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MEDICAL COLLEGE OF VIRGINIA QUARTERLY
VOLUME EIGHT • NUMBER ONE • 1972

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hyoscine hydrobromide	0.0065 mg.	0.0065 mg.	0.0195 mg.
phenobarbital	(¼ gr.) 16.2 mg.	(½ gr.) 32.4 mg.	(¾ gr.) 48.6 mg.

Brief summary. Side effects: Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction as in prostatic hypertrophy. Contraindicated in patients with acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

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Advances in Reproduction

The Department of Obstetrics and Gynecology is honored to have been asked to develop the 43rd Annual McGuire Lectures. We were especially pleased that this meeting coincided with the Annual Meeting of the H. Hudnall Ware, Jr. Society which is composed of the former residents of our Department of Obstetrics and Gynecology.

The purpose of the program was to summarize major areas of recent advances in the field of reproduction. This intellectual time capsule could serve us well as a point of reference by which we may measure past and future progress. We chose as our speakers and panelists those persons who could speak with unquestioned authority in each specific field. We are grateful to the participants, especially Dr. Allan C. Barnes, our McGuire Lecturer, for assembling in Richmond to honor the memory of Dr. McGuire.

This issue of the MEDICAL COLLEGE OF VIRGINIA QUARTERLY includes most of the lectures given at the symposium. The two McGuire Lectures have been summarized by the editorial staff, as Dr. Barnes did not wish to have them published in their entirety.

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Chairman, Department of Obstetrics and Gynecology
Medical College of Virginia

Current Knowledge of Gonadotrophin Releasing Factor(s)*

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In discussing the subject of gonadotrophin releasing factor(s) I cannot help but feel that for once in my life I am really in tune with the times, since the same subject was recently given a lengthy and serious airing on a popular morning television program and has also been mentioned in the lay press. It seems safe to predict that practitioners of gynecological medicine will soon be asked, possibly even deluged with demands, to explain the significance of the releasing factor(s) to their patients. I am not sure that what I am going to say here will be exactly what these patients should be told, or what they will want to hear, but I think it should form the basis for a frank representation of the facts.

What I want to discuss are not the potential therapeutic or diagnostic uses of these newly available compounds, which are really very speculative, but certain features of the physiological control system in which these compounds are thought to play key roles. Any rational system of therapeutics or prophylaxis involving these releasing factors, or their derivatives, must be based on an adequate understanding of (1) the physiological roles of these compounds, (2) how these roles may be altered by disease, and (3) the effects specific disturbances of the control system(s) in which they operate can be expected to have on reproductive processes.

I will not review the background for my remarks in detail, since I have recently done that elsewhere (Bogdanove, 1972). The relevant literature is quite extensive but efforts to synthesize meaningful interpretations of it have not been lacking. There are several recent or imminent review articles,† and even an entire book (Meites, 1970), which would be particularly helpful as keys to this literature.

It is generally accepted today that many if not all of the secretory functions of the anterior pituitary gland are controlled, at least to some extent, by the central nervous system. The vascular link between the brain and the pituitary, the hypothalamic-pituitary portal system of veins, is viewed as the final common pathway of neural control. The idea that the neural influences are mediated by "neurohumors,"‡ transmitted to the gland by these portal veins, was first suggested by Friedgood (Friedgood, 1936) and later placed on a solid experimental footing by G. W. Harris and his associates (Harris, 1948; Harris, 1955; Harris and Campbell, 1966).

A large body of experimental evidence indicates that the pituitary gland secretes little or no LH and FSH if it is deprived of contact with the hypothalamus, but that the injection of hypothalamic extracts can cause these two hormones to be secreted. Similar partial or complete dependence upon hypothalamic "neurohumoral" support is characteristic of the secretion of TSH, growth hormone, and ACTH. However, the hypothalamic influence upon the secretion of prolactin has been asserted to be inhibitory, rather than stimulatory, since the isolated pituitary secretes a lot of prolactin and treatment with hypothalamic extracts can suppress this hypersecretion.

* Presented at the 43rd Annual McGuire Lecture Series, December 2, 1971, at the Medical College of Virginia, Richmond.

† McCann and Porter, 1969; Burgus and Guillemin, 1970; Schally and Kastin, 1970; Gay, 1972; Saffran, 1972; Schally, Kastin, and Arimura, 1972.

‡ Although these compounds do originate in the nervous system, there is no evidence that they are secreted by neurons. The possibility that they may be secreted by glial elements (Knigge and Scott, 1970) cannot be overlooked.

The active component(s) of the crude hypothalamic extracts have been frustratingly elusive, so much so that some investigators (Schreiber, 1967) have occasionally wavered in their faith that they would eventually be isolated. Those who held the faith, however, (as well as the rest of us) have been rewarded by the recent isolation and identification of several of the releasing factors* and the consequent preparation of pure molecules by organic synthesis.

The two releasing factors which now appear to be important for reproduction are TRH and GnRH. It is the identifications of these two molecules which have been hailed (quite rightly) as achievements worthy of the attention of the news and propaganda media. It is on these two compounds, about which the lay public (ever ready to plumb the mysteries of sex) will soon be demanding information, that my remarks will focus.

The term "releasing factor" (RF) was coined by McCann (McCann, Taleisnik, and Friedman, 1960) to signify the effect such a compound could exert on the cells of the anterior pituitary gland—causing them to release a portion of their hormonal content. Over the past 10 years, this term—RF—has largely supplanted the earlier name for this group of neurohumoral factors—"hypophysiotrophins" or "hypophysiotrophic agents" (Guillemin and Rosenberg, 1955)—which had a broader denotation. The physiological process involved in hormone secretion can be considered to have two principal phases: *synthesis* (or production) of the hormone and *release* of the hormone into the circulation (Gay and Bogdanove, 1968). In the case of several of the hormones produced by the adenohypophysis, both phases of the secretory process depend upon the integrity of the hypothalamic-pituitary unit. If either the hypothalamus or the vascular connection between the hypothalamus and the anterior pituitary lobe is damaged, both release and synthesis of the pituitary hormones are impaired (Bogdanove, 1972). Under acute experimental conditions, it is much easier to determine whether something has stimulated release of a hormone than whether the processes of hormone synthesis have been influenced.

Figure 1 diagrams the relationship of the rates of synthesis and release to the amount of hormone stored in the gland (compartment I), as well as the relation between the rate of release from the pituitary and the levels of the hormone in the circulation. These levels, or titers, are the result of input and output rates. When hormone enters the blood faster than it leaves, titers increase; when the entry rate is less than the exit rate, titers decline. The concept is deceptively simple, however, since the size of compartment II, which represents the serum or plasma volume *plus* the summed volumes of a series of extravascular compartments in equilibrium with the blood, is not known. Consequently, it is not yet possible to establish the *amount* of hormone which has to be added or removed to produce a given change in serum hormone *concentration*. Conversely, it is not yet possible to quantitate, from measurement of changes in serum hormone concentrations, the causative inequality between the entry rate (rate of release of hormone from the pituitary into the blood) and the exit rate (the combined rates of destruction and excretion of circulating hormone) during the time that serum hormone titers were changing. One can merely infer that the inequality existed. However, since exit rates from the blood do not seem to vary as widely as rates of entry into the blood, major changes in serum hormone levels must reflect major changes in release rates.

Since the volume of compartment I can be measured by simply weighing the pituitary (at least in an experimental animal), it should be a very simple matter to relate quantitative changes in intrapituitary hormone stores to transient inequalities of synthesis (entry) and release (exit) rates. The catch lies in establishing changes in intrapituitary hormone stores, which poses a number of practical problems. The foremost is that sampling of intrapituitary hormone levels requires removal of the gland. This procedure, in contrast to blood sampling, cannot be repeated. However, in theory at least, any *change* in intrapituitary hormone content during a finite period of time would have to represent the product of that period of time and the algebraic sum of the synthesis (entry) and release (exit) rates.

Thus, *release* of a pituitary hormone can be detected, if not measured, solely by observing an increase in the concentration of the hormone in the circulation. To establish a concomitant change in hormone *synthesis* would be far more difficult. Since our conceptions tend to reflect our fields of vision, we tend to speak of "releasing factors" simply because effects on hormone release are more visible than effects on hormone synthesis.

In using the pituitary as a model for illustrating

* Nair, *et al,* 1970; Burgus, Dunn, *et al,* 1969; Baba, *et al,* 1971.

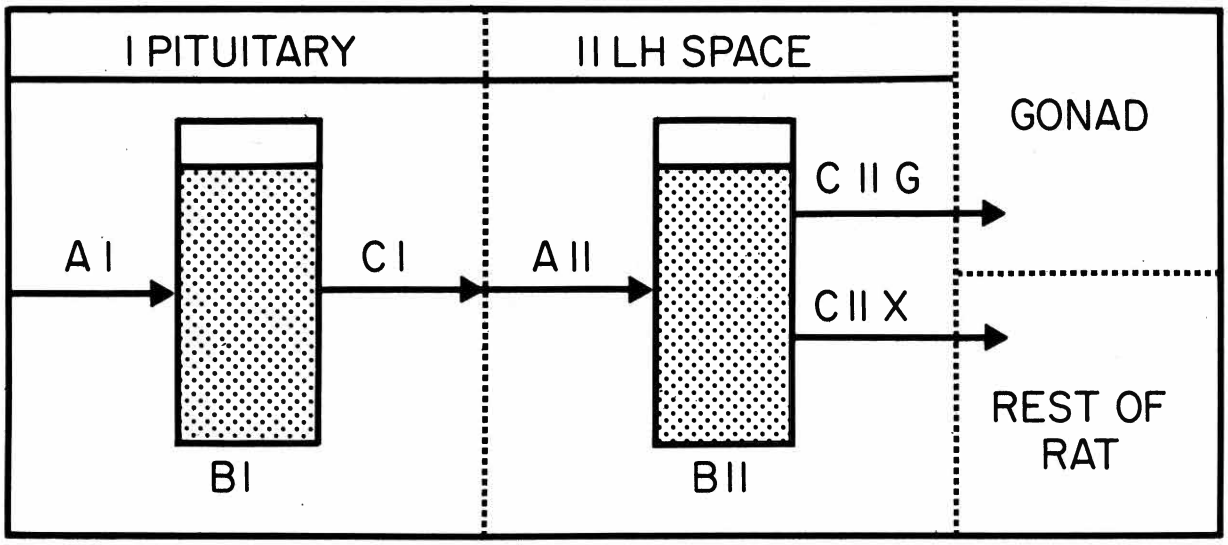

Fig. 1—Simplified, 2-compartment model illustrating LH secretory dynamics but convertible to any endocrine subsystem. Arrows labeled A (I or II) represent rates of entry into each of the 2 adjacent compartments. Arrows labeled C (I, II_G or II_X) represent total or fractional rates of exit. In compartment I, the pituitary (in the case of LH), AI is the *net* rate of LH *synthesis* and CI is the rate of LH *release*. Compartment II, the LH "space" (which seems to approximate the plasma volume) corresponds to the "inner pool" of Tait and Burstein. The rate of LH entry from the hypophysis into the LH space (AII) must, at all times, be commensurate with CI, the LH release rate. Rates CII_G, and CII_X, especially the latter, might better be drawn as $\rightleftarrows$ to indicate that they represent *net* flux between the LH space and the gonad (CII_G) and all other extrahypophysial spaces (CII_X). If entry of LH into the plasma from these extrahypophysial spaces (Tait and Burstein's "outer pool") were substantial, the apparent rate of exit (CII_X in our studies) would be slower than the true rate and the decay curve would not be described by the simple formula we have used.

If, in one of the compartments, a transient disequilibrium between A and C occurs, a change in B (stores or content) must result, according to the relationship $A - C = \Delta B$, where ΔB is the change in stores during the unit of time selected to express rate. BI can be measured (as concentration $\times$ weight-content) and BII can be calculated from the concentration of the hormone in the plasma, if the distribution volume is known (concentration $\times$ distribution volume = total circulating hormone). None of the rates (AI, CI, AII, CII_X, or CII_G) has ever been measured. However, in a relatively steady state, as in a rat castrated 4 or more weeks previously (where $CII_G = 0$), $\Delta BI \backsimeq \Delta BII \backsimeq 0$ and therefore $AI \backsimeq CI = AII \backsimeq CII_X$.

In the "stop-entry" experiment (acute removal of compartment I by hypophysectomy) AII instantaneously becomes zero but CII_X, the rate of exit from the plasma, slows gradually. Although CII_X immediately starts to decrease, seemingly as an exponentia function of BII, its instantaneous initial (*zero* time) value, which must about equal the steady state (pre-hypophysectomy) values of AI, CI and AII, can easily be calculated, as explained in the text. (Redrawn with permission from Gay and Bogdanove. *Endocrinology* 82:359, 1968.)

the input-output relationships involved in the processes of secretion, I have focused on the most meaningful accessible index of activity—changes in serum hormone concentration (ΔBII in Fig. 1). If we wish to focus on *hypothalamic* secretory activity, the problems become very much greater. Figure 2 illustrates a current concept of how the hypothalamus and pituitary are integrated with other organs involved in the control of reproductive activity. The evolution of this model has been reviewed elsewhere (Bogdanove, 1972). For our present purpose, a discussion of current knowledge of gonadotrophin releasing factor(s), this model is presented merely to locate the subject of discussion.

As shown in Fig. 2, arrow 3 represents the *secretion* of releasing factors, which act (either alone or in conjunction with other internal environmental influences) to induce secretion of pituitary gonadotrophic hormones (arrow 4). It is precisely this stimulus-response relationship which has provided the existing operational definitions of the so-called releasing factors. Thus, a factor which released FSH was called FSH-RF (Igarashi and McCann, 1964). One which released LH was called LH-RF, or LRF (McCann, Taleisnik, and Friedman, 1960). A factor thought to affect FSH synthesis (Corbin and Milmore, 1971) or prolactin synthesis (Nicoll and Fiorindo, 1969) was given still another name. One which decreased the rate of prolactin release was dubbed PIF or PRIF, for prolactin release inhibiting factor. In every case, the definition was *indefinite*—there was never any valid reason for believing that LRF and FSH-RF were separate entities (despite published conclusions to that effect which will not be cited here). As long as the changes in pituitary hormone release rates which were ob-

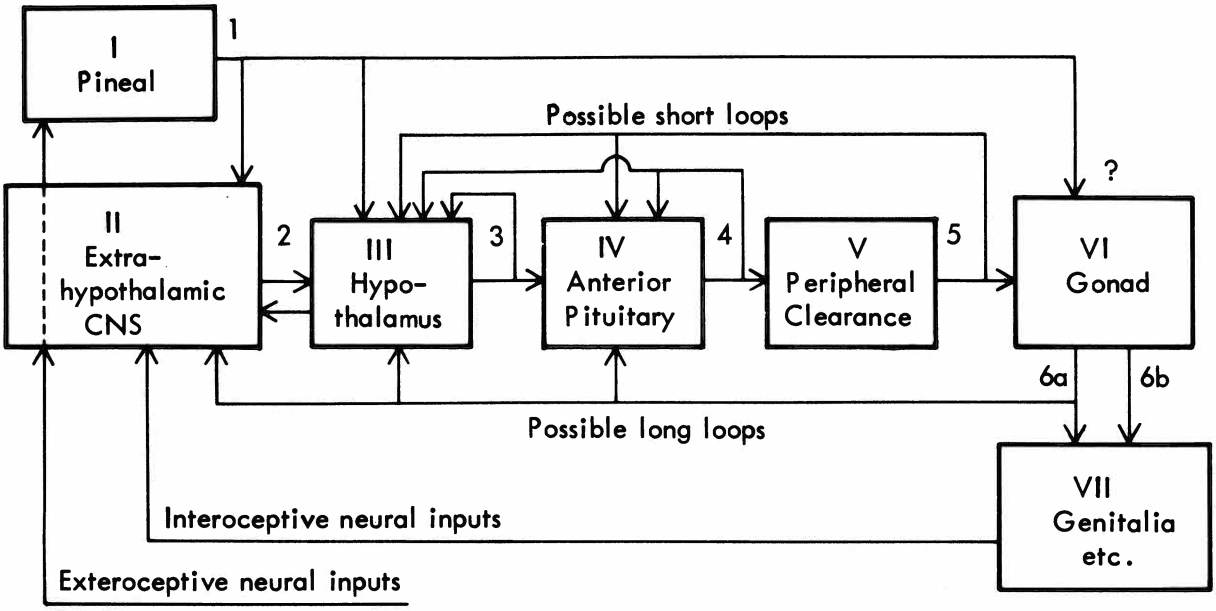

Fig. 2—Major components of brain-pituitary-gonadal control system. Boxes represent *loci* (generally organs) of physiological responses to stimuli. Arrows, which constitute responses as well as stimuli, represent signals (which may be either neural or humoral). To conserve space, some arrows have been numbered: 1) pineal secretion (melatonin?); 2) afferent and efferent neurons; 3) hypophysiotrophin secretion; 4) LH, FSH, etc. in portal venous blood; 5) LH, FSH, etc. in peripheral blood; 6a) gonadal steroid secretion; 6b) eggs or sperm.

served—plus any changes which may also have occurred without being detected—had been induced by administration of crude, or even partially purified, hypothalamic extracts, it was simply impossible to attribute the response(s) to specific components of the extracts. Thus, acid extracts of rat (or sheep, or pig, or steer) hypothalamus could release TSH, growth hormone, ACTH, LH, FSH, and, under some conditions, MSH (melanophore-stimulating hormone, or *intermedin*). At the same time, they could inhibit release of prolactin. The extent to which these, and other, effects could be attributed to the presence of specific hypophysiotrophic molecules in these extracts still remains to be determined. Until all such demonstrations have been reproduced, using "RFs" of unequivocal purity, our views of how the hypothalamus might exert its effects on pituitary secretory activity will have to remain indefinite.

