nine of the patients had also been taking other drugs including tranquilizers, anorectics, thyroid extract, and cortisone.

For the oral contraceptive users, distinctive vascular lesions in association with thrombosis were widely distributed in the arteries and veins. These lesions were characterized by the following intrinsic vascular changes: (*a*) three-layered thrombi of at least 1 week's duration, with underlying structural and microscopic changes (19 instances); (*b*) endothelial proliferation and intimal thickening, with no changes in the media and adventitia (four instances); and (*c*) focal nodular thickening of the intima, media, and adventitia (one instance). Vascular lesions without occlusive thrombi were present in four patients, indicating that the changes in the blood vessels were primary.

In the control group, intrinsic vascular lesions resembling those found in the oral contraceptive users were found in only one patient. This woman had suffered from pulmonary hypertension associated with congenital heart disease. Further inquiries concerning this patient revealed that she had been taking an oral contraceptive for 6 weeks prior to her death.

It was concluded from this study that although further studies were necessary to establish a causal relationship between the ingestion of steroid hormones and intimal hyperplasia, the consistent finding of these vascular changes in oral contraceptive users, as opposed to a lack of such changes in nonusers, suggested that such a relationship might indeed exist.

Opposed to the findings of the retrospective, epidemiologic British studies, results from other studies have been in agreement with the conclusions of Drill and Calhoun (104) that there is no causal relationship between oral contraceptive use and thromboembolism. For example, in 1970, Fuertes-de la Haba *et al.* (115) reported the results of an investigation of the causes of death in a group of women in Puerto Rico. From July 1961 to May 1969, 9633 women participated in the study (Project Population).

The women were distributed randomly into an oral contraceptive group (4846) and a vaginal group (4787). The oral group received norethynodrel 5 mg. with mestranol, and those in the vaginal group were provided with a vaginal contraceptive method excluding any form of intracervical or intrauterine device; pertinent instructions were given. The women also were given a comprehensive medical survey which included: (*a*) medical and socioeconomic history; (*b*) complete physical examination; (*c*) urinalysis; (*d*) cervical cytologic study; and (*e*) determination of hematocrit, 17-ketosteroids, and sulfobromophthalein (BSP) retention. The women returned at 2 month intervals, following the initial survey, to receive more contraceptive material and a health questionnaire. At yearly intervals they received a physical examination, urinalysis, and blood study, unless some abnormality had developed which demanded more frequent attention.

As of May 1969, 87% of the patients were under active supervision. Of the remainder, some had moved to other areas of Puerto Rico or the United States, some had been contacted but had not returned for the followup examination, and others had died from a variety of causes. Of the 30 verified deaths, 12 had occurred in the oral group and 18 in the vaginal group. When the death rates were averaged over the 8-year period of the study, the rate for the oral group was 4.0/10,000 and that for the vaginal group was 7.5/10,000. Therefore, the authors concluded that the data did not support the contention that mortality increases among oral contraceptive users.

Several months later, Fuertes-de la Haba *et al.* (116) published a subsequent paper involving the same individuals discussed previously (115), but data from 265 additional women were included. This report concerned the occurrence of thrombophlebitis in users and nonusers of oral contraceptives. Lists of patients diagnosed as having thrombophlebitis since the start of the experiment were obtained from all hospitals where the clinics were located and matched against the patient list of the study. Forty-eight cases of thrombophlebitis were found, and these patients were interviewed and examined by an internist. Of these, 31 were excluded for various reasons, including: (*a*) having no verification of the disease, (*b*) having had the disease before entering the study, and (*c*) being postpartum. Of the remaining 17, eight were from the control (vaginal) group and nine from the oral group.

The incidences of thrombophlebitis in the oral group and in the control group were 1.8/1000 and 1.6/1000, respectively; the difference was not statistically significant. The relative risk also was calculated after controlling for variables such as age and number of cycles of treatment; again no significant difference was found between the oral and control groups with respect to the risk of developing thrombophlebitis.

To determine the effect, if any, that oral contraceptives might have on the occurrence of pulmonary embolism in supposedly healthy persons, Zimmerman *et al.* (117) reviewed the records concerning this condition, of the Cuyahoga County Coroner's Office, for two periods of time: January 1, 1951, to July 31, 1962, before oral contraceptive use was introduced, and August 1, 1962, to December 31, 1969, during which period these drugs were used extensively. Persons included in the study were of either sex and between the ages of 15 and 45 years at the time of death; persons who had had a condition that might have led to pulmonary embolism were excluded.