This was the urgent reason for the intense and sustained efforts of the several laboratories which were engaged in the great releasing-factor hunt of the last decade. The task of collecting and extracting hundreds of thousands of hypothalamic fragments from sows and cows and ewes, in order to obtain, at the end of nearly 10 years, the smidgins of purified materials needed to define the chemical structures of the RFs, can truly be described as epic. The results of these Augean labors have finally begun to be visible. The structure of the thyrotrophin releasing hormone TRF (or TRH, using Schally's nomenclature†) was discovered barely a year before that of the single decapeptide molecule which appears able to release both LH and FSH (Baba, *et al,* 1971). The name of this molecule has not yet been settled. Schally has given it the quasi-acronym "LH-RH/FSH-RH," but the same molecule is being prepared synthetically, by Abbott Laboratories, under the name of GnRH (for gonadotrophin-releasing hormone).

Far more important than what this compound should be called is the question of what it can do;

† Schally has proposed (Schally, Arimura, *et al,* 1968) that the RFs be called RHs (for releasing hormones) on the basis that they ought to be recognized as full-fledged members of the community of hormones. Guillemin and others (Burgus and Guillemin, 1970; Bogdanove, 1972) have objected to Schally's terminology, for several reasons. Still other nomenclature has recently been proposed (Saffran, 1972). Debate about etymological propriety seems pointless since these compounds will be known best by the names under which they are distributed by the pharmaceutical companies which undertake to mass produce them. Therefore, despite my previous objections, I will refer to Schally's LH-RH/FSH-RH as GnRH.

first for the physiologist and subsequently for either the physician and his individual patient, or for the public health and agricultural scientists who are concerned with the control of human and animal fertility on a larger scale. I think the answers to these questions are of vital importance, but I do not think I or anyone can give them just yet.

We do not yet know whether GnRH is the only factor secreted by the hypothalamus which can influence LH and FSH secretion. As a matter of fact, we do not even know that GnRH is secreted by the hypothalamus, but only that it can be extracted from it. [The increased LH and FSH releasing *activity* which can be shown in portal venous blood after the hypothalamus has been stimulated (Kamberi, *et al,* 1971), may or may not be due to increased GnRH concentration in that blood.] If GnRH is, in fact, the *only* gonadotrophin-releasing factor, what is its physiological role? Is it involved in disease? How can we make use of it?

Much work lies ahead before these questions will be answered. If we pause to think for a moment about some of the major steps toward our present understanding of the pituitary hormones involved in reproduction, we may be able to glimpse some parallels in the problems which lie ahead in the study of hypothalamic hormones.

The demise of the Aristotelian notion of pituitary function came about when pioneer surgeons and physiologists (Crowe, *et al,* 1910; Smith, 1927) removed the gland from the living animal and discovered that the major resultant deficiencies were not in the production of nasal mucus, but in somatic growth (particularly at epiphyseal plates) and the activities of what we have now come to know as the pituitary target glands: thyroid, adrenal cortex, and gonads. (Similar deficiencies result from damage to the hypothalamus or isolation of the pituitary from hypothalamic influence.) The next steps were to show that injection of extracts of anterior pituitary tissue could remedy these various deficiencies and that specific fractions of such extracts could selectively restore specific functions. It is through this *substitutive* approach that the existence of seven distinct adenohypophyseal hormones was finally defined: LH, FSH, TSH, ACTH, prolactin, growth hormone, and MSH. It took a long time to accomplish this partial goal, primarily because it took so long to determine the actual structures of the macromolecules secreted by the anterior pituitary. (In this respect, the oligopeptidic hypothalamic hormones should present much less of an obstacle.)

As sufficiently pure pituitary hormones became available it was found that FSH alone could produce follicular growth, but not estrogen secretion, in hypophysectomized rats (Greep, 1968). If a little LH was administered with the FSH, steroid secretion also resulted. Ovulation and formation of a corpus luteum required a rapid surge of a large amount of LH, superimposed on this "priming" of the follicle by FSH and a trace of LH. Physical maintenance of the corpus luteum appeared to require nothing from the pituitary (corpora lutea survived for months in hypophysectomized rats) although *secretion* of luteal hormones did. In the rat and mouse, but not in other animals, prolactin was found to be luteotrophic, capable of activating the secretory machinery of the corpus luteum. In the male, FSH alone has been credited with a role in the production and maintenance of spermatogenesis (Steinberger, 1971), while LH is clearly capable of stimulating androgen production by the Leydig cells. (The androgen, in turn, stimulates spermatogenesis.) The male does not seem to use prolactin as a gonadotrophin, although he can use it as a lactogenic hormone under certain conditions.

We are now entering a comparable phase of *substitutive* investigation of the releasing factors. So far, only two or three facts have emerged which merit comment. One is that the response of the pituitary to GnRH can be influenced, at least quantitatively, by steroid feedback (long-loop arrow, probably to box IV, in Fig. 2), as well as by either genetic sex or some consequence thereof. The evidence for this is that when identical doses of natural porcine GnRH were injected into male and female castrated rats which had been pretreated with ovarian steroids or testosterone (four groups in all), the response in the spayed rats which had been pretreated with ovarian steroids greatly exceeded that in the similarly pretreated orchidectomized rats and in the testosterone-pretreated castrates of either sex (Rennels, *et al,* 1971). Evidence that steroids can act directly at the pituitary level to qualitatively alter pituitary activity, presumably by modifying the effects of either GnRH or some other hypophysiotrophic agent(s), has been presented recently (Kingsley and Bogdanove, 1971). These two facts suggest, but do not establish, that it may not be necessary to postulate neural participation in every change in LH or FSH secretory rate, or every shift in the LH:FSH ratio, which may occur under physiological or experimental conditions. Thus, although changes in LH and FSH secretion may result from changes in GnRH secretion, it is also pos-

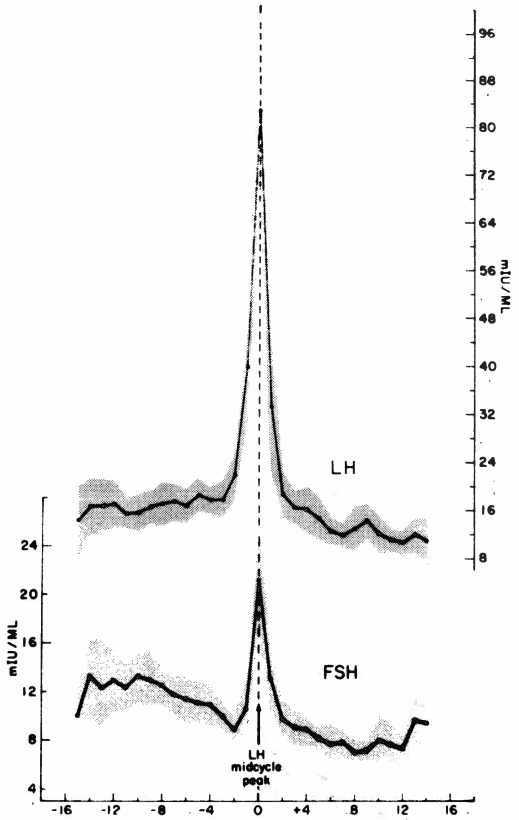

Fig. 3—Patterns of radioimmunoassayable serum LH (upper graph) and FSH (lower graph) in women during 16 presumptively ovulatory cycles. Shaded areas represent 95% confidence limits of means. (Reprinted with permission from Ross, et al. *Rec. Progr. Horm. Res.* 26:1, Academic Press, 1970.)

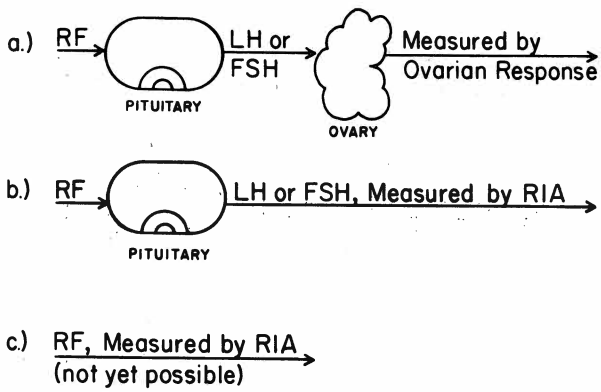

Fig. 4—Components of RF assays: a. "Double" bioassay, in which end-point is an ovarian response; b. "Single" bioassay, in which end-point is a pituitary response determined by radioimmunoassays (RIA) of serum; c. Direct assay, which does not involve any biological response. (This type of assay is not yet available.)

sible that changes in the pituitary output of these two hormones can occur without any precedent change in hypothalamic secretory activity. For this to happen, it would be necessary for pituitary responsiveness to GnRH to vary under the influence of one or several control agents other than GnRH. It remains to be determined whether sex steroid feedback, which apparently can influence pituitary responsiveness to releasing factor(s) under experimental conditions, can also do so under physiological conditions.

Another fact is noteworthy. Both human (Gual, *et al,* 1972) and rat (Tashjian, *et al,* 1971) pituitary cells can be stimulated to release prolactin by TRH! The mechanism of this unexpected finding remains to be established.

As additional synthetic releasing factors become available, and additional clever or lucky experiments are carried out, a considerable body of data will develop. From it, the physiologist will venture, about the secretion of endogenous releasing factors, opinions for which today there is not yet a sufficient foundation. The aim of substitutive research must be to supply the pituitary gland deprived of hypothalamic control with a sufficiently elaborate replacement for its natural releasing factor input (a sort of prosthetic hypothalamus), so that its behavior will mimic that seen when neural controls are allowed to operate. Even from such substitution studies, however, conclusions should be drawn with caution. The *caveat* I would stress is that substitution studies reveal only what a hormone *can* do, not necessarily what it *does*. As an example of the distinction, consider the impression—based on the demonstrable proportionality between the amounts of FSH injected and the resultant sizes of ovarian follicles—that the progressive growth of the follicle during the preovulatory phase of the cycle reflects a progressive increase in the rate of FSH secretion. This impression, derived from substitution studies, has not been borne out by direct observation. Figure 3 shows the patterns of LH and FSH in the serum during the menstrual cycle in the human. Note that, during the follicular phase of this cycle, serum FSH levels do *not* increase, but actually seem to *decline*. This finding would not have been anticipated on the basis of substitution experiments.

Ultimately, it is always necessary to follow the substitutive approach with an analytical one, aimed at characterizing patterns of secretion by direct observation. However, I think it will be some time before direct analysis of releasing factor secretion becomes a possibility. For most of the past 10 years,

measurements of releasing factor activity have required a "double bioassay" (Fig. 4) in which the effect of the factor on the pituitary (a biological response) could be assessed only by bioassay (involving a second biological response). The order of error in such an assay system was usually, perhaps always, sufficiently enormous that conclusions had to be based on intuitive selection among several possibilities. The advent of radioimmunoassay methods for measuring pituitary hormones very precisely has reduced error by eliminating the second, but not the first, biological response. I think at least some of what has been reported on the basis of double bioassays will not bear careful scrutiny using single bioassays.

Total elimination of a bioassay step, through development of radioimmunoassays for releasing factors, is of course desirable. However, some interesting calculations by Gay (Gay, 1972) are noteworthy. These calculations, based on substitution studies, suggest that the concentrations of releasing factor(s) in the hypothalamic-pituitary portal circulation would have to be 2 to 3 orders of magnitude below the limits of sensitivity of any known radioimmunoassay. In peripheral blood, they would be lower still. When this is considered together with the fact that there are no methods available for sampling portal venous blood in an unanesthetized animal, the chances for characterizing patterns of spontaneous releasing factor secretion by direct observation still seem very remote.

If I have drawn too dismal a picture, I am sorry. Schally's gift of GnRH is a great one, and a beautiful scientific achievement. To the physiologist, it is a find comparable to the Rosetta stone, without which the system depicted in Fig. 2 could never be understood. To the physician, it may prove to be a useful diagnostic tool and perhaps even, ultimately, to have some therapeutic value. But its primary value is that of the key to an unsolved puzzle. While the key may now be at hand, the solution(s) to the puzzle must still be worked out.

REFERENCES

BABA, Y., MATSUO, H., AND SCHALLY, A. V. Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analysis. *Biochem. Biophys. Res. Commun.* 44:459, 1971.

BOGDANOVE, E. M. Chapter 1: Hypothalamic-hypophysial interrelationships: Basic Aspects. *Reproductive Biology* (Balin and Glasser, eds.), Amsterdam: Excerpta Medica, 1972.

BURGUS, R., DUNN, T. F., DESIDERIO, D., AND GUILLEMIN, R. Structure moléculaire du facteur hypothalamique hypophysiotrope TRF d'origine ovine: mise en évidence par spectrométrie de masse de la séquence PCA-His-Pro-NH_2. *C. R. Acad. Sci. Paris* 269:1870, 1969.

BURGUS, R. AND GUILLEMIN, R. Hypothalamic releasing factors. *Ann. Rev. Biochem.* 39:499, 1970.

CORBIN, A. AND MILMORE, J. E. Hypothalamic control of pituitary follicle stimulating hormone synthesis. *Endocrinology* 89:426, 1971.

CROWE, S. J., CUSHING, H., AND HOMANS, J. Experimental hypophysectomy. *Bull. Johns Hopk. Hosp.* 21:127, 1910.

FRIEDGOOD, H. B. The nervous control of the anterior hypophysis (first presented at the Harvard University Tercentenary Celebration, 1936). Reprinted in *J. Reprod. Fertil., Suppl.* 10:3, 1970.

GAY, V. L. The Hypothalamus: Physiology and clinical use of releasing factors. *Fertility and Sterility* 23:50, 1972.

GAY, V. L. AND BOGDANOVE, E. M. Disappearance of endogenous and exogenous luteinizing hormone activity from the plasma of previously castrated, acutely hypophysectomized rats: An indirect assessment of synthesis and release rates. *Endocrinology* 82:359, 1968.

GREEP, R. O. The saga and the science of the gonadotrophins. *Persp. Bio. Med.* 12:124, 1968.

GUAL, C. A., KASTIN, A. J., AND SCHALLY, A. V. Clinical experience with the use of hypothalamic releasing factors. *Rec. Progr. Horm. Res.* 28, 1972 (in press).

GUILLEMIN, R. AND ROSENBERG, B. Humoral hypothalamic control of anterior pituitary: a study with combined tissue cultures. *Endocrinology* 57:599, 1955.

HARRIS, G. W. Neural control of the pituitary gland. *Physiol. Rev.* 28:139, 1948.

HARRIS, G. W. (ed.). *Neural Control of the Pituitary Gland.* London: Arnold, 1955.

HARRIS, G. W. AND CAMPBELL, J. J. Chapter 4: The regulation of the secretion of luteinizing hormone and ovulation. *The Pituitary Gland* (Harris and Donovan, eds.), Vol. 2, p. 99. Berkeley: University of California Press, 1966.

IGARASHI, M. AND MCCANN, S. M. A hypothalamic follicle stimulating hormone-releasing factor. *Endocrinology* 74:446, 1964.

KAMBERI, I. A., MICAL, R. S., AND PORTER, J. C. *Pituitary* portal vessel infusion of hypothalamic extract and release of LH, FSH, and prolactin. *Endocrinology* 88:1294, 1971.

KINGSLEY, T. R. AND BOGDANOVE, E. M. Direct androgen-pituitary feedback. *Fed. Proc.* 30:253, 1971.

KNIGGE, K. M. AND SCOTT, D. E. Structure and function of the median eminence. *Am. J. Anat.* 129:223, 1970.

MCCANN, S. M. AND PORTER, J. C. Hypothalamic pituitary stimulating and inhibiting hormones. *Physiol. Rev.* 49:240, 1969.

MCCANN, S. M., TALEISNIK, S., AND FRIEDMAN, H. M. LH releasing activity in hypothalamic extracts. *Proc. Soc. Exp. Biol. Med.* 104:432, 1960.

MEITES, J. (ed.), *Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry*. Baltimore: Williams and Wilkins, 1970.

NAIR, R. M. G., BARRETT, J. F., BOWERS, C. Y., AND SCHALLY, A. V. Structure of porcine thyrotropin releasing hormone. *Biochemistry* 9:1103, 1970.

NEUMANN, F., BERSWORDT-WALLRABE, R. VON, ELGER, W., STEINBECK, H., HAHN, J. D., AND KRAMER, M. Aspects of androgen-dependent events as studied by antiandrogens. *Recent Progr. Horm. Res.* 26:337, 1970.

NICOLL, C. S. AND FIORINDO, R. P. Hypothalamic control of prolactin secretion. *Gen. Comp. Endocr.* Suppl. 2:26, 1969.

RENNELS, E. G., BOGDANOVE, E. M., ARIMURA, A., SAITO, M., AND SCHALLY, A. V. Ultrastructural observations of rat pituitary gonadotrophs following injection of purified porcine LH-RH. *Endocrinology* 88:1318, 1971.

ROSS, G. T., CARGILLE, C. M., LIPSETT, M. B., RAYFORD, P. L., MARSHALL, J. R., SCOTT, C. A., AND RODBARD, D. Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. *Rec. Progr. Horm. Res.* 26:1, 1970.

SAFFRAN, M. Chemistry of the hypothalamic hypophysiotropic hormones. *Handbook of Physiology—The Pituitary and its Control Mechanisms* (Knobil and Sawyer, eds.). *Am. Physiol. Soc.*, Washington, D. C., 1972 (in press).

SCHALLY, A. V., ARIMURA, A., BOWERS, C. Y., KASTIN, A. J., SAWANO, S., AND REDDING, T. W. Hypothalamic neurohormones regulating anterior pituitary function. *Recent Progr. Hormone Res.* 24:497, 1968.

SCHALLY, A. V. AND KASTIN, A. J. The role of sex steroids, hypothalamic LH-releasing hormone, and FSH-releasing hormone in the regulation of gonadotrophin secretion from the anterior pituitary gland. *Advances in Steroid Biochemistry,* Vol. II, p. 41 (Briggs, M. H., ed.). New York: Academic Press, 1970.

SCHALLY, A. V., KASTIN, A. J., AND ARIMURA, A. FSH-releasing hormone and LH-releasing hormone. *Vit. and Horm.* 30, 1972 (in press).

SCREIBER, V. Thyrotrophin-releasing factor: purification, mechanism, and blockage of action (a Review). *Neuroendocrinology* 2:175, 1967.

SMITH, P. E. The disabilities caused by hypophysectomy and their repair. The tuberal syndrome in the rat. *J. Am. Med. Ass.* 88:158, 1927.

STEINBERGER, E. Hormonal control of mammalian spermatogenesis. *Physiol. Rev.* 51:1, 1971.

TASHJIAN, A. H., JR., BAROWSKY, N. J., AND JENSEN, D. K. Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. *Biochem. Biophys. Res. Commun.* 43:516, 1971.

Artificial Insemination in the Human*

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The idea that impregnation might occur without coitus has aroused interest since ancient times. In the second century A.D., there was recorded a hypothetical discussion concerning a woman who had been inseminated by semen previously deposited in the bath water in which she bathed. In 1322, an Arab used artificial insemination with horses. A wad of wool was introduced into the vagina of a mare and left overnight. It was then held over the nostrils of a stallion, and with this stimulus the stallion ejaculated on a cloth held in readiness. The ejaculated material was then introduced into the vagina of a mare, which foaled after the appropriate length of time.

During the 1700's, isolated instances of human artificial insemination were reported, almost always using the husband's semen. In the United States, human artificial insemination using donor's semen was first practiced by Dr. Robert L Dickinson in 1890. His work was initially done in secrecy, although subsequently he did much to train others in the technique and to gain public acceptance of the procedure.

Artificial Insemination Using Donor's Sperm. The most common indication for artificial insemination using donor's semen is sterility of the husband. This may be manifest as total azoospermia or severe oligospermia, demonstrated on repeated examinations. The husband should, of course, have a thorough medical and urologic examination to be certain that his sterility is not secondary to a treatable condition. Considerable judgment is required to decide when to treat cases of oligospermia this way. In most instances, this is done when appropriate medical therapy has failed to improve the quality of the ejaculate and artificial insemination using the husband's semen has not resulted in pregnancy.

When Rh incompatibility has already resulted in the birth of an erythroblastotic infant, and particularly when the husband is homozygous Rh positive, insemination of the wife with semen from an Rh negative donor will prevent the recurrence of erythroblastosis. Fortunately, the availability of Rh_0 (D) immune globulin (human) has dramatically reduced the number of Rh negative women who have been sensitized, and this is becoming a less common indication for artificial insemination.

When the husband has a family history of genetic disease which makes fatherhood inadvisable or when the couple has had affected offspring indicating abnormal recessive genes with the likelihood of producing serious congenital defects in subsequent pregnancies, artificial insemination with donor's semen may be used. Examples of this are Tay-Sachs disease, and cases where both husband and wife have AS hemoglobin.

The presence of agglutinating antibodies against the husband's sperm but not against donor sperm may occur in a woman with prolonged and otherwise unexplained infertility (Dukes and Franklin, 1968). The test for this is not an absolute one, as some women who agglutinate their husband's sperm have been of high fertility. In a situation where antibodies are present, in some cases the use of condom for a time to protect against repeated exposure to antigen will result in disappearance of agglutinating antibodies followed by pregnancy. Where condom therapy fails, artificial insemination using donor's sperm which are not agglutinated by the wife's antibodies may be used. The ABO relationship to infertility is even more unclear, although there seems to be a statistically significant effect when an ABO incompatibility exists and the husband is a "secretor," in which case donor semen from a male of appropriate blood type may result in pregnancy.

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Donor insemination has special emotional connotations, and both husband and wife should be aware of the possible emotional effect of the procedure on their future relationship. Both husband and wife should be reasonably well-adjusted individuals, and they should feel that they have a stable marriage. It is extremely important not to do this procedure to "save the marriage" as this is far too big a job to place upon the shoulders of an individual who weighs only seven pounds or so. Both husband and wife should have a desire for children, and the man should not feel that he is being coerced into this procedure in order to provide fulfillment of his wife's maternal instincts. In most cases, it is advisable to wait for a while after the husband has found out about his sterility problem before carrying out artificial insemination because he will need time to recover from the shock that such news is to every man. When it has been decided to use insemination with donor's semen, both husband and wife should sign a consent form.

In the past when there was no shortage of babies for adoption, we did not encourage artificial insemination, but did it only when the couple specifically requested it, feeling that adoption solved two social problems, that is, providing a family to the child and a child to the family. With the recent increase in therapeutic abortions, the supply of infants for possible adoption has so dwindled that artificial insemination is more often mentioned to the couple as a solution to their infertility or genetic problems. Aside from suggesting the availability of donor's semen to such couples, we do not try to encourage this where either partner expresses any hesitancy.

A fertility investigation is done for each couple seeking artificial insemination, if this has not been previously accomplished. As a minimum, patency of the wife's fallopian tubes is established by a Rubin's test using carbon dioxide, and an endometrial biopsy is done to confirm ovulation. The patient is also instructed to begin keeping basal temperature charts, in order that the most optimal time for insemination may be selected. After reviewing temperature charts for several months, a day is selected. This day will likely be one or two days prior to the basal temperature rise. It has been our practice to perform insemination once a month for the first three months, and if pregnancy has not occurred in this time, then to perform several at two-day intervals until the temperature rise has definitely occurred. Some physicians use a rat ovary hyperemia test to predict the time of ovulation. This test is said to be capable of determining the time of ovulation within six to twelve hours (Farris, 1948). More recently, the use of rapid radio immunoassay procedures for LH, in order to detect the pre-ovulatory LH peak, has been suggested as an aid in timing the deposition of sperm, whether it be by natural or artificial means.

Kleegman has found there is a sex differentiation in infants related to the time of artificial insemination and ovulation (Kleegman, 1967). She utilized basal temperatures somewhat to determine the time of ovulation, but put more emphasis on mittelschmerz (sometimes elicited with a "bounce test") and mucorrhea. Using this as evidence of ovulation, she has noted that exposure 2–24 hours before ovulation was more likely to result in a male infant and that exposure 36 or more hours before ovulation or 2–8 hours after ovulation was more likely to result in a female infant. She was able to predict sex in 77% of cases utilizing this method. She also noted that men who have severe oligospermia but whose wives have still become pregnant, have a preponderance of female children and that rhythm failures with conception occurring on cycle days 4 to 7 usually result in female infants. Certain other investigators have been unable to reduplicate this accuracy in prediction (Cohen, 1966), but in fairness to Kleegman, it should be noted that most have used only basal temperature as an index of ovulation and have not relied on mittelschmerz or cervical mucus changes.

Most of the donors which we use are either medical students or house officers, who are married and are already fathers of normal children. These men report no knowledge of hereditary disease and are of above average intelligence. If the wife has Rh negative blood, an Rh negative donor is also chosen. We do not make any effort to match blood types otherwise, because any good laboratory could readily establish the fact that the woman's husband is not the father of the child by utilizing other blood groups such as the MN, Kidd, Duffy, and Kell which establish a "fingerprint." We make an effort to match donor to husband by somatic type, skin, hair, and eye coloration, but this is not always possible. We would not, however, use a short, black-haired donor of Mediterranean origin for a couple where both husband and wife were tall, blonde, and fair-skinned.

Our experience has been entirely with fresh semen, and the donor is instructed to obtain a specimen by masturbation into a clean glass con-

tainer shortly before the couple's appointment. The donor then delivers the semen to a designated point, and it is then transferred to the patient area by other personnel. It is important that the donor not deliver the specimen himself to this office, in order to exclude the possibility that he and the recipients might meet. In order to preserve anonymity, the couple always pays the donor in cash. Our practice is to keep no record of which donor is used for insemination, and this precludes using the same donor for subsequent pregnancies. It is of note that some physicians do not even record in the patient's chart the fact that insemination was done.

On the selected day, the patient lies on an examining table, and an unlubricated vaginal speculum is inserted. The donor's semen is drawn into a syringe which has a small length of polyethylene tubing attached to it. The husband then comes to the examining room. The husband holds the syringe and deposits 0.1 to 0.5 ml. of semen about 1 cm. within the endocervical canal. The polyethylene tubing and speculum are then removed, and a plastic cap with an attached tubing is placed over the cervix. The remainder of the donor's semen is then inserted into the cervical cap by the husband. We feel that this active participation in the insemination of the wife by the husband is very important for him psychologically. The cervical cap is sometimes difficult for the patient to remove, so while the husband is in the examining room, we instruct both him and his wife on the removal of the cervical cap, and to date, between the two of them, this has always been accomplished at home.

We do not advocate the mixing of husband's ejaculate with donor's semen or that the couple have intercourse immediately prior to coming to the office for insemination. A cervical mucus-spermatozoa incompatibility is not alway due to a cervical factor, and one case was reported where the donor's sperm but not the husband's sperm readily entered the wife's cervical mucus (Kunitake and Davajan, 1970). When the husband's sperm was separated from the seminal plasma and resuspended in seminal plasma from a donor who had had a vasectomy, good penetration of the wife's cervical mucus was obtained. Unless this seminal plasma factor is checked for, it would be inadvisable to mix husband's semen with donor's semen. After the cervical cap has been in place for six hours or so, the couple is advised to remove it, and it is suggested that they then have intercourse. By this time, active sperm should be well on their way up the female reproductive tract and any lethal activity in the seminal plasma would be of no consequence.

When a woman has somewhat irregular ovulation, she is given an injection of 5000 international units of human chorionic gonadotrophin to act as an LH surge, hopefully to cause an impending ovulation to occur while viable sperm are still present (Fuchs, *et al,* 1966).

The effectiveness of insemination using donor semen has been shown in many studies. In an analysis of seven reported series which included 630 couples who had used artificial insemination with donor semen, the proportion becoming pregnant varied from 55–78%. Of the women who eventually became pregnant, between 31 and 46% became pregnant during the first month of insemination. The number of inseminations per menstrual cycle appeared to be more significant in obtaining an early pregnancy than the method of semen deposit (Potter, 1958). About 90% of the women who became pregnant did so in 6 months, and if a pregnancy has not resulted within 12 months, the chances of success are very remote.

Artificial Insemination Using Husband's Semen. Indications for insemination using husband's semen include failure to deposit the semen in the posterior vaginal fornix, inadequate cervical invasion, and a moderate but irreversible degree of male infertility.

The failure to deposit the semen may be due to penile or vaginal malformations, impotence, or retrograde ejaculation. Penile hypospadias was one of the first-recorded indications for artificial inseminations, and this is still valid. The cases of impotence are best individualized, and decisions should be made only after consultation with the husband's psychiatrist. The therapy for male impotence is psychiatric, and in some instances a pregnancy in the wife and impending fatherhood will exert a beneficial psychiatric effect, while in others this is best postponed until psychotherapy has progressed further. Retrograde ejaculation may follow injury to the internal bladder sphincter due to surgery such as transurethral resection. However, retrograde ejaculation may also be due to congenital anatomical abnormalities or neurologic problems, including diabetes and chemical sympathectomy achieved with guanethidine. Typically, there is not only azoospermia but also markedly reduced semen volume, although an ejaculatory sensation is experienced with orgasm. To corroborate the diagnosis, the patient empties his bladder and

then ejaculates in one glass, after which he collects in a separate glass whatever urine is in the bladder.

Artificial insemination using husband's semen is also sometimes used in an attempt to bypass the cervical mucus when it is found that normal sperm cannot migrate through this or when postcoital tests reveal no living sperm in the presence of apparently normal mucus and a good sperm count. The achievement of pregnancy in these last two instances is quite rare in our hands, although Kleegman reported a success rate of around 85% in the small group where the only abnormal factor was a cervical secretion impenetrable to sperm (Kleegman and Kaufman, 1966). She stated that most cases of cervical impenetrability are due to endocrine dysfunction rather than cervicitis, and the results of husband insemination in this group are poor. Others have found consistently poor results in all attempts to bypass the cervix (Balin, 1967).

When intrauterine insemination is done, the cannula is placed in the endometrial cavity and only 0.5 ml. or less of semen is instilled. This is done very slowly with practically no pressure. The physician must be careful not to inject the semen through the fallopian tubes into the peritoneal cavity where it will cause a peritonitis, and he must be ready to stop the installation if the patient complains of any cramping.

Insemination with husband's semen may be tried when the wife is of apparently good fertility and the husband's sperm consistently has a low count with the postcoital test showing only a few sperm in the cervical canal. This may also be utilized when the husband's sperm count is within normal limits, but the motility of the sperm is less than normal. The split ejaculate offers a concentrated number of better sperm, and is often employed when husband's semen is to be inseminated.

Split ejaculates are obtained simply by using one clean container for the first part of the ejaculate and another for the remainder. It is most useful when the volume of the ejaculate is over 2 ml. In one study of 86 husband donors, the count was significantly higher in the first portion than in the second portion of the total specimen in 88%, equally distributed in both in about 8%, and significantly higher in the second portion than in the first in 6%. Sperm motility was greater in the first portion in 77%. In 25 cases of increased viscosity of the total ejaculate, the second portion was consistently the most viscous (Amelar and Hotchkiss, 1965).

Biochemical studies of the split ejaculate have indicated that the first half contains the main bulk of acid phosphatase, which comes from the prostate, along with the products of the testes, epididymis, and vas deferens. Since lactic acid accumulates as an end product of the metabolism of spermatozoa, it is also found in greater concentration here. In the second half of the split ejaculate, the higher concentration of fructose is found, a substance specific to the seminal vesicles (Amelar and Hotchkiss, 1965; MacLeod and Hotchkiss, 1942).

Centrifugation of the full specimen concentrates the sperm, but frequently depresses the motility and also concentrates the debris and mucus. Concentration of the sperm can be obtained using the split ejaculate without injuring the cells and without concentrating debris also.