During the first period, there were 28 cases of unexpected death due to pulmonary embolism: 10 in men,

13 in nonpregnant women, and five in pregnant women; figures for the second period were five, 10, and two, respectively. The crude death rate based on the 1960 census figures were as follows: for the first period, 2.55/1,000,000 for men, 3.04 for nonpregnant women, and 4.20 for all women; for the second period the figures were 2.16/1,000,000, 3.96, and 4.76, respectively. No significant difference existed between the frequency in men and nonpregnant women during either period, nor between the death rates of nonpregnant women when the two periods were compared. The authors concluded that this study did not reveal a significant increase in death due to pulmonary embolism in nonpregnant women since the time of introduction and widespread use of oral contraceptives.

Goldzieher (118) examined 23,217 subjects who were using a sequential type of oral contraceptive. The subjects were required to make monthly visits to clinics or Planned Parenthood Centers to obtain their contraceptive supplies and were followed intensively through 363,469 cycles, an average of 16 months per patient. Seventeen instances of thrombophlebitis occurred, producing an incidence of 0.56/1000 women/year. As the author pointed out, this figure was strikingly similar to the figure given by Drill and Calhoun (104) for the control population and for the incidence found in users of the combination type of oral contraceptives.

In 1970, Goldzieher (80) critically reviewed the evidence associating oral contraceptives with thromboembolism. He stated that statistical studies of the type presented in the British and American retrospective, epidemiologic studies did not establish causal relationships but simply established strengths of correlation, a fact generally recognized by statisticians and clinical investigators.

In 1972, Drill (119) reported a study which included data in addition to those reported previously (104). This report summarized data concerning the incidence of superficial and deep vein thromboembolic disease of the lower extremity in untreated (normal incidence), postpartum, and antepartum women and in users of oral contraceptives. Women with and without predisposing conditions were included.

Based on data concerning hospital admissions obtained from several countries, the average normal incidence of the disease in women of childbearing age was 0.9 (range 0.71–1.08)/1000 women/year. When the incidence was based on patients' visits to the physician, the rate was 2.2 cases/1000 women/year. For idiopathic cases alone, the rate was 0.65/1000 women/year.

In postpartum patients the frequency of these diseases was elevated. Based on 199,730 pregnancies, the total incidence was 9.8 cases/1000 women; this figure was divided between 5.3 for superficial vein involvement and 4.5 for deep vein involvement.

During the antepartum period, the incidence was reduced. Based on 350,661 pregnancies, the incidence of superficial and deep vein disease was 0.46 case/1000 women/9-month pregnancy. When this figure was extrapolated to 12 months, it became 0.61: 0.36 for deep vein disease and 0.31 for superficial vein involvement. The rate of 0.61 was not above that found for nonpregnant women, suggesting that the increased secretion of estrogen during the antepartum period did not result in an increased incidence of thromboembolism.

The incidences of thromboembolic disease reported between 1963 and 1970 in 15 large-scale and 53 small-scale prospective studies of oral contraceptive users were compiled. In the large-scale studies involving 66,915 women treated for 68,616 woman-years, the average rate was 0.92 case/1000 women/year. The difference between this figure and that for normals (2.2) was not statistically significant. The small-scale studies totaled 13,162 woman-years of experience. The average rate was 1.22 cases/1000 women/year, and again the difference between it and the nonuser figure was not statistically significant. When the two types of studies were totaled, 81,778 woman-years of experience were represented, and the average rate was 0.97 case/1000 women/year.

The large-scale prospective studies also were utilized to determine the incidences of deep vein and superficial involvements separately. Based on 32,668 woman-years of use, the rate for deep vein disease was 0.21 case/1000 women/year and it was 1.04 for superficial involvement. These rates were not above the control values.

It was also determined from this extensive study that five cases of pulmonary embolism were associated with oral contraceptive use during 65,281 woman-years, yielding a rate of 7.7/100,000. Drill (119) also quoted from the unpublished data of Dr. R. L. Burket that the incidence of pulmonary embolism in 15–44-year-old nonpregnant women in 12 Cincinnati hospitals during 1959 was 15.9/100,000 and that the rate for idiopathic cases alone was 10.8.

Drill summarized his review by listing six salient points:

1. The increased output of hormones during pregnancy was not associated with an increase in the incidence of superficial and deep vein thromboembolic disease.