Kleegman reports that with 100 consecutive women who had intrauterine insemination of the split ejaculate in cases where there was subnormal husband's sperm quality, 17 women became pregnant (Kleegman and Kaufman, 1966). Other reports include 15% pregnancies in 86 women with insemination of husband's semen (Kaskarelis and Comninos, 1959). A number of women who failed with insemination conceived subsequently with normal intercourse.

Insemination with husband's sperm suspended in donor's seminal plasma may be considered in the rare cases where the husband's own seminal plasma contributes to cervical mucus-spermatozoa incompatibility or where there is excessively low fructose in the husband's seminal plasma (Moon and Bunge, 1968).

Frozen Semen. Much has been written about the practical applications of frozen human semen, with the establishment of human semen banks. For example, by utilizing such a bank, couples could be suitably matched according to many physical characteristics and by blood type, regardless of the time and place. Another possible use of frozen semen entails its use in insemination with husband's semen where the husband is oligospermatic. Several split ejaculates could be pooled, and then delivered to the wife at the calculated fertile time. It has been proposed that men might deposit semen in a bank for possible future use prior to having a vas ligation. Other possible uses of semen banks sound like science fiction stories. They include (1) having a supply safely stored behind thick walls in case of nuclear attack which might sterilize the entire male population of the country, (2) making it possible for a man to sire offspring

many years after he had died; and (3) complete population control where all pregnancies are from bank sperm and only those sperm known to transmit characteristics thought to be useful to the state are dispensed, thus enabling the development of either a "super race" or a "leader-slave culture."

In any event, various techniques have been used to freeze sperm. Most employ glycerol as a protecting medium. One study from the University of Michigan used a protective medium of egg yolk, glycerol, glucose plus sodium citrate solution, and glycine, which was buffered at pH 7.3. The semen sample was mixed with the protective medium, cooled slowly to −80°C and then stored at −196.5°C. Thawing was performed in a 37°C. water bath, and inseminations were then done within 30 minutes of thawing the samples (Behrman and Sawada, 1966).

A conception rate of 40–50 % may be expected when frozen semen is used, and this compares unfavorably with the 75% which may be achieved by using fresh specimens for donor insemination. This would be an indication that the fertilizing capacity of the frozen-preserved sperm is relatively low. In the experience which was reported from the University of Michigan in 1968, conception had never been achieved when a frozen-preserved specimen had been administered more than 24 hours prior to ovulation as determined by basal temperature records. This was thought to indicate a relatively short term of viability. Human sperm have an apparently good motility after thawing, but despite this, fertility potential is reduced. Ackerman was able to demonstrate a pronounced effect upon sperm metabolism by cold-shock and believes that the metabolic changes occurring in frozen-preserved human sperm may account for its lowered fertility capacity (Ackerman, 1968).

Pedersen and Lebech found that most cells showed varying and often pronounced ultrastructure changes after a routine method of freezing for semen storage (Pedersen and Lebech, 1971). The most conspicuous changes were found in the acrosome region, the anterior segment of the acrosome becoming increasingly swollen, the content thinned out, and the outer acrosome membrane becoming tortuous and discontinuous. As a result, the anterior end of the spermatozoa loses the cell membrane and the anterior segment of the acrosome. Changes in the mitochondrial matrix and in the endpiece were also noted. As the acrosome has previously been shown to contain enzymes which probably play a role in lysing the zona pellucida during penetration, it seems probable that morphologic changes in this region result in enzyme changes. It is possible that the changes reported here were influenced by the protective substance in which the spermatozoa were mixed rather than by the freezing itself, nevertheless, the structural changes did follow an accepted method of sperm preservation.

Because of the lowered conception rate and the ultrastructure changes associated with frozen semen, we do not think that this technique is ready for routine clinical use, but believe that its present usefulness is in an investigational setting.

Artificial insemination in appropriate cases, using fresh human semen from either husband or donor, will result in a high pregnancy rate and will successfully treat infertility.

REFERENCES

ACKERMAN, D. R. The effect of cooling and freezing on the aerobic and anaerobic lactic acid production of human semen. *Fertil. Steril.* 19:123–128, 1968.

AMELAR, R. D. AND HOTCHKISS, R.S. The split ejaculate. Its use in the management of male infertility. *Fertil. Steril.* 16:46–60, 1965.

BALIN, H. *Artificial Insemination, Diagnosis and Treatment of Menstrual Disorders and Sterility,* 5th Ed. S. L. Israel (ed.). New York: Harper & Row, 1967, pp. 570–579.

BEHRMAN, S. J. AND SAWADA, Y. Hetrologous and homologous inseminations with human semen frozen and stored in a liquid-nitrogen refrigerator. *Fertil. Steril.* 17:457–466, 1966.

COHEN, M. R. Differentiation of sex as determined by ovulating timing. International Congress Series No 133. Proceedings of the Fifth World Congress on Fertility and Sterility, Stockholm, June 1966. Edited by B. Westin and N. Wiqvist. Amsterdam. Exerpta Medica Foundation, 1967, pp. 1196–1202.

DUKES, C. D. AND FRANKLIN, R. R. Sperm agglutinins and human infertility: Female. *Fertil. Steril.* 19:263–267, 1968.

FARRIS, E. J. The prediction of the day of human ovulation by the rat test as confirmed by fifty conceptions. *Am. J. Obstet. Gynec.* 56:347–352, 1948.

FUCHS, K., BRANDES, J. M., AND PALDI, E. Enhancement of ovulation by Chorigon for successful artificial insemination. *Int. J. Fertil.* 11:211–214, 1966.

KASKARELIS, D. AND COMNINOS, A. A critical evaluation of homologous artificial insemination. *Int. J. Fertil.* 4:38–41, 1959.

KLEEGMAN, S. J. Can sex be planned by the physician? International Congress Series No 133. Proceedings of the Fifth World Congress on Fertility and Sterility, Stockholm, June 1966. Edited by B. Westin and N. Wiqvist. Amsterdam. Exerpta Medica Foundation, 1967, pp. 1185–1195.

KLEEGMAN, S. J. AND KAUFMAN, S. A. *Infertility in Women.* Philadelphia: F. A. Davis Company, 1966, pp. 160–178.

KUNITAKE, G. AND DAVAJAN, V. A new method of evaluating infertility due to cervical mucus-spermatozoa incompatability. *Fertil. Steril.* 21:706–714, 1970.

MACLEOD, J. AND HOTCHKISS, R. S. The distribution of spermatozoa and of certain chemical constituents in the human ejaculate. *J. Urol.* 48:225–229, 1942.

MOON, K. H. AND BUNGE, R. G. Observations on the biochemistry of human semen. *Fertil. Steril.* 19:186–191, 1968.

PEDERSEN, H. AND LEBECH, P. E. Ultrastructural changes in the human spermatozoon after freezing for artificial insemination. *Fertil. Steril.* 22:125–133, 1971.

POTTER, R. G. JR. Artificial insemination by donors. *Fertil. Steril.* 9:37–53, 1958.

Our Growing Numbers*

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According to Dr. Barnes: There is no one problem more immediately pressing or as crucial as the questionable future of man's existence of terms of our expanding population.

To stress the immediacy of the problem, Dr. Barnes vividly illustrated by statistics the magnitude of the world's population growth. The exponential curve yielded by calculations of this rate of growth leads Dr. Barnes to state, unequivocably, that ". . . we have about six hundred years at the present rate of growth before every man, woman, and child on this globe has one square foot in which to stand. This calculation includes the polar caps, northern Siberia, and southern Siberia."

Because we live in a finite world, Dr. Barnes points out that infinite population expansion is impossible. Analogous to the life-support system for the astronauts, "We are locked in a little capsule that has been thrown up into the air and we are orbiting the sun. *Nothing* that is not already here is going to be delivered to our door." Presently, elements critical for man's existence, such as calcium and nitrogen, cannot be replaced once depleted. Consequently, "Zero population [growth] is going to become a necessity. And the later the year at which we achieve it, the lower will be our standard of living."

Recent observations made by Dr. Barnes during travels to several underdeveloped nations reveal that the problem there is not so much a problem of living space, as ". . . that they cannot maintain the supporting structures for what we call society." That is, the existing facilities to cope with their present population are so inadequate as to be nearly nonexistent. When this predicted population over-growth occurs, it will be impossible for a self-sustaining nation to retain its socio-economic equilibrium.

Statistics quoted by Dr. Barnes indicate that the United States now faces a precarious situation. Presently, our population doubling time is approximately 75 years, but worse, our pollution factor is even greater. In addition, America is the consumer of 55% of everything that is produced on the globe. One obvious solution, states Dr. Barnes, is that "We must start immediately to lower our standard of living so that we will have more to send out to those around us." At the moment, however, even if we agreed on family replacement (two children per set of parents), statistically, ". . . we would still almost double our numbers before we brought a halt to our continuing burgeoning growth."

Fervently, Dr. Barnes supports the belief that the medical profession should exert leadership in the field of population control. His reasoning is threefold: *First,* physicians should be advocates of preventive medicine. "America doesn't practice preventive sociology, it practices crisis sociology . . . the physician who is delivering groups of illegitimate children, who is watching unwanted pregnancies continue, has not fulfilled his obligation when he sews up the last episiotomy. He has not fulfilled it until he advocates a national policy toward [abolishing] unwanted pregnancies and national enforcement that will assist those individuals who choose not to be so afflicted." *Second,* ". . . it is our responsibility because it is caused by us." As Dr. Barnes illustrates, medical advances in the areas of vaccination, antibiotics, and nutritional changes each have had traceable affects upon our population growth. And *last,* ". . . it is our problem, at least for the moment, because the weapons that will cure it are in our possession."

The weapons Dr. Barnes refers to are: contraception, sterilization, and abortion. It is important to note that he is primarily concerned with their use on a global basis. "Of contraception," he feels, "the ideal method is not at the present time at hand. With the introduction of the ovulatory suppressant steroids and with the reintroduction of the intrauterine device, there is a tendency to relax that has proven

* Summary of the first McGuire Lecture presented on December 2, 1971, at the Medical College of Virginia, Richmond. Doctor Barnes did not wish to have speech published in its entirety.

unwarranted in its optimism." Doctor Barnes supports the continuing need for research in this field; for example, "We need an oral abortifacient . . . a simple medication that rules out the need for the operating room, abortions, sterile instruments, and so forth . . . and I would hope that we're within five to eight years of that." Above all, however, ". . . in the research field we need *dignity* given to this topic . . . particularly in the face of the size of the problem."

It is Dr. Barnes's view that "Progress in research . . . is progress in reaching the ignorant, the uneducated, and the lowly motivated." That is, if we can provide the underdeveloped nations with a simple, inexpensive, readily available procedure, we can hope to lower the percentage of conceptions. A sizable failure rate in preventing conception, whether it be 8% or 1%, should be considered a positive indication. "On a global basis," continued Dr. Barnes, "you have to look at the risk-benefit ratio . . . America has fallen into the trap of *not* [establishing] a balance between risk and benefit, and only looking at the risk as far as its medical program." In other words, ". . . you can use a considerably less safe method and still be well ahead of the death rate when no method at all is used. In the United States, no form of contraception is as dangerous as pregnancy."

Doctor Barnes points out that at the present time we have the pill, the intrauterine device, and the condom for global use. The comparative complexity of the pill limits its usefulness to a minority of the people on a global basis. The intrauterine device, on the other hand, has the advantage that people will have to be motivated only once a year. When insertion of this device is possible by paramedical personnel, as Dr. Barnes expects it will be, reaching the lowly motivated will not be such an arduous task. As for male methods of contraception (i.e. withdrawal and the condom), statistics reveal that they have been more effective than female methods of contraception.

The use of surgical sterilization and abortion on a global basis, Dr. Barnes adds, requires too many sterile instruments, trained personnel, and scrubbed rooms. In a modern society such as ours, Dr. Barnes advocates sterilization and abortion on demand. Globally, however, his advice is ". . . do all you can as fast as you can, but I'm afraid it isn't going to have a statistical recognizable effect."

The contributions obtainable from prostaglandins, according to Dr. Barnes, are still uncertain. Presently, their cost is too high to enable consideration on a global basis. "It takes about forty dollars' worth of prostaglandins to serve as an interrupter of early pregnancy or as a [luteolytic] agent, and that multiplies out to too much when you're beginning to talk about the percentage of people we need to reach."

Because of the mounting statistical evidence, Dr. Barnes cannot understand why concern for population expansion does not overshadow every other problem facing today's world. It is his contention that in our crisis society, people tend to ignore that which either does not touch them personally, or is not immediately grave or perilous. In order to bring proper attention to the problem, people must be reached on a personal basis. As a means of demonstrating the seriousness of the problem, Dr. Barnes suggests giving the public something to focus on; possibly a poster campaign with a "population baby" as the central theme, somewhat similar to the campaign mounted by the March of Dimes or Cerebral Palsy.

Even in the event of immediate public response and worldwide mobilization to counter the problem, Dr. Barnes is skeptical. "What is the solution? I'm not sure I know," said Dr. Barnes, ". . . it is entirely possible that anything we do may be too little and too late."

MCV/Q STAFF

Fetal Abnormalities of Metabolic Origin*

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The subject of my discussion is prenatal diagnosis of genetic disorders of a *metabolic* rather than a *chromosomal* nature. Whereas the chromosomal defects are the result of either the transmission of a translocated chromosome from a single parent to its offspring or an error in meiosis or mitosis, almost all *metabolic* disorders are inherited in an autosomal recessive fashion. Therefore, except in rare instances, the only clue to the possible presence of an inborn error of metabolism (IEM) in the fetus is that a previous child of the parents has had the disorder. This history identifies both mother and father as heterozygotes for the deleterious gene and thus forewarns that each subsequent pregnancy is at a 1:4 risk of yielding the disorder again. By studying the amniotic fluid of the at-risk fetus, it is now possible to determine if the disorder is present, and if so, to prevent its occurrence by therapeutic abortion. My main purpose is first to review the status of the art of amniocentesis and biochemical analysis of the material thus obtained, and then to examine the impact this procedure will have on the incidence and management of the IEM.

Amniocentesis in the *second* half of pregnancy for the diagnosis and treatment of erythroblastosis has been widely used for almost two decades with a minimum of morbidity or mortality to the mother or fetus. Theoretical risks of the procedure (fetal abortion, puncture, or induced malformation, and maternal bleeding, infection, or sensitization) had not been reported when the accumulated experience of 500 amniocenteses during the *first* half of pregnancy were reviewed one year ago (Nadler: *BD,* 1971). Prior to the 10th week of gestation there are less than 30 cc. of amniotic fluid present, and the uterus has not yet risen outside of the pelvis. Because of these facts, a transabdominal approach at this time is usually unsuccessful and the transvaginal route is attendant with a high risk of complications (especially abortion) (Fuchs, 1971). For these reasons the procedure is usually withheld until the 13th to 14th week, at which time approximately 100–120 cc. of amniotic fluid are present, and the uterus can easily be positioned to the anterior midline by bimanual examinations. Most investigators do not use placental localization, and they accomplish the procedure on an out-patient basis. In the combined experience of four investigators only 11% of 353 taps had to be repeated in order to get adequate material; initial failure in the majority of cases was due to contamination by gross blood (Nadler: *BD,* 1971).

Almost all of the cells present in the amniotic fluid are of fetal origin (either from amnion or skin), and some are viable. If the removed fluid is placed in a siliconized container, the cells will not adhere to the walls, and they can be shipped by mail over a 48-hour period or stored overnight in a refrigerator. When these cells are isolated from the amniotic fluid by centrifugation and placed in appropriate tissue culture media, their growth and multiplication rapidly ensues. After two subcultures and a total of four weeks of growth, hopefully a sufficient number of cells is present for analysis. Depending upon the disorder, the biochemical test performed is the measurement of either specific enzyme activity or of possible accumulation of a substance (such as mucopolysaccharides). Unfortunately, tissue sufficient in quantity to perform the desired test can be obtained only 75–90% of the time (Nadler: *SMJ,* 1971); with chromosomal studies, success rates of 95–99% have been achieved (Nadler: *BD,* 1971; *SMJ,* 1971).

The major premise utilized with this procedure is that these cultured amniotic cells accurately reflect the specific characteristics of the disorder in question. Previous studies of skin fibroblast cultures have proven that these cells *do* reflect the deficient state of the whole organism in most disorders.

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When the amniotic fluid cells are first removed they have the morphological appearance of epithelial cells, but in culture they take on the biochemical and morphological characteristics of fibroblasts. However, many factors affecting the enzyme activity of these cells must be considered before proper interpretation of results is possible (for example, stage of gestation, stage of growth of cells, degree of cell confluency). If one also remembers that these techniques must be refined to the point where the investigator can accurately differentiate the heterozygote from the homozygote, then hopefully he is left with the highest regard for the complexities of this diagnostic procedure.

In addition to the use of *cultured* amniotic fluid cells, biochemical analysis of *uncultured* amniotic fluid cells or of the amniotic fluid itself may provide sufficient information to make an accurate prenatal diagnosis. Although presently less than a half-dozen disorders can be detected by these methods (Nadler: *BD,* 1971), we hope further refinements will serve to increase this number, because such progress would obviate the minimum 4-week delay required to culture. Using cultured cells to establish the diagnosis leaves only a precious week or two to effect any needed abortion, as there are substantial medical, if not legal, restrictions to performing a therapeutic abortion much after the 20th gestational week.

Now that we have briefly described the capabilities and limitations of the procedure itself, let us examine how its usage can affect our present management of the IEM. The various modalites of treatment presently utilized (for example, limiting substrate, providing deficient end product), for the most part, do not attack the basic problem—that of decreased enzyme activity. Recently, there has been much discussion regarding the feasibility of gene therapy, that is, the isolation of some of the patient's somatic cells, the alteration of their genetic endowment *in vitro,* and their replacement in the individual. Other possible methods of providing the deficient enzyme include organ transplantation or specific enzyme replacement, but none of these approaches (particularly gene therapy) appears feasible in the next few years.

The only *preventive* measures available in the past were genetic counseling and abortion of male fetuses of mothers carrying a serious sex-linked disease such as muscular dystrophy (the sex of the fetus can be fairly accurately established by a study of the sex chromatin status of uncultured or cultured amniotic fluid cells). Genetic counseling is a good preventive measure because the majority of parents given a 1:4 recurrence risk will be deterred from future pregnancies, especially where the disorder is lethal or uncontrollable and where there are already one or more normal children in the family. But what of the parents whose *first* child has a serious inborn error of metabolism and who wish a normal child but are rightfully fearful of the 1:4 recurrence risk? This is where the amniocentesis and subsequent determination of the specific enzyme content of fetal cells is especially useful. I presently have two sets of young parents, each with an infant with Hurler's syndrome, who wish to have their first normal child. Only 3–4 laboratories in the country have developed the necessary techniques for the *in utero* diagnosis of this condition. Should one of these laboratories agree to monitor the pregnancy, and if the parents consent to an abortion if studies show an involved fetus, either the amniotic fluid (with the cells) or already cultured cells would be mailed to that laboratory.

At the present time there are less than twelve laboratories active in the prenatal diagnosis of the IEM—most, if not all, of these operate in a research rather than service capacity. A coordination of these various centers is needed because no one laboratory can perform all the available tests. As of one year ago there had been less than 25 therapeutic abortions performed in the United States for proven fetal metabolic disorders (Nadler: *BD,* 1971). This number would have been much higher had the biochemical determinations been more readily available.

What effect will this development have in reducing the actual number of infants born with metabolic disorders? One should remember that at present we must have an index case before knowing that we should monitor future pregnancies. Therefore, the birth of a majority of patients with IEM will not be prevented. Making the assumption that the goal of a family is to have two normal children, Motulsky has calculated that performing therapeutic abortions on all fetuses found to have a metabolic disorder (after one sibling involvement) will only reduce the incidence of that disorder by 12.5–34% (Motulsky, 1971).

In order to substantially reduce the incidence of these disorders we would need to identify which parent pairs are heterozygotic for the same deleterious gene *before* the delivery of their first affected child. It would be impractical to screen all prospective parents for heterozygosity for most of the

over 150 autosomal recessive IEM, because either the disease is so rare, or the test is too difficult to perform on a mass basis. However, it *is* presently feasible to screen certain high-risk groups for certain disorders—for instance, sickle trait testing for Blacks and the measurement of serum hexosaminidase A (for Gaucher's disease) in Ashkenazi Jews. Also, because of its prevalence in Caucasians, mass screening for the gene for cystic fibrosis should be performed once a simple reliable test is developed.

Many ethical, moral, legal, and theological issues are raised with our ability to define the metabolic status of the fetus. Time does not permit any in-depth discussion of these matters, but let me pose two questions as examples:

1. Should one abort a fetus who has galactosemia, pyridoxine-responsive homocystinuria, or phenylketonuria? These disorders are severe if untreated but the prognosis is good with dietary restriction or vitamin supplementation.
2. Should one induce an abortion where only one of nonidentical twins has an untreatable metabolic disorder?

At present it is felt that the final decision must be left to the parents, with the obstetrician and geneticist providing informed but impartial counsel. Perhaps shortly, each center involved in prenatal diagnosis will establish a board of physicians and lay people to reach a consensus in each given case.

In summary, it is now theoretically possible to diagnose approximately 40 different inborn errors of metabolism in a fetus early enough in gestation to perform a therapeutic abortion. However, most of these disorders are extremely rare, the techniques laborious, and diagnosis possible only after one sibling is already involved. For now, higher priority should be given to the development of procedures for massive heterozygote screening and intrauterine diagnosis of more common recessive diseases such as cystic fibrosis and sickle cell anemia.

REFERENCES

FUCHS, F. Amniocentesis and abortion. *Birth Defects:* Original Article Series 7:18, 1971.

MOTULSKY, A. G., FRASER, G. R., AND FELSENSTEIN, J. Public health and long-term genetic implications of intrauterine diagnosis and selective abortion. *Birth Defects:* Original Article Series 7:22, 1971.

NADLER, H. L., Indications for amniocentesis in the early prenatal detection of genetic disorders. *Birth Defects:* Original Article Series 7:5, 1971.

NADLER, H. L. Prenatal detection of inborn errors of metabolism. *So. Med. J.* 64:92, 1971.

Fetal Abnormalities of Viral Origin*

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The high incidence of acute infections known to occur in pregnant women contrasts with the relative rarity of fetal damage and attests to the efficiency of the mechanisms by which the fetus is protected *in utero*.

The events shown in Fig. 1 summarize some of the obstacles which a virus must overcome in order to reach the fetus and produce defects. An effective exposure is required to allow the virus to multiply locally in the maternal tissues at the portal of entry. If a viremic phase results, the virus becomes disseminated and the placenta may be infected; depending on the type of infection, placental damage may or may not result. If the virus successfully crosses the placental barrier, the fetus may become infected either by way of fetal viremia or by contiguous spread through the membranes. Since maternal viremia is necessary to initiate this chain of events, the fetus is naturally protected against viruses which seldom invade the blood stream. Most of the viruses causing respiratory diseases fall into this category. The maternal immunologic system provides significant protection by circulating antibodies stimulated by earlier natural or vaccine induced infection. Even if the mother is susceptible and the infection is associated with viremia, the virus may still be effectively stopped by the placental barrier; this has been shown to occur commonly in both rubella and cytomegalovirus (CMV) infection. If, on the other hand, the virus reaches the fetus, to produce a teratogenic effect the virus must have a delicately controlled pathogenicity allowing a chronic infection to be established compatible with a period of continued growth and development. Only two or perhaps three human viruses have been documented to have the ability to produce such infections. These include rubella virus, human cytomegalovirus, and, possibly, herpesvirus hominis (herpes simplex virus).

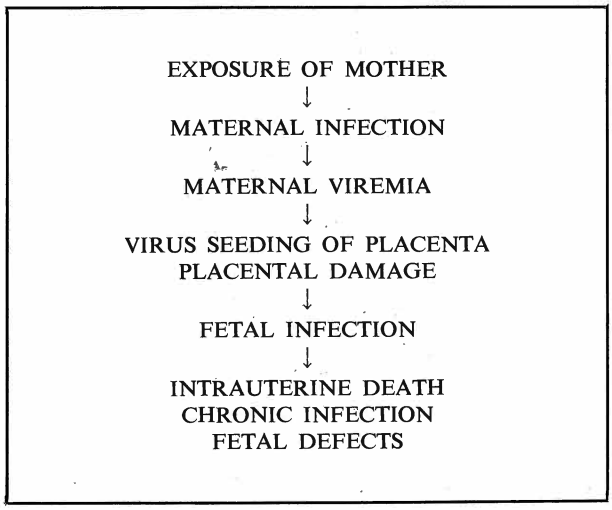

Fig. 1—Events leading to fetal infection and disease.

Of these three human viruses, the teratogenic effects of herpes simplex virus are the least documented. There are 5 reports in the literature suggesting that genital herpes infections acquired early in pregnancy may be associated with congenital malformations. Defects seen in these 5 infants included microcephaly, microphthalmus, and chorioretinitis. Skin lesions were present either at birth or soon thereafter in 3 cases and encephalitis was present in 1 instance. Investigation of the infants with microcephaly for other possible infectious etiologies including cytomegalovirus and toxoplasmosis infection gave negative results. The association of herpes simplex virus with eye and central nervous system (CNS) defects in these cases is highly suggestive that this virus may on occasion be teratogenic; the role of herpes simplex virus in this regard deserves further investigation (Nahmias, *et al*, 1970).

As the result of a decade of intensive interest, rubella represents the most adequately studied

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congenital infection producing fetal defects (Krugman, 1969). It also represents the only teratogenic virus infection currently controllable through the use of vaccine.

Rubella is recognized to occur every year in sporadic outbreaks covering restricted areas. Since 1928 major epidemics of the disease involving the entire country or large sections of the country have occurred at 6- to 9-year intervals. Rubella shows the same prominent seasonal pattern of other diseases transmitted by the respiratory route. The maximum incidence of the disease occurs during the winter and spring months, with very low rates in summer and fall. In a nonepidemic year, it has been estimated that the occurrence of rubella in pregnant women is in excess of 8/10,000 pregnancies. In an epidemic year, this rate may increase 30 to 40 times.

The clinical features of natural rubella virus infection vary from the clinically inapparent to an illness of sufficient severity to be confused with measles. Children before the age of puberty tend to have a mild illness consisting typically of 3 days of low-grade fever, seldom exceeding 101°F, rash, and generalized lymphadenopathy. Nodes are especially prominent in the posterior cervical region. In adults, particularly women, the illness is often more severe. The earliest evidence of infection is lymphadenopathy. The nodes may be tender and the enlargement often persists for several weeks after the onset of symptoms.

The rash is variable in appearance. Ordinarily, it first becomes apparent on the face and spreads rapidly to the trunk and upper extremities. It may have begun to fade on the face by the time it becomes fully developed on the lower extremities. Prominent respiratory tract symptoms do not occur. Women with rubella sometimes also complain of headache, malaise, myalgia, painful gums, and paresthesias, particularly of the hands.

Rubella is the only viral infection in this country which is frequently associated with arthralgia and arthritis. Age and sex have a marked effect on the frequency and severity of these symptoms. In a recent retrospective survey following an epidemic, 25% of prepubertal children experienced joint-related symptoms. After puberty there was a sharp increase in the incidence of arthralgia and arthritis, and with increasing age between menarche and menopause the frequency and severity of involvement increased progressively. Commonly the joint symptoms followed the rash by several days, although in some instances arthritis preceded the rash or was delayed until several weeks after the onset of infection. The joints involved, in order of frequency, were the proximal interphalangeal joints, the knees, wrists, ankles, and the metatarsophalangeal joints. Symptoms ordinarily subsided in 1 to 14 days but some patients, notably older women, experienced recurring symptoms lasting for a year or more. Even in such instances symptoms have cleared and there has been no residual deformity. There is no evidence that these reactions predispose to the development of chronic arthritis.

In the typical case, virus may be recovered from blood and respiratory secretions one to one and a half weeks prior to the onset of rash. Viremia disappears shortly after the onset of rash in association with the appearance of circulating antibodies. Presence of virus in the respiratory tract diminishes more slowly; virus may continue to be recoverable for several weeks. Neutralizing and hemagglutination-inhibiting (HAI) antibodies develop promptly after the exanthem appears and persist for years, probably for life. The CF antibody response is slightly delayed and may decline over a period of years. The widespread availability of the HAI test has made the rubella serodiagnosis of critical cases available to everyone. Establishing an accurate diagnosis is nowhere more important than in the instance when a pregnant woman is exposed to rubella. Under such circumstances, an initial serum specimen should be obtained as soon as possible after exposure and tested for antibodies to determine the immune status of the patient. Subsequent samples should be collected in early convalescence if disease develops and in asymptomatic patients at 4 and 6 weeks after exposure. These later samples tested in parallel with the earlier specimen will document if infection has occurred.

In 1940, a communication appeared in a prominent medical journal protesting interest in rubella as inappropriate since it was "an entirely inconsequential disease." This misconception was laid to rest within the next year by the astute observations of an Australian ophthalmologist. This was the contribution of the late Sir Norman Gregg, who opened the field of viral teratogenesis as a result of his discovery of the association between maternal rubella, congenital cataract, and heart defects. Over the years since Gregg's original observations, it has become apparent that virtually any organ system of the developing fetus may be damaged.

The fetal infection may be severe enough to

cause spontaneous abortion or stillbirth. Congenital involvement often present in the newborn period included deafness, heart disease, retardation, meningoencephalitis, microcephaly, eye defects, including congenital cataract, retinopathy and glaucoma, purpura, anemia, hepatosplenomegaly with hepatitis and jaundice, pneumonitis, and bone lesions.

The earlier in pregnancy maternal rubella occurred, the more likely it was that the defects would be multiple and severe. Follow-up data indicated that infection during the first 2 months of gestation was associated with the highest incidence of heart, eye, and CNS defects; fetuses infected in the 3rd month had significantly fewer heart and eye defects. All types of malformations declined in those babies who had been infected during the 4th month (Cooper, *et al,* 1969). It has been estimated that the chances of anomalies when maternal infection occurs during the first month is in excess of 50%, in the second month about 22%, and in the 3rd through 5th months, 6% to 10%.

In the pathogenesis of congenital rubella, viral invasion of the placenta occurs during the period of maternal viremia. Replication of virus in the placenta produces focal areas of mild inflammatory response and scattered damage of the endothelium of the chorionic blood vessels. Extension of the infection to the fetal circulation permits the virus to reach the developing organs where a persistent infection ensues. This infection may be localized or widely disseminated throughout the fetal tissues. Virus shedding in pharyngeal secretions and urine and the presence of virus in the tissues can be demonstrated throughout the neonatal period; in those infants with severe rubella defects, particularly, virus shedding may continue sporadically into the second year. The presistence of virus is of epidemiological importance since rubella infected infants can transmit their infection to nursery personnel and other contacts; thus it is important to limit the contact of such infants with pregnant women in the hospital and after discharge.

Antibody responses in maternal rubella, as with most infectious diseases, consist of the early appearance of transient IgM antibody, promptly followed and eventually replaced by the IgG class of antibodies. It is the latter which are responsible for the long-term persisting rubella antibodies. The situation in the infected fetus is more complex. Maternal IgG is transplacentally transferred. The infected fetus begins to produce his own immune substances, in the form of IgM antibodies, at some period during the second trimester. At birth the titer of antibodies in maternal and infant sera is very nearly the same. The infant's antibodies are made up of both transplacental IgG and endogeneous IgM. As many as 80% of rubella infants will have an elevated IgM serum protein fraction during the neonatal period. During the first year of life, passively acquired IgG declines. The production of infant IgG appears during the early months of life, and late in the first year replaces IgM as the predominant rubella antibody.

At the present time, short of therapeutic abortion, there are two methods for averting congenital rubella: (1) passive immunization of exposed pregnant women using commercially available immune serum globulin (gamma globulin); and (2) the use of live attenuated rubella virus vaccine for active immunization of nonpregnant subjects.

Gamma globulin has been commonly used for many years for the prevention of rubella infection in exposed pregnant women. The efficacy of this procedure has been a matter of controversy. There is no question that large doses of globulin given *prior to exposure* are effective in decreasing the incidence of infection and the clinical manifestations among those who acquire infection. In the ordinary circumstance in which globulin is used, however, exposure has already occurred several days earlier. In such cases the beneficial effect of globulin is less well documented. In fact, children with rubella syndrome defects have been born to mothers given appropriately timed adequate doses of gamma globulin. Carefully performed virologic studies, designed to mimic the usual post-exposure situation encountered clinically, failed to show protection against experimental infections, although there was some effect in diminishing the duration of viremia. From these data, it is obvious that globulin is unreliable as a measure for the prevention of congenital defects. On the other hand, it is impossible to be certain that globulin, through reducing the duration or level of viremia, might not have some effect in reducing the risk of fetal infection. On this basis, and because it represents the only currently available measure which might be of help in this situation, our feeling is that the continued use of large doses (20 ml of gamma globulin) is justifiable in circumstances where interruption of pregnancy is not desired.