2. Neither large-scale nor small-scale studies of oral contraceptive users demonstrated an increased incidence of the diseases.

3. If a relationship between oral contraceptives and thromboembolism existed, a high proportion of cases should occur during the first few months of use; this point would not be true if the relationship depended on a cumulative effect of the drugs. However, studies such as those of Vessey and Doll (106) and Sartwell *et al.* (110) suggested that duration of use, at least up to 2 years, had no effect on the incidence of thromboembolism.

4. If a dose–response relationship existed between oral contraceptive use and thromboembolism, increased evidence for the relationship should have been obtained when higher doses of the compounds were administered. Drill cited evidence that large doses of oral contraceptive preparations had been given without adverse effects occurring. Some inconsistencies in this respect do exist, however (*vide supra*); the answer might be related to whether the large doses refer to estrogenic and/or progestagenic activity.

5. If a relationship existed between oral contraceptives and thromboembolic disease, patients with a

history of the disease might be expected to be sensitive to these drugs. Drill pointed out that the data available, although limited in amount, did not demonstrate an increased recurrence of thrombophlebitis when oral contraceptives were used.

6. Women who have developed thrombophlebitis have responded to therapeutic measures without stopping the use of oral contraceptives. Again, however, the data are quite limited.

Drill concluded that the extensive prospective studies he had reviewed demonstrated that oral contraceptive use did not increase the incidence of venous thromboembolic disease of the lower extremities.

At the same time, Drill and Calhoun (120) reported a review of data from prospective studies concerning the relationship between the dose of estrogen in oral contraceptive preparations and thromboembolic disease of the lower extremities. This study had two goals: (*a*) to determine whether the disease rate might be above normal at any dose of either mestranol or ethinyl estradiol and (*b*) to determine whether a statistically significant dose-response curve could be found between the estrogen dose and the incidence of thromboembolic disease.

The prospective studies included for analysis were those in which a definite statement had been made regarding the presence or absence of superficial and deep vein thromboembolic disease, associated with the use of a specific oral contraceptive product for which the number of woman-years of drug use had been stated. Data from these studies then were analyzed in three groups: (*a*) large-scale studies involving 1000 or more woman-years of use, (*b*) small-scale studies involving less than 1000 woman-years of use, and (*c*) a group wherein the data from the large- and small-scale studies were combined after it had been shown that there was no difference between the two sets of data.

The data collected involved a study of 72,075 women for 902,446 cycles during a total of 69,502 woman-years of use. The oral contraceptive preparations studied contained one of the following six doses of estrogen: 50 or 100 mcg. ethinyl estradiol or 75, 80, 100, or 150 mcg. mestranol. No information was given concerning the progestagen(s) in the preparations.

There was no significant difference between the thromboembolic disease rates associated with the two doses of ethinyl estradiol, and these incidences were not significantly different from the normal rate of 2.2/1000 women/year. Neither did the disease rates differ among the four doses of mestranol, nor did they differ significantly from the normal rate.

The authors critically discussed the study of Inman *et al.* (111), which initiated the idea that a relationship might exist between the risk of thrombophlebitis and the dose of estrogen. They offered criticisms of the study similar to those discussed previously by Nanni (112). Because of these fallacies, Drill and Calhoun (120) questioned the conclusions drawn by Inman *et al.* (111). Nevertheless, the latter workers at least attempted to consider the possible effects of the progestagenic components, a point apparently ignored by Drill and Calhoun.

Meanwhile, additional articles, each containing a small number of case reports concerning thromboembolic phenomena in oral contraceptive users, appeared in the recent literature. The phenomena observed include: aorto-iliac occlusion (121), dural sinus and cerebral venous thrombosis (122–124), cerebral arterial occlusion (125–128), cerebral ischemia (129), vascular occlusion of the colon (130), Budd–Chiari syndrome (131), mesenteric vascular thrombosis (132, 133), myocardial infarction (134–137), and pulmonary embolism (138–140). As mentioned previously, the usefulness of these case reports in establishing a cause-and-effect relationship is nil. However, their importance is manifested in the fact that by uncovering and reporting such information, others can be made aware of their possible occurrence. This awareness can lead to similar observations which, when numerous enough, may become meaningful and deserving of case-control epidemiologic and prospective studies. For example, some investigators (128, 130, 133, 141) found that certain thromboembolic disorders originated at the time that oral contraceptive therapy started but that the symptoms regressed spontaneously a short time after terminating their use. This type of observation suggests a degree of involvement between drug and effect.