Live rubella vaccines are prepared in cell cultures and induce immunity by producing a modi-

fied rubella infection in susceptible persons (Meyer and Parkman, 1971). The 3 vaccines currently available are produced in duck embryo cells by Merck, Sharp, and Dohme (Meruvax®), in canine renal cells by Philips Roxane, Inc. (marketed by them as rubella virus vaccine, live) and by Parke Davis and Co. as Rubelogen®, and in rabbit renal cells by Smith Kline and French, Inc. (Cendevax®). In children, vaccine induced infections are usually asymptomatic, while adults may develop rubella like symptoms. Such symptoms may consist of an evanescent rash, lymphadenopathy, and transient peripheral nerve and joint involvement. The most troublesome complaints have been related to occurrence of arthralgias, arthritis, and paresthesias; these symptoms follow a pattern of distribution similar to that seen in the natural disease. They appear within 3 to 5 weeks after immunization, but on occasion may be delayed until 8 weeks postvaccination. Reaction rates in women of childbearing age range from 10% to 60% and as in the natural disease are in part related to the age of the woman; reaction rates have been highest in the older age groups. The immunogenicity of the vaccine used also affects the likelihood of reactions. The canine renal cell prepared vaccine which produces higher antibody levels was also responsible for higher reaction rates than vaccines prepared in duck or rabbit cells. The joint manifestations are usually mild; however, occasionally a patient may continue to have residual subjective complaints lasting for weeks or months. Persons with arthritic manifestations have not shown the development of later chronic joint disease.

Rubella vaccines stimulate antibodies in 95% to 100% of recipients between the 2nd and 4th weeks postvaccination. Thus far, these antibodies have been shown to persist without significant decline throughout the 5-year period of observation since the earliest vaccinations were performed.

In the United States, efforts of mass vaccination programs have been directed toward the immunization of children. This approach is designed to reduce the opportunities for spread of virus by decreasing the susceptible childhood population most responsible for initiating and maintaining epidemic rubella. At the same time, the desirability of providing protection for the susceptible adult women is also recognized. Immunization of this group presents special problems. Arthritic reactions are more common in this age range; most important, however, is the risk of immunizing women in unrecognized early pregnancy or women who may become pregnant immediately after vaccination. In such situations, it has been shown that in susceptible women the attenuated virus can produce chronic placental infection which may be transmitted to the fetus. Generally, when vaccinations have been performed early in gestation, therapeutic abortion has been performed. Too few pregnancies have proceeded to term to determine the possible consequences of fetal infection with vaccine virus. Thus it is highly important when vaccinating adult women, to insure that the patient is not pregnant and will not become pregnant for at least two months after immunization. Pretesting of women for HAI antibodies is recommended and will exclude the necessity for using vaccine in immune women.

If vaccines are used extensively and if the immune status of the population can be maintained at a high level, it seems possible that rubella control can be achieved.

In comparison with the abundance of information available about rubella, knowledge of the epidemiology and pathogenesis of congenital CMV infections is limited. Thirty to sixty percent of childbearing age women have detectable antibodies. Infection during the reproductive years may be quite common. Serologic responses occurred in 3% to 5% of pregnant women in two urban centers studied. Information is not currently available concerning the frequency with which maternal infection is transmitted to the fetus. It is also unclear what proportion of fetal infections are (1) inapparent, (2) silent at birth but produce clinical manifestations later, or (3) result in classical cytomegalic inclusion disease. The reason for this poor documentation is in part attributable to the fact that maternal infections are subclinical. Rarely acute CMV infection in the adult may mimic infectious mononucleosis. Diagnosis of maternal CMV infection by virus recovery is hampered by the observation that virus may be shed in the urine for periods up to 2 years following initial infection; thus recovery of virus from a pregnant woman does not necessarily indicate acute disease.

The occurrence of the majority of congenital CMV infection in babies born to young primiparous mothers, suggests that intrauterine infection is most commonly acquired by the fetus during the viremic phase of primary maternal CMV infection. Fetal infection may occur in the first trimester (Davis, *et al,* 1971) and perhaps later in pregnancy as well. As in rubella, it has been shown that placental infection may occur without infection of the fetus.

Congenital CMV infection in 2 siblings born within 8 months of one another has been recently reported (Emil, *et al,* 1970). The possibility of such an occurrence should not be overlooked in the follow-up of women who have borne a CMV affected child.

Adding to the complexity of the CMV problem is the observation that multiple antigenic variants of the virus exist; the significance of this variation in the virus for immunity and the possibility of reinfection is unknown.

The features of classical congenital cytomegalic inclusion disease include, like rubella, defects in CNS, eye, and hepatic and bone marrow function. Microcephaly and hydrocephaly, encephalitis, cerebral calcifications, convulsive disorders, chorioretinitis, blindness, hepatosplenomegaly with jaundice, thrombocytopenia with petechiae, anemia, and interstitial pneumonia all can occur. Unlike the rubella syndrome, cataracts and heart defects are not seen.

As in congenital rubella, virus can be recovered from throat, urine, and blood of affected live born children and from multiple tissues of aborted or stillborn fetuses. Virus recovery from clinical specimens, unless accomplished in the immediate newborn period, cannot be taken as certain indication of an etiologic relationship between defects and CMV infection since these viruses can be recovered with some frequency from urine of asymptomatic children. The demonstration of elevated serum IgM and the persistent occurrence of specific CMV IgM antibodies in the serum of newborn infants provides convincing evidence of intrauterine infection (Sever and White, 1968; Alford, *et al,* 1967).

Aside from the experimental use of chemotherapeutic agents which inhibit replication of DNA viruses in children with the congenital disease, no methods of prevention or treatment are currently available.

What new developments are likely to occur which might be important from the standpoint of congenital infections? Several recent reviews have explored the status of other viral agents in producing abortions, stillbirths, and neonatal disease. It has been speculated on the basis of epidemiologic studies that other agents including varicella, vaccinia, mumps, influenza, and measles viruses may possibly induce fetal defects (Sever and White, 1968; Hardy, 1965). The etiologic relationship of these viruses with congenital defects are not in hand, but with additional study might yet be proven. Perhaps other mechanisms than those active in rubella and CMV infections will be shown important. An even more interesting possibility relates to the cancer virus field. Impressive evidence is available indicating that many tumors of amphibians, birds, rodents, and cats are caused by viruses. The study of animal model systems in chickens and mice indicates that RNA leukemia viruses may be transmitted "vertically," that is, directly from mother to embryo. The incidence of malignant disease in the congenitally infected animal is higher than that seen in the adult host infected by contact. Could such situations exist in man? Enough parallels exist between the human and animal diseases to make this a tantalizing possibility. Perhaps as techniques for searching out human oncogenic viruses become more sophisticated, similar agents may one day be recognized as causing highly important congenitally acquired infections.

REFERENCES

ALFORD, C. A., SCHAFFER, J., BLANKENSHIP, W. J. *et al.* A Correlative Immunologic, Microbiologic and Clinical Approach to the Diagnosis of Acute and Chronic Infections in Newborn Infants. *New Eng. J. Med.* 277:437–449, 1967.

COOPER, L. Z., ZIRING, P. R., OCKERSE, A. B., *et al.* Rubella: Clinical Manifestations and Management. *Am. J. Dis. Child.* 118:18–29, 1969.

DAVIS, L. E., TWEED, G. V., STEWART, J. A., *et al.* Cytomegalovirus Mononucleosis in a First Trimester Pregnant Female with Transmission to the Fetus. *Pediat.* 48:200–206, 1971.

EMIL, J. A., OZERE, R. L., AND HALDANE, M. B. Congenital Cytomegalovirus Infection in Two Siblings from Consecutive Pregnancies. *J. Pediat.* 77:417–421, 1970.

HARDY, J. B. Viral Infection in Pregnancy: A Review. *Am. J. Obstet. Gynec.* 93:1052–1065, 1965.

KRUGMAN, S. (ed.). Proceedings of the International Conference on Rubella Immunization. *Am. J. Dis. Child.* 118:3–410, 1969.

MEYER, H. M., JR. AND PARKMAN, P. D. Rubella Vaccination: A Review of Practical Experience. *JAMA* 215:613–619, 1971.

NAHMIAS, A. J., ALFORD, C. A., AND KORONES, S. B. Infection of the Newborn with Herpesvirus Hominis. *Advances in Pediatrics.* 17:185–226, 1970.

SEVER, J. AND WHITE, L. R. Intrauterine Viral Infections. *Ann. Rev. Med.* 19:471–486, 1968.

Psychological Abnormalities of Sexual Identification*

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The normal process of development of sexual identification slides almost unnoticed through well-defined, but overlapping stages to a definite end point. That end point is an individual who senses that his core gender, male or female, is consistent with the body morphology, the external genitalia, the chromosomal configuration, and the hormonal balance. Also, there must be the development of personality traits, masculine or feminine, consistent with the sense of core gender. Finally, although somewhat outside the scope of our present discussion, there must be the establishment of a role or life style in adulthood in accordance with the first two steps. The end point normally is sexual behavior acceptable to both the individual and to society, that is, heterosexual behavior in an individual who is comfortable with himself.

There are, then, three steps to mature sexual identification. The first and the most basic is the establishment of core gender, a sense of maleness or femaleness. The second is the establishment of personality traits, masculinity or femininity, which are layered upon the core gender but, as we shall see, are not necessarily consistent with it. The final step is the establishment of a life style or role which produces that obscure thing we call adult maturity. Let us take a closer look at the steps with emphasis upon the female.

The Core Gender. Core gender is the inner sense of being male or female and is not synonymous with masculinity or femininity (Stoller, 1965). Maleness/femaleness and masculinity/femininity are entirely different concepts, and in abnormal situations they may be in conflict with each other. Core gender is a basic concept universally recognized in all cultures. The sense of masculinity and femininity largely determines the use the individual makes of the core gender and is far from being universal (Mead, 1955). It varies markedly from culture to culture and may even determine specific subcultures in a given segment of society. This is not true of core gender wherein the sense of maleness or femaleness does not change.

The most important and the most accurate somatic sign of core gender to an individual and to his social milieu is the configuration of the external genitalia. One of the first things noted about the newly delivered baby is its genitalia. This quick and automatic examination is the first step, and in the vast majority of cases the only necessary step, toward determining the direction of that infant's development of a sense of core gender. When the parents are told the sex of a newborn infant, a complicated series of attitudes, feelings, and activities are set in motion in the parents, and later in the total social group. These factors are of primary importance in deciding whether or not that given individual will sense itself as male or female in adulthood.

Somatic gender and psychological gender do not always coincide in a small percentage of cases. Nature's errors have shown us that there are several variables which may enter the picture. These errors which produce congenital abnormalities have furnished us a natural laboratory in which to study the process of sexual identification (Money, Hampson, and Hampson, 1955). The variables which go into the picture are: chromosomal sex, gonadal sex, hormonal sex, internal reproductive organs, external genitalia, and finally, the emotional set of the parents.

The identity of core gender for practical purposes appears derived from three major sources: (1) the anatomy and physiology of the external genitalia; (2) the attitudes of parents, siblings, and peers toward the child's gender role; (3) a biological force that may modify or counter the effects of the first two in rare cases. These factors cannot

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be totally dissected from each other. The third factor, the biological force, is theorized by Robert Stoller from those rare cases which develop a core gender identity at variance with both the anatomy of the genitalia and the apparent attitudes of the parents toward the child (Stoller, 1964).

By far the most significant factor in the development of gender identity is the attitude which the parents associate with the gender assigned to the child at birth. This original assignment is made upon the appearance of the genitalia. The complicated maze of cues and signals which come from this include name, personal pronouns, type of dress, haircut, toys, amount of time the baby is handled, and more subtle attitudes. However, there are many cases in which a definite gender assignment has not been made at birth, and the child has been reared in an ambiguous situation. This allows for three possible gender assignments at birth: male, female, and no definite assignment. A child who has been reared to be unequivocally male or female appears firmly fixed in this gender role by the age of two and one-half years, but the child reared by parents who are uncertain of its gender role may see itself as neither male nor female at any year of life (Money, Hampson, and Hampson, 1957; Money, Hampson, and Hampson, 1955). This means that except for those rare, confused cases, core gender identity is fully established before two and one-half years of age, and that the sense of maleness or femaleness then becomes increasingly difficult if not impossible to change. Stoller has found that almost every successful report of changing sexual assignment after this age has involved an individual whose parents have not been able to see it as definitely male or female (Stoller, 1964).

Money and his co-workers verified this in their study of intersexed patients (Money, Hampson, and Hampson, 1955). In their first series of 76 cases raised with a gender assignment contrary to the variables of somatic sex, only four had not accepted the assigned sex. They found that an intersexed individual may establish a gender role opposite to the appearance of the external genitalia even though this is the most important sign to the parents and to the patient in determining gender identity.

There are many other studies which support the conclusion that a clearcut assignment to a core gender in early infancy leads to a psychological gender identity which remains fixed, even when that gender is incompatible with the somatic measures of sex. The practical implication is that a baby born with sexual anomalies of any sort should be diagnosed as rapidly as possible so that the proper sexual assignment can be made. Much attention and importance should be placed upon the external genitalia and upon the ease with which the abnormalities can be surgically reconstructed to be consistent with the diagnosed sex. Reassignments of sexual role after the third year should be undertaken only when it can be determined that the child has not accepted the role of either male or female. If surgical revision is feasible, these patients may make the switch without undue emotional turmoil. These will be the children whose parents have raised them in no particular gender assignment and communicated this ambiguity to the child.

These observations of nature's errors—intersexed patients—have received support from the imprinting studies of Konrad Lorenz and from H. Harlow's experiences with monkeys (Lorenz, 1952; Harlow, 1962). These studies on animals have shown that the early experiences of the animals determine how they will function sexually when they become adults. There is no evidence that imprinting in this manner occurs as specifically in the human being as in its primate relatives, but the phenomenon of this permanent identification supports the concept that certain sensory experiences may influence the development of reaction patterns in the central nervous system.

Such confused sense of core gender can occur when there are no abnormalities of a morphological or physiological nature, and when it does, the condition is called transsexualism which simply means the mind or psyche of one sex trapped in the body of another. Christine Jorgenson made this a respectable medical study in this country some 20 odd years ago when she became a woman after many years of maleness. Transsexualism probably represents a form of imprinting in which the individual is programmed to the core gender opposite to the morphological and genetic sex (Benjamin, 1966).

An example of this condition is a young lady who a few years ago requested that she be given a male voice and a beard. She stated that she had difficulty holding down her job as a carpenter once they found out she was not a man. Dressed in male clothing and with a short haircut, she looked much like a boy in his late teens although she was 30 years of age. Her story was as follows:

On the same day in a rural town two sisters

delivered babies. One sister was married, and one was single. The married sister delivered a male child who died immediately at birth. The unmarried sister delivered a normal girl child who was given to the married sister to replace her dead infant and raised as a male child. This girl baby was named William and called Bill. As an only child, she became Daddy's helper around the small ranch and had her first dress on at the age of 13 when this was enforced at the high school level. At the age of 30, Bill could describe the acute embarrassment of being forced to wear female clothing.

Bill sensed herself to be a male although she had a normal menstrual cycle, and complete endocrinological and chromosomal work-ups revealed a perfectly normal XX individual. She had even forced herself to have sexual intercourse in her early 20's in an attempt to become "different" but had found it disgusting.

Bill wanted simply to function as best as possible in accordance with how she sensed herself—as a man. She was a transsexual in the fullest sense of the word, but whereas it is possible to replace the penis and the scrotum with a vagina, the opposite is surgically a bit difficult.

Thus for the first phase of sexual development (core gender), transsexualism is the psychological abnormality sometimes seen, and it appears to be relatively unchangeable after two and one-half years of age.

The second aspect of sexual identification which may go awry is the sense of masculinity or femininity. This is layered upon but not necessarily identical with the unalterable core gender. The concepts of masculinity and femininity are complicated developmental processes that are culturally and socially determined and which modify and cover over the core gender but do not change it (Mead, 1949). Thus, a male homosexual may have serious doubts about his masculinity, but he does not doubt his maleness at any time. He may not like being a male, but he definitely knows that he is one. Similarly, the female homosexual may abhor her core gender to the extent of removing every external vestige of it, but this does not alter her knowledge of her femaleness.

Masculine and feminine identities may change throughout life, but the most significant aspects may be well established by the age of 6 to 7. Social, cultural, and parental factors play determining roles in differentiating and establishing the individual's psychological concepts of sexuality. At about three years of age, there is the beginning of increased attention to the genital area as a mark of progressing physiological and biological maturation. The child perceives new sensations which lead to a marked increase in curiosity about its body and the bodies of others. Children are apt to notice that there are sexual differences, that the outline of one body is not the same as another. This is a momentous discovery for a child and may lead it to ruminate about these differences and to concoct various explanations for them. The little girl must deal with her discovery of having no external genitalia comparable to that of the male. Whether or not she perceives herself as being defective will depend upon, in most cases, how her mother views the role of female and whether or not the mother shows a preference for that which is masculine.