In summary, the evidence for the existence of a cause-and-effect relationship between thromboembolism and oral contraceptive use is tenuous. It consists for the most part of data from retrospective, epidemiologic, survey-type studies which, as previously stated, cannot prove such a relationship. On the other hand, the large volume of data from prospective studies from which one is better able to establish such a relationship indicates that such a relationship may be nonexistent.

While retrospective and prospective studies have been, and continue to be, carried out in an effort to determine conclusively whether or not there is a cause-and-effect relationship between oral contraceptives and thromboembolism, concurrent investigations have attempted to elucidate factors that might be involved in the development of thromboembolism in certain users of oral contraceptives. Specifically, these investigations have involved blood types and clotting factors.

In 1969, Jick *et al.* (142) reported the results of a cooperative study involving three countries. Initially, data collected for a prospective drug surveillance program in Boston had revealed that among male and female patients receiving anticoagulants for venous thromboembolism, there was a deficit of patients with type O blood. These results stimulated the cooperation of interested groups in Sweden and the United Kingdom. The three groups then conducted retrospective studies to determine the blood types of young white women who had developed thromboembolism: (*a*) while taking oral contraceptives, (*b*) during pregnancy or the puerperium, or (*c*) while neither pregnant nor taking oral contraceptives. In addition, the proportions of blood types A, B, AB, and O in control populations were determined. The percentages for the three countries ranged from 37 to 44% for type A, from 8 to 12% for B, from 3 to 6% for AB, and from 40 to 47% for O.

The numbers of patients representing blood groups B and AB were too small to permit valid comparisons with the control population. However, a trend could be

seen when comparisons were made between the proportions of thromboembolic patients and controls having blood types A and O. Specifically, the percentage of patients having type A ranged from 47 to 61; for type O, the percentage ranged from 16 to 38. These observations, which applied to all three groups of young white women, suggested that individuals with type A blood were more likely to develop thromboembolism and/or that those with type O were less likely to do so.

In 1971, Mourant *et al.* (143) recalculated the data of Jick *et al.* (142) after including some additional data from other studies. They determined the relative incidence of blood group A by dividing [(number of A patients)/(number of O patients)]/[(number of A controls)/(number of O controls)]. In women taking oral contraceptives, the estimated mean incidence of group A was 3.12; in pregnant and puerperal women, it was 1.85. For both conditions, the difference between cases and controls was statistically significant; however, no conclusion could be drawn as to relative liability to thrombosis of women taking oral contraceptives as opposed to those who were pregnant or puerperal.

Additional supportive data were supplied later in 1971 by Westerholm *et al.* (144) and Allan (145). In addition, Allan offered the hypothesis that hormonal factors might intensify what appeared to be a basic association between thromboembolism and blood group A. He listed some indirect evidence that could lend support to his hypothesis, including: (*a*) oral contraception raises the serum cholesterol level, which is already higher on the average for blood group A_1 than for O; and (*b*) oral contraception lowers the antithrombin-III level, which is already lower on the average for A_1 than for O. Furthermore, Fagerhol *et al.* (146) found that pulmonary embolism and diffuse intravascular coagulation also are associated with lowered levels of antithrombin-III; in contrast, levels of the latter appear to be increased during oral anticoagulant therapy.

Although precipitating and contributory factors have long been identified, the basic mechanism of thrombosis is unknown (147). Nevertheless, studies of moieties, such as antithrombin-III, involved in the blood coagulation or fibrinolytic systems have been undertaken because of the possible relationship between oral contraceptives and thromboembolism. It has been presumed that a balance is normally maintained in the blood between coagulative and fibrinolytic activities (148). It has been suggested, furthermore, that oral contraceptives might upset this balance by causing an increase and/or decrease in one or more of the components of these systems.

Many factors of the coagulation and fibrinolytic systems have indeed been shown to be affected by oral contraceptives. For example, electrophoretic behavior of blood platelets from women taking combined or sequential oral contraceptives, but not progestagen-only oral contraceptives, became abnormal due to increased sensitivity to adenosine diphosphate; the sensitivity to noradrenaline, however, remained normal (149). This pattern of platelet behavior was similar to that shown by platelets from patients with arterial disease.

Factors II (prothrombin) (150, 151), VII (150, 152, 153), X (150–153), and fibrinogen (151), all components of the coagulation system, have been shown to increase in women taking oral contraceptives of the combined type. Similar increases in Factors VII and X have been seen in women using the sequential type (152). In contrast, other investigators reported no significant change in fibrinogen levels in women using oral contraceptives of the combined (148, 154), sequential (148), and progestagen-only (154) types, nor in those using combined and progestagen-only injectable contraceptives (154). Nevertheless, blood viscosity, which is partly a function of fibrinogen concentration, and hematocrit were shown to be increased in eight of 47 women using combined and sequential oral contraceptives (155). Furthermore, cryofibrinogen, a cold-precipitable fibrinogen found in disease states such as those associated with intravascular coagulation, was found in the plasma of 17 of 68 users of combined and sequential oral contraceptives, seven of 55 users of an intrauterine contraceptive device, and five of 59 untreated women (156).

Antithrombin levels (*vide supra*) have also been shown to be depressed in women taking oral contraceptives of unspecified and combined types (157–160). Moreover, it has been suggested (159) that of all the clotting changes reported to occur in women using oral contraceptives, the loss of antithrombin-III activity might be the one most significant from a clinical point of view. On the other hand, Hedlin and Monkhouse (148) found no significant changes in antithrombin levels in women using combined and sequential preparations.

Reports concerning fibrinolytic activity and plasminogen levels, as well as interpretations of increases in the latter, in users of steroid contraceptives have been even more conflicting. Most workers (148, 150, 154) reported an increase in fibrinolytic activity in women using oral preparations, suggesting the possibility of a tendency toward hypocoagulability. Brakman *et al.* (154), however, found no change in this parameter in women given a parenteral estrogen–progestagen preparation and decreased fibrinolytic activity in women given an injectable progestagen preparation.

Plasminogen levels also have been reported by most workers (150, 151, 154, 158) to be increased in women using oral contraceptives. However, Brakman *et al.* (154) reported that in earlier studies they had found either no change or decreased levels of plasminogen in oral contraceptive users; these workers also reported no change in plasminogen levels in women given parenteral preparations. The ranges of values presented by these workers did overlap among the controls and various treatment groups, however, and, unfortunately, no levels of statistical significance were given to support the conclusions drawn.

With respect to the clinical significance of increased plasminogen levels, Ambrus *et al.* (151) interpreted this phenomenon as an indication of increased activity in the fibrinolytic system. On the other hand, Peterson *et al.* (158) interpreted low plasminogen levels as evidence of increased fibrinolytic activity. Conversely, high levels suggested to them the possibility of an increased risk of thrombosis. Presumably, they felt that

increased levels signified that the plasminogen was not being converted to plasmin (fibrinolysin). Indeed, Ygge *et al.* (150) reported high plasminogen levels and low spontaneous fibrinolytic activity in patients 1–2 weeks postoperatively, *i.e.*, during a period in which there is a high incidence of thromboembolism.

The question remains, however, as to what significance these changes in clotting and fibrinolytic factors may have with respect to thromboembolism and the use of oral contraceptives. First of all, results from different studies obviously are in disagreement. Brakman *et al.* (154) suggested that these discrepancies could have been caused by differences: (*a*) among assay procedures, (*b*) in the composition of the various hormone preparations used, (*c*) among the dosages of the various hormones, and/or (*d*) in the duration of treatment. Finally, individual treatment and control groups in some studies occasionally contained five or fewer women.

More importantly, it is still necessary to determine whether a certain pattern in blood coagulation and fibrinolysis may really be related to an increased incidence of thromboembolism (150). Nilsson and Astedt (161) maintained that there are no common characteristic changes in the coagulation factors, fibrinolytic system, and platelet aggregation or adhesiveness in patients with thrombosis, although they agreed that a normal fibrinolytic activity in vessel walls is important for counteracting thrombosis. Wolf (162) also questioned the association of clotting factor changes with thrombosis, maintaining that there is no definite distribution pattern of antithrombin-III in patients with thrombosis and that measurement of Factor VII activity, for example, shows much overlap between normal controls and patients with thrombosis.