The little girl's perception of what it takes to be feminine is controlled largely by whether or not she senses femininity as desirable. The father figure plays a major role here. The little girl must conceptualize whether or not the father figure approves of femininity and all that it entails. If not, she is apt to find it difficult to accept a role which, more or less, excludes father's approval. Normal psychological development of sexuality in this period requires that the mother be a desirable figure after whom to model oneself, and that simultaneously the father figure approve of this role.

We are talking about what Freud called the Phallic phase and the Oedipal period. The most important requirement of the Oedipal period is that the child experience a sustained relationship to a mother and a father in their sexually differentiated roles. The lack of a parent of the same sex or an undesirable parent of the same sex during this phase makes it extremely difficult to establish a proper identification compatible with the core gender. The lack of a parent figure of the opposite sex, relative or otherwise, is equally discouraging to the development of adequate heterosexual relationships in the future.

The daughter of a harsh, demanding, and rejecting father or in a home situation in which masculinity reigns supreme and all things feminine are depreciated, can hardly see herself obtaining desired approval by developing feminine traits. She is apt to become an adult who sees feminine characteristics not only as without positive value, but also as attributes which produce discomfort and anxiety.

Whereas maleness and femaleness are definite all-or-none phenomena, masculinity and femininity are not. The latter lie upon a continuum which produces at one end a high level of homosexuality, and at the other end, a high level of heterosexuality. Most people fall somewhere in between. If we confine our discussion to the female, we can avoid speaking of the many deviations of sexuality which occur in the male. True deviations, with the exception of homosexuality, are relatively rare in females.

Not so rare in the female are the many aberrations of sexuality due to defective identification as to the feminine aspect. For example, these aberrations almost always will be manifested as aberrations of the personality. Over-aggressivity or over-passivity may mean the same thing. Difficulties in relating to the opposite sex may stem largely from deficient identification as feminine. The various conditions such as frigidity, dyspareunia, promiscuity, and even menstrual abnormalities may be symptomatic of deficiencies in identification. The entire attitude of a woman toward being a wife and a mother depends largely on this facet of the personality.

Psychological problems can be manifested as physiological changes. The hypothalamic areas are in charge of the pituitary, and therefore, of much of endocrine functioning. Just as severe stress or fear can change menstrual function, so can prolonged identification problems produce abnormal physical manifestations directly through the autonomic nervous system and indirectly through the endocrines (Sturgis, 1962).

Let us look briefly at one example of an aberration of the development of femininity. A young lady came for psychiatric consultation at the age of 25, two years after her marriage to a young lawyer. Both of her parents were physicians. Her mother was a very competent, cold, and efficient professional, and the father was a very outgoing, warm, and well-liked individual. Her fantasies of her father included this statement, "I bet he 'made' every woman he met during his younger years." She said this with a look of distinct pleasure and pride.

This lady came into treatment primarily because she was being extremely promiscuous. She enjoyed sexual intercourse with her numerous partners, but she could barely tolerate it with her husband. She had, upon several occasions, become violently ill because of the necessity to have intercourse with him. She followed this by a hot shower to cleanse herself symbolically.

None of this occurred when she slept with other men. She did not have orgasms, but she enjoyed the act immensely and never had any feeling of dirtiness or guilt. She wanted help for the problem, because she recognized that such a life eventually would become greatly complicated, and that it was foreign to how she really felt at some level.

This girl identified strongly with the warm, outgoing father. The cold and aloof professional mother simply was not a model for her. Her core gender was perfectly normal; she knew quite definitely that she was female. However, she was far from being convinced of her femininity. She had seen femininity as a little girl as something not desirable. She had conceptualized being like her father, the outgoing, likeable, masculine image, as being very desirable. The promiscuity was following the example of how she thought her father must have acted when he had been her age, but it also was a way of proving to herself that she really was desirable. She could not tolerate sexual intercourse with her husband because this represented to her intercourse with her own father. This is a kind of reverse of the Madonna-Prostitute Syndrome, sometimes called the Messalina Syndrome (Mathis, 1971). It represents an abnormality of the sexual identification at the second step.

The third and almost final step of sexual identification occurs in the teens with the establishment of a role. Maleness and femaleness are fixed, masculinity and femininity are fairly definite, but just how one is to use these aspects in living can still vary greatly. We will not go into this, since the cultural aberrations are many and are beyond the scope of this talk. It is, however, in the early teens that the psychological abnormalities of sexual development become openly manifest. Always they will have been obvious to the astute observer at the earlier years, but now they are inescapable. The onset of puberty and the blossoming of secondary sex characteristics may come as a great shock to a little girl who does not like to face the fact that she is a woman. She may see menstruation and a budding breast as vulgar signs of something that is undesirable. Not infrequently this leads to great anxiety which is manifested by delinquent behavior—almost always sexual in nature. Her anti-social sexuality may have many meanings, but one is to misuse or to degrade that part of her which she does not like. On the other hand, this

behavior may be used as a constantly recurring attempt to prove that she really is feminine, something that actually is beyond her reach at some level.

Heterosexual maturity requires an unambiguous knowledge of core gender and a mature identification of the masculine and feminine roles in the society in which an individual lives. The process by which this is obtained is complex and fraught with innumerable dangers. Fortunately, by far the greatest majority of people make it to the desired maturity level quite well. A large number, however, make up the patients which you see, primarily because they have deviated at some point in this developmental process.

REFERENCES

BENJAMIN, HARRY. *The Transsexual Phenomenon.* New York: Julian Press, 1966.

HARLOW, H. The heterosexual affectional system in monkeys. *Amer. Psychol.* 17:1, 1962.

LORENZ, K. D. *King Solomon's Ring: New Light on Animal Ways.* New York: Thomas Y. Crowell Co., 1952, p. 40.

MATHIS, J. L. The Madonna-Prostitute Syndrome. *Med. Aspects of Human Sexuality.* 5(1):202–209, Jan., 1971.

MEAD, MARGARET. *Male and Female.* New York: William Morrow and Company, 1949, p. 245.

MONEY, J., HAMPSON, JOAN, AND HAMPSON, JOHN. An examination of some basic sexual concepts: The evidence of human hermaphroditism. *Bull. Hopkins Hosp.* 97:301, 1955.

MONEY, J., HAMPSON, JOAN, AND HAMPSON, JOHN. Imprinting and the establishment of gender role, *Arch. Neurol.* (Chicago) 77:333, 1957.

STOLLER, R. J. A contribution to the study of gender identity, *Int. J. Psychoanal.* 5:220, 1964.

STOLLER, R. J. Gender role change in intersexed patients. *JAMA* 188:684, 1964.

STOLLER, R. J. The sense of maleness. *Psychoanal. Quart.* 34:207, 1965.

STURGIS, SOMERS. *The Gynecological Patient.* New York: Grune and Stratton, 1962.

The Surgical Approach to Problems of Sexual Identification*

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At the level of chromosomes recognized morphologically in every cell, two sexes are a basic biological phenomenon. At the level of the total organism, these sexual differences may be seen anatomically and endocrinologically and perhaps in a less precise way psychologically, especially as measured by behavior. In the human, various errors in the anatomical development of the sex organs due to genetic, environmental, or unknown causes have been recognized and described under the general heading of intersexuality. Poorly treated victims of these aberrations may experience confusion, or even crises, in sexual identification. For the most part, such psychological difficulties can be related to the anatomical ambiguity of the genital organs.

However, there are other individuals who, by ordinary anatomical, endocrinological, and chromosomal examination, are quite consonant with respect to one or the other sex but who, nonetheless, consider themselves to be of the other sex. In its full-blown clinical expression, such individuals manifest with consistent and persistent conviction the desire to live as a member of the opposite sex and progressively take steps to do so. Furthermore, psychological evaluation of such individuals reveals many characteristics of the sex opposite to their anatomical sex. Such individuals may be labeled as transsexual, and it is to the therapy of such patients that the role of surgery has been evaluated on a small number of patients at The Johns Hopkins Hospital over the last several years.

While transsexualism is a disorder which seems to affect both anatomical sexes, the gynecologist plays his principal role in the therapy of the male transsexual, i.e., the anatomical male who considers the difficulty to be that of a female psyche trapped within a male body. It is only with these male patients with a female gender orientation that this discussion will deal.

Unfortunately for the therapist, the diagnosis of transsexualism is not easy and must be distinguished from other disorders which have, or seem to have, expressions of incongruent gender behavior. Among these are transvestism, homosexuality, psychotic individuals with confusion in sexual identification and gender role, neurotic sexual problems, and exhibitionism.

The gynecological surgeon should not, and indeed cannot, dispense with the wholehearted help of psychiatrists and psychologists in sorting out these sometimes overlapping entities. As with most behavioral disorders, there are no objective criteria, no specific physical findings, no laboratory test, no pathonomonic sign by which a specific diagnosis can be made. Nonetheless, it cannot be overemphasized that no pains must be spared to make an accurate diagnosis. Experience to date has made it abundantly clear that surgery is unlikely to be appropriate and may be devastating for those disorders which might be confused with true transsexualism but which, nevertheless, exhibit signs of deviant gender role.

Thus, the male transvestite is a "cross-dresser" at intervals for the relief of an insistent tension that builds up between cross-dressing. However, he is usually heterosexual in his relation with women and, if married, takes the position that his wife and children understand him and are not devastated by his actions and cross-dressing. It would obviously be an error to think of reconstructive surgery of the genitalia for the transvestite.

The effeminate male homosexual is erotically attracted exclusively to men. He may wear wo-

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men's clothes, but apparently the compulsion to do so is less than that of the male transvestite. He is not heterosexual, as is the transvestite. However, and most important, he seems to have no problem with his own sexual identification and has no desire for transsexual surgery, except in temporary situations such as the break up of homosexual partners.

The true male transsexual exhibits many phases of a female personality. This includes not only the desire to repudiate men's clothing and strictly male activities, but expresses itself to the surgeon as a desire to be rid of the male genitalia which serve as a constant reminder of a sexual symbol and status which are regarded with repulsion as inaccurate. Also, it is generally true that when a male transsexual is offered the option of having a vagina constructed, which is the only female organ which can be surgically offered, she will invariably elect to have that procedure carried out.

The undesirability of offering operations to psychotic individuals with confusion in sexual identification and gender role should be obvious. The other disorders which might be of some confusion, such as neurotic sexual problems and the like, are not only too trivial for surgical reconstruction but are amenable to psychiatric therapy.

One of the main reasons for evaluating surgery in the treatment of this disorder is the fact that transsexual individuals are very resistant to psychiatric help for their disturbance. While the gynecologist obviously cannot speak with great understanding and experience on the psychiatric approach to disease, it is perhaps not inaccurate to observe that psychiatric failure to offer help to the transsexual stems from the fact that the psychiatrist necessarily wishes to aid the transsexual by reorganizing the psyche to conform to the anatomical sex. This the transsexual resists and does not want. The psychiatrist, therefore, has no handle on the transsexual and does not have a sympathetic patient, for the patient regards with suspicion anyone who denies what the male transsexual believes to be the basic problem, that is, a female psyche trapped within a male body. It is because of this basic impasse in psychiatric therapy that endocrine and surgical manipulation might have a role in bringing the patient to terms with her basic conflict.

The Selection of Patients for Surgery. About five years ago at The Johns Hopkins Hospital, a gender identity clinic was established for the purpose of studying the problem of transsexuality and the role of surgery in the therapy of patients with transsexualism. This clinic consists of several individuals from various disciplines. Psychiatry is very heavily represented, and a psychiatrist is chairman of the committee. Representatives of plastic surgery, endocrinology, urology, and gynecology and obstetrics regularly attend the clinic. Since the establishment of the gender identity clinic in 1966, there have been over 1200 inquiries by mail or individual application of individuals who considered themselves candidates for surgery. However, several of these applicants eliminated themselves from further consideration by failure to answer follow-up questionnaires about certain details of their disorder. In the third year of its existence, the clinic mailed additional questionnaires to all applicants and has a waiting list of approximately 500 individuals who might be candidates for further evaluation. It has never been the primary object of the clinic to service all comers, but rather it has been hoped that its principal objective would be a mature and careful consideration of the problem, especially of the relation of surgery to therapy.

Suitable patients are interviewed by a psychiatrist in a preliminary interview and if, on this occasion, the various confusing disorders mentioned above can be tentatively eliminated, the patient is then admitted for what is termed a full-scale evaluation. By this is meant an examination by each of the various members of the gender identity clinic during which time the patient is given not only a physical examination but an extensive psychiatric and psychological work-up. Patients to be considered candidates for reconstructive surgery must have fulfilled as a minimum the following requirements:

1. Insofar as can be determined by the various psychiatric and psychological tests, the patient must be a true transsexual and not suffering from any of the allied disorders mentioned above which have deviant sexual identification as part of the syndrome.
2. The patient must be at least 21 years of age.
3. The patient must have no police record of a serious crime or misdemeanor.
4. The patient must have lived in the female sex role for a minimum period of twelve months. During this time she must have proved her ability to be gainfully employed

as a female and to function satisfactorily in society as a female.

5. The patient must be unmarried.
6. The patient must be available for follow-up.
7. A responsible member of the family, the parents if possible, must be completely aware of the situation and enthusiastically support the possibility of surgical sex reassignment.
8. The patient must receive estrogen for a minimum of twelve months prior to the operative procedure.

It is obviously wrong, therefore, to think of surgical sex reassignment as changing the sex of the individual. The operation cannot be looked upon as a sex change operation. It is simply one step in the total rehabilitation of the patient. Since it is an irreversible step, it is of the greatest importance that no error be made in the diagnosis. Furthermore, it is highly desirable, but difficult of achievement, that the period of surgical sex reassignment not be the final contact of the patient with the clinic, so that the members of the clinic can be as useful as possible in aiding the further rehabilitation of the patient in her continuing female sex role.

Results of Surgery. The postoperative behavior and adjustment of transsexual patients is the key to an evaluation of the role of surgery in the treatment of transsexualism.

The postoperative course of 17 male transsexuals who have had endocrine and surgical sex reassignment has been the subject of a study by Money and Ehrhardt of our clinic (Money and Ehrhardt, 1970). These 17 patients were followed from one to thirteen years. As a group, they expressed a willingness to undergo surgery again and all of them considered that they had achieved improved status. Eight of the patients had an employment status which was essentially the same as that before operation, but nine of the patients had an improved employment and economic status. Six of the patients, prior to the adoption of a policy which would exclude such individuals at the present time, had a police record. Two of these continued to have difficulties with the police after surgery, and it is for this reason that such patients are no longer considered for surgery. The marriage status of the individuals was as follows: two were married preoperatively only as males, seven were married postoperatively only as females, two were married preoperatively as males and postoperatively as females, and six were never married.

It seems clear that surgical reassignment does not harm carefully selected patients, does offer considerable improvement but, by the same token, does not automatically result in the cure of various problems not directly related to the problem of gender identification.

Postoperative male transsexuals living as women do not lose the capacity for erotic genital sensation. Orgasm is not unusual. There seems to be a particularly erotic area in the region of the prostate.

In general, follow-up of transsexual patients who have been successfully operated on is difficult because the more successful the treatment of the patient, the less they wish to maintain contact with the clinic and the more they wish to disappear into the new society they have found and created.

Our experience would lead us to believe that endocrine and surgical therapy of carefully selected male transsexuals has a role in the contemporary management of such individuals. However, it cannot be overemphasized that the proper diagnosis is not simple, requiring the dedicated cooperation of psychiatrists and psychologists who are knowledgeable in the field and who are willing to devote the necessary time and energy to add their skills to those of the endocrinologists and surgeon; all of whom as a team can make the only reasonable approach to this problem.

REFERENCE

MONEY, J. AND EHRHARDT, A. A. Reassigned transsexuals: marriage, employment, police records, psychotherapy, and subjective satisfaction. *Tendenzen der Sexualforschung.* Gunter Schmidt, Volkmar Sigusch, and Eberhard (eds.). Stuttgart: Ferdinand Enke, 1970, pp. 72–87.

Chemical Changes in Amniotic Fluid*

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The scope of my talk as represented by its title encompasses a very large field which time does not allow me to cover in its entirety. Therefore, I will limit my remarks to one very specific area where chemical changes in amniotic fluid could be used to predict fetal maturity.