At any rate, it has been stated (147) that increased levels of clotting factors in the nonactivated form could possibly predispose to thrombosis, but that they could not *per se* initiate intravascular coagulation. However, if together with a loss in antithrombin-III activity, for example, the homeostatic coagulation equilibrium is further unbalanced by events such as vascular endothelial damage, vasculitis, prolonged stasis, increased platelet adhesiveness, or postsurgical hypercoagulability, intravascular clotting could possibly be precipitated (159). Finally, Peterson *et al.* (158) suggested that perhaps only an occasional individual may be unable to handle the changes that appear to be induced by the oral contraceptives. Whether or not such patients could be screened out prior to or during oral contraceptive therapy is not known, but the measurement of clotting factors in users and potential users of these agents would seem to be worthwhile.

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RESEARCH ARTICLES

Drug Transfer across Intact Rat Intestinal Mucosa following Surgical Removal of Serosa and Muscularis Externa

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Abstract □ The *in vitro* absorption kinetics for salicylate ion were followed through rat intestinal membranes from which the serosa and longitudinal and transverse layers of the muscularis externa had been removed. Techniques are described where up to 15 cm. of intestine may be stripped of musculature so that absorption studies may be carried out utilizing commonly employed *in vitro* methods such as the everted sac, the Crane–Wilson technique, and a perfusion apparatus. A histological examination of the stripped intestinal mucosa showed that the section consisted of epithelium, underlying lamina propria, muscularis mucosa, and submucosa. The latter two layers do not remain when mucosa is isolated by scraping off the mucosal surface with a glass slide, but they are necessary for maintaining an intact epithelial membrane during *in vitro* transport studies. Transfer rates were measured using 10 cm. of everted intestine in a perfusion apparatus and 5 cm. of everted intestine made into a Crane–Wilson sac. Absorption rates for salicylate through stripped intestinal segments were found to be 1.5–1.8 times greater than those found with nonstripped segments.

Keyphrases □ Drug transfer—*in vitro–in vivo* correlations improved, serosa and muscularis externa removed from intact rat intestinal mucosa □ Transfer rates—procedure for removal of serosa and muscularis externa from intact rat intestinal mucosa preparations, improved *in vitro–in vivo* drug transfer correlations □ Absorption kinetics, *in vitro*—salicylate ion through rat intestinal membrane stripped of serosa and muscularis externa, improved correlation with *in vivo* data □ Intestinal mucosa preparation, rat—procedure for stripping serosa and muscularis externa to improve *in vitro–in vivo* drug transfer correlations

Since many intestinal absorption studies in the pharmaceutical sciences are done employing *in vitro* techniques, the obvious question arises: What correlation can be attached to observations made from *in vitro* studies and events occurring *in vivo*? Ideally, one would hope that the information gained from studying absorption mechanisms can be applied ultimately to the design of more predictable and more effective oral drug dosage forms.

As pointed out by Turner *et al.* (1), a number of studies in the literature showed large differences between *in vitro* results and those expected upon the basis of *in vivo* hypotheses. While one cannot expect *in vitro* preparations to provide a direct reflection of *in vivo* events, one can hope to gain insight into mechanisms that are difficult or impossible to elucidate *in vivo*. However, the reliability of *in vitro* observations in providing meaningful information depends upon experimental conditions conducive to optimum physiological activity. Up to the present time, three commonly employed *in vitro* techniques have been utilized in studying drug absorption: the everted sac method of Wilson and Wiseman (2), the Crane–Wilson method (3), and a perfusion apparatus (4, 5) where transmembrane potentials are neither measured nor short circuited. Some of the problems inherent to these *in vitro* methods that may be contributory to *in vitro versus in vivo* absorption discrepancies are: (*a*) maintenance of biological viability, (*b*) maintenance of structural integrity, and (*c*) artifactual influences due to unnatural absorption barriers.

A number of workers have investigated the extent of viability of excised gut preparations. Under controlled conditions, rather constant metabolic activity for 2–3 hr. was demonstrated with the rat intestine by Bramford (6) and Jordana and Ponz (7) through oxygen consumption studies and by Robinson and Felber (8) with L-methionine and L-phenylalanine uptake. Duration of viability during drug absorption studies would be affected by drugs that are toxic to, or interfere with, metabolic mechanisms and/or the use of bathing solutions (especially buffered solutions) that are not of optimum physiological compatibility.

The problem of structural integrity has been considered by some (9, 10) to be even more crucial to the absorption processes than viability, especially where passive mechanisms seem to predominate. Alteration in structural relationships certainly would have a major influence upon the passive absorption of drugs and, consequently, the ques-