An ever present dilemma in modern obstetrical management is the proper timing of delivery of the fetus in situations where complications either threaten its own life *in utero* or significantly affect maternal morbidity. In such situations the obstetrician is confronted with the equivocal choice between premature delivery and the high risk to the fetus of continued intrauterine existence. In addition, he is placed in a unique position where any sort of direct communication with one of his "patients," namely the fetus, is nearly impossible. In the past, the delivery of obstetrical care to the fetus and the monitoring of its well-being were possible only through the agency of the maternal organism. To improve upon this situation, methods had to be found that would give a more direct and accurate reflection of fetal status. Hence, as complete a collection as possible of accurate indices relating to fetal maturity is of great importance to the obstetrician. Some of the notable complications of pregnancy in which these indices would be of value are diabetes, toxemia, erythroblastosis fetalis, and previous poor obstetrical history: for example, cases requiring repeat cesarean sections or abruptio placentae.

In the late fifties (Jeffcoate and Scott, 1959), it was recognized that the fetus contributed metabolic products and secretions to the amniotic fluid, and this encouraged many researchers to investigate a possible correlation between the status of the fetus and the amniotic fluid constituents. At that time, however, the technique for amniocentesis had not been generally accepted, although it has been known for over two decades, and the progress of research was slow. With the acceptance of amniocentesis as an aggressive means of diagnosis, the procedure for withdrawal of amniotic fluid led to several investigations into fetal maturity indices. Amongst these were the measurements of amniotic fluid creatinine (Pitkin and Zwirek, 1967; Liley, 1961; Mandelbaum, *et al,* 1967), bilirubin (Droegemueller, 1969), and the examination of the cytology of cells stained with Nile Blue Sulfate (Brosens and Gordon, 1966; Anderson and Griffiths, 1968). These indices are now used in a fairly routine manner in the management of obstetrical patients, and I shall not go into any detail about them. A further and more specialized offshoot of this research has been the measurement of certain other specific amniotic fluid constituents that might lead, in the near future, to a more reliable picture of fetal well-being or distress in addition to fetal maturity.

In spite of all the advances that have been made, there still exists an appreciable degree of perinatal mortality today, the major contributor being respiratory distress due to premature delivery. An estimated 25,000 newborn deaths annually in the United States are attributed to this cause (Gluck, 1971). The stage of fetal lung maturity at the time of delivery is of prime importance as it most often entirely determines the survival or death of the neonate. For the neonate to survive, its lung has to assume immediate function with the change from an intra to an extrauterine environment. Thus, in the case of a pregnancy complicated, for example, by the presence of maternal diabetes or toxemia, the obstetrician has to walk a tightrope where he must maintain, to the best of his ability, a critical balance between neonatal death due to respiratory distress (hyaline membrane disease) and the threat of intrauterine demise caused by maternal disease. The frustrating decision that has to be made is, "When

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is the earliest the obstetrician can deliver a healthy baby that will survive?"

At the moment, what seems to be a ray of hope on the horizon for the obstetrician has resulted from research that was started in the early fifties on the phospholipid content and its patterns in the fetal lung. As early as 1929, von Neergaard (von Neergaard, 1929), demonstrated that surface forces contribute significantly to the retractive pressure of the lung. At that time he postulated a lung coating to explain these observations. In 1955, Pattle (Pattle, 1955), showed that pulmonary edema foam contained some substance, or substances, that stabilized the tiny bubbles seen characteristically in such foam, and in 1957, Clements (Clements, 1957) and Avery and Mead (Avery and Mead, 1959) identified a surface tension lowering substance in lung tissue. These observations, subsequently confirmed by several workers, led to the conclusive identification of this surface tension lowering compound, called a surfactant, as being α,β-dipalmitoyl lecithin.

I am now going to highlight some of the significant laboratory findings that have led us to the point at which we are today. In 1961, Klaus and his co-workers (Klaus, *et al,* 1961) showed that bovine lung foam contained a mixture of several surface active compounds, the major constituent being dipalmitoyl lecithin. The other surface active compounds he found were sphingomyelin, phosphatidyl inositol, phosphatidyl dimethylethanolamine, and lysolecithin. The chemical structures of some of these compounds are depicted in Fig. 1. During the sixties, significant details of the surfactant system were gleaned through the work of Morgan (Morgan, *et al,* 1965), Fujiwara (Fujiwara, *et al,* 1968), and Gluck (Gluck, Kulovich, *et al,* 1967, 1970) all working independently. Morgan and his group worked on the identification of the various components of the surface active system in alveolar and whole lung homogenates in the dog. They found that of the total lipids found in dog lung, phospholipids constituted the major group at 74.1%. Of this group, phosphatidyl choline was the major component. Phosphatidyl ethanolamine, lysophosphatidyl choline, which is lysolecithin, and sphingomyelin were also found, but in much smaller quantities. These are all surface active along with dipalmitoyl lecithin or phosphatidyl choline, but phosphatidyl choline seems to be by far the major component. As seen in Fig. 1, the lecithins are characterized by fatty acid groups R_1 and R_2 on the α and β positions of the triglyceride backbone. Fujiwara and his associates

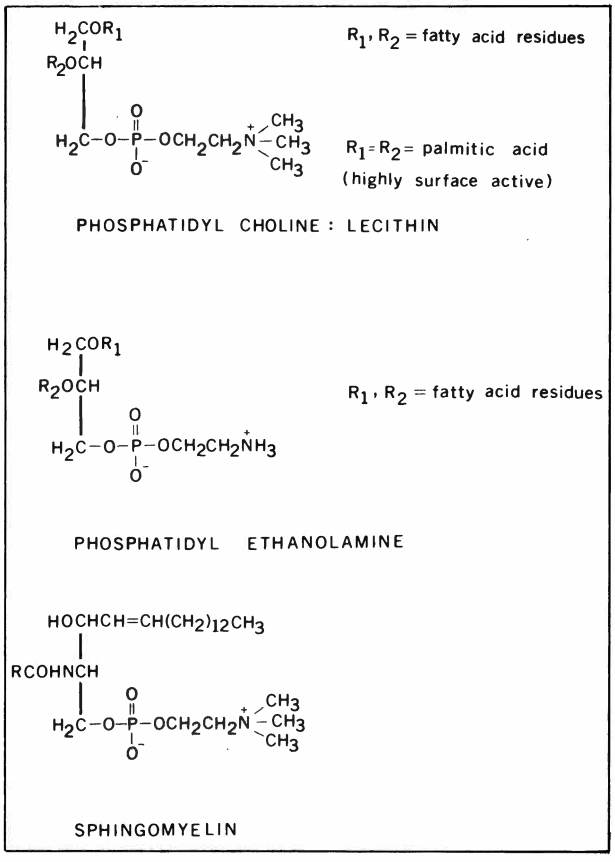

Fig. 1—Chemical structures of some phospholipids.

(Fujiwara, *et al,* 1968) reported on the significance of these fatty acids, which are normally C_{14} to C_{18} fatty acids, and the total concentration of individual phospholipids in the developing fetal lamb lung. He took fetuses at three different ages of gestation; immature (99–119 days), transitional (120–134 days) and term (135–145 days). Gestational age in sheep is about 140 days. He found that as gestation proceeded toward term, the amount of phospholipids, both on a weight basis and on a total phospholipid basis, increased. He also found that phosphatidyl choline increased while phosphatidyl ethanolamine decreased, and this will be an important factor when we talk about the biosynthetic pathways. The significance of the fatty acids was demonstrated by the observation that the percentage of saturated fatty acids in the whole molecule increased towards term, whereas the percentage of saturated fatty acids at the β position stayed fairly constant. This means that now we have two parameters by which the state of development of the fetal lung can be assessed; in general, the total amount of lecithin present and specifically, the

amount of lecithin esterified with saturated fatty acids. To test the hypothesis that lung maturity is reflected by an increase in saturated lecithins, and vice versa, Brumley and his colleagues measured the surface tension and phospholipid content of normal lungs and lungs from infants with respiratory distress syndrome (Brumley, *et al,* 1967). Brumley's data is shown in Table 1. As I mentioned earlier, what the surfactant does is lower surface tension and thus produce more elasticity in the lung. In adults the surface tension of trachial aspirates is somewhere of the order of 6 dynes/cm., and the total phospholipid content is 16.8 mg/gm wet weight. The content of phosphatidyl choline, as a percentage of total phospholipids, is about 45.8%. In the infant with no respiratory distress syndrome these values are very close to those in the normal adult. With respiratory distress syndrome the surface tension of trachial aspirates becomes much higher. The amount of phospholipid is considerably lower compared to that of adults and infants with no respiratory distress syndrome, as are the amounts of phosphatidyl choline and saturated fatty acid. As the neonate recovers from the respiratory distress situation, these values slowly start returning to normal. This means that there is a valid justification for implicating the amount of phospholipid, specifically phosphatidyl choline, in the development of fetal lung. Gluck and his group (Gluck, 1971; Gluck, Kulovich, *et al,* 1967, 1970) reported extensively in the middle and late sixties on the biochemistry of surfactant production in the lung. They measured the amount of dipalmatoyl lecithin and sphingomyelin in amniotic fluid in humans (Gluck, 1971), with the results shown in Fig. 2. Gluck found that early in gestation, the amount of lecithin is slightly lower than that of sphingomyelin but that they parallel one another and continue to do so until about the 25th week of gestation where lecithin starts rising slightly more rapidly than sphingomyelin. At about the 30th to 34th week there is a sharp increase in lecithin concentration till term, whereas sphingomyelin plateaus and starts going down again. The reason for this dramatic rise in lecithin biosynthesis at about the 32nd to 34th week can be partially explained by the picture of the biosynthetic pathways of these phospholipids (Fig. 3). The lecithins are synthesized mainly by two routes. In pathway 1 (the choline incorporation pathway) an α,β-di-substituted diglyceride reacts with cytidine-diphospho choline (CDP-choline) thereby incorporating choline into the molecule which results in phosphatidyl choline, or lecithin. In pathway 2 (the methylation pathway) the α,β-diglyceride incorporates ethanolamine through the agency of cytidine-diphosphoethanolamine (CDP-ethanolamine) resulting in phosphatidyl ethanolamine (PE) which by subsequent methylations by S-adenosylmethionine gives phosphatidyl methyl ethanolamine (PME), phosphatidyl dimethyl ethanolamine (PDME), and finally lecithin. These two pathways need to be studied in greater detail than they have been, but certain facets about them are known. It has been shown that the dramatic surge in lecithin at the 33rd to 34th week appears because of the activation of pathway 1, that is, the choline incorporation pathway. Gluck has evidence, primarily clinical in nature, that the methylation pathway is the primary mode of lecithin biosynthesis in early gestation, but that on or after about the 34th week, the choline incorporation pathway becomes the more important (Gluck, 1971). He found that when he analyzed respiratory distress syndrome cases in prematures, most of them were acidotic and/or hypothermic. He also found that neither

TABLE 1

Phospholipid Analysis of Lung Tissue*

Condition	Phospholipid (mg/g wet weight)	Phosphatidyl choline (as % of total phospholipid)	Saturated fatty acids (as % in phosphatidyl choline)	Surface Tension (dynes/cm)
Infants with respiratory distress	10.4	46.4	64.7	23
Infants recovered from respiratory distress	17.4	56.9	70.4	3.0
Infants without respiratory distress	22.3	58.8	84.2	6.0
Normal adults	16.8	45.8	80.8	6.0

* Data adapted from Brumley, Hodson, and Avery, 1967.

acidosis nor hypothermia were associated with clinical respiratory distress syndrome in the full-term infant. When trachial aspirates were obtained from prenatal, or from premature infants while they were still hypothermic, he found little or no phosphatidyl dimethyl ethanolamine which meant that the methylation pathway was not functioning. As the infants were put into an incubator and temperature brought up, increasing amounts of PDME were found in trachial aspirates which indicated that the heat supplied to them was activating this pathway. These findings suggested that the methylation pathway is vulnerable to external insult. Yet if this were the case, then some full-term infants should also show respiratory distress, which they do not. Gluck's explanation for this is based on his evidence that early in gestation the methylation pathway is the more important but at about the 35th week the choline incorporation pathway takes over causing the surge of lecithin. As the choline incorporation pathway is not vulnerable to external insult, it is producing sufficient quantities of surfactant for the full-term infant not to have respiratory distress. Thus the premature infant is at a disadvantage because at around 30–34 weeks gestation when the methylation pathway is dominant and vulnerable to external insult, the choline incorporation pathway has not yet assumed maximal function, and hence, sufficient surfactant is not being produced. The only lab finding that Gluck reports in support of his hypothesis is that *in vitro,* the methylation pathway is markedly inhibited below a pH of 7.2; *in vivo,* exposure to cold or hypothermia or hypoxia causes acidosis in the neonate, which could then inhibit the methylation pathway.

With all this data, we now have the basis for developing a method for the prediction of respiratory distress in the neonate by measuring lecithin and sphingomyelin in amniotic fluid. Although lecithin and sphingomyelin are measured individually, the results are reported in terms of the ratio of lecithin to sphingomyelin. Once the ratio starts getting higher than 1 or 2, one might be able to give some indication about whether respiratory distress could be predicted in that fetus were it delivered at that time. The method we have developed and are using at present is a modification of Gluck's original method (Gluck, Kulovich, *et al,* 1971) and is outlined in Fig. 4. Three mililiters of amniotic fluid are taken to which are added 3 ml of methanol and 6 ml of chloroform in a centrifuge tube. The whole is extracted once, centrifuged, and the lower

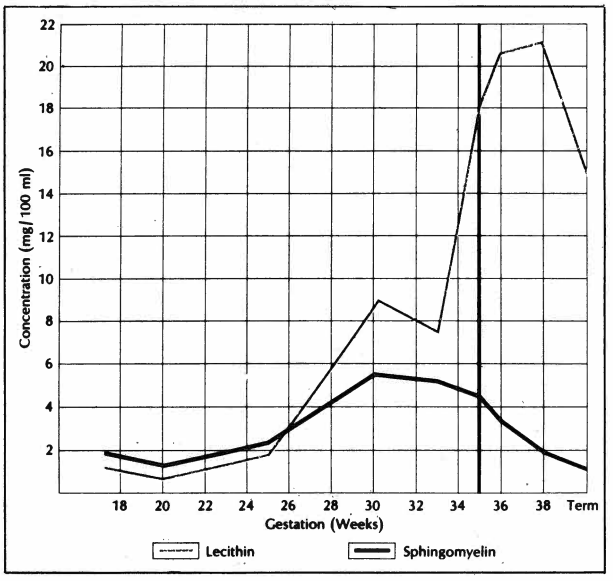

Fig. 2—Concentrations of sphingomyelin and lecithin in amniotic fluid during gestation. (Reprinted with permission from Gluck, *Hosp. Pract.* 6:45, 1971.)

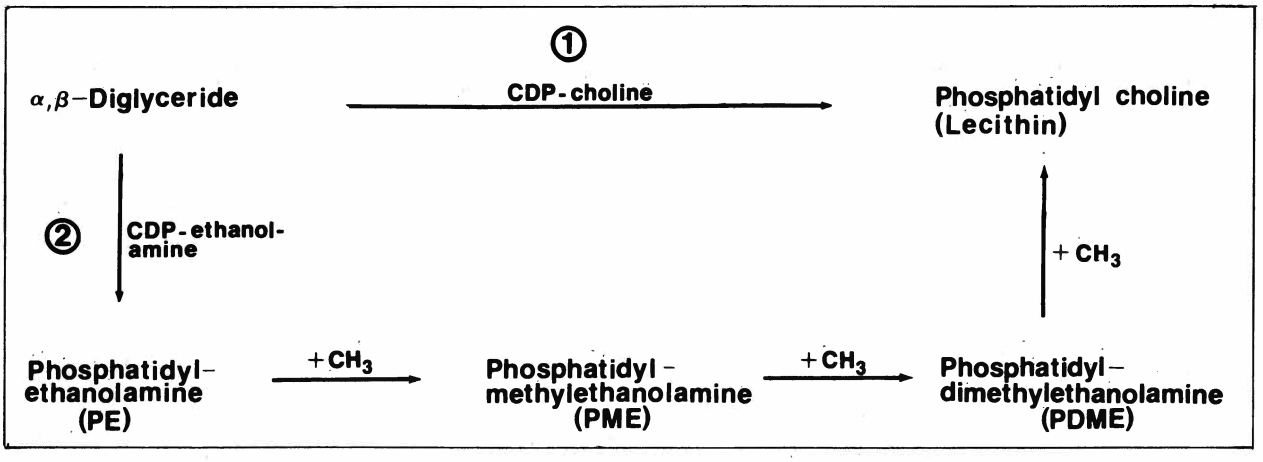

Fig. 3—Two postulated pathways for lecithin biosynthesis.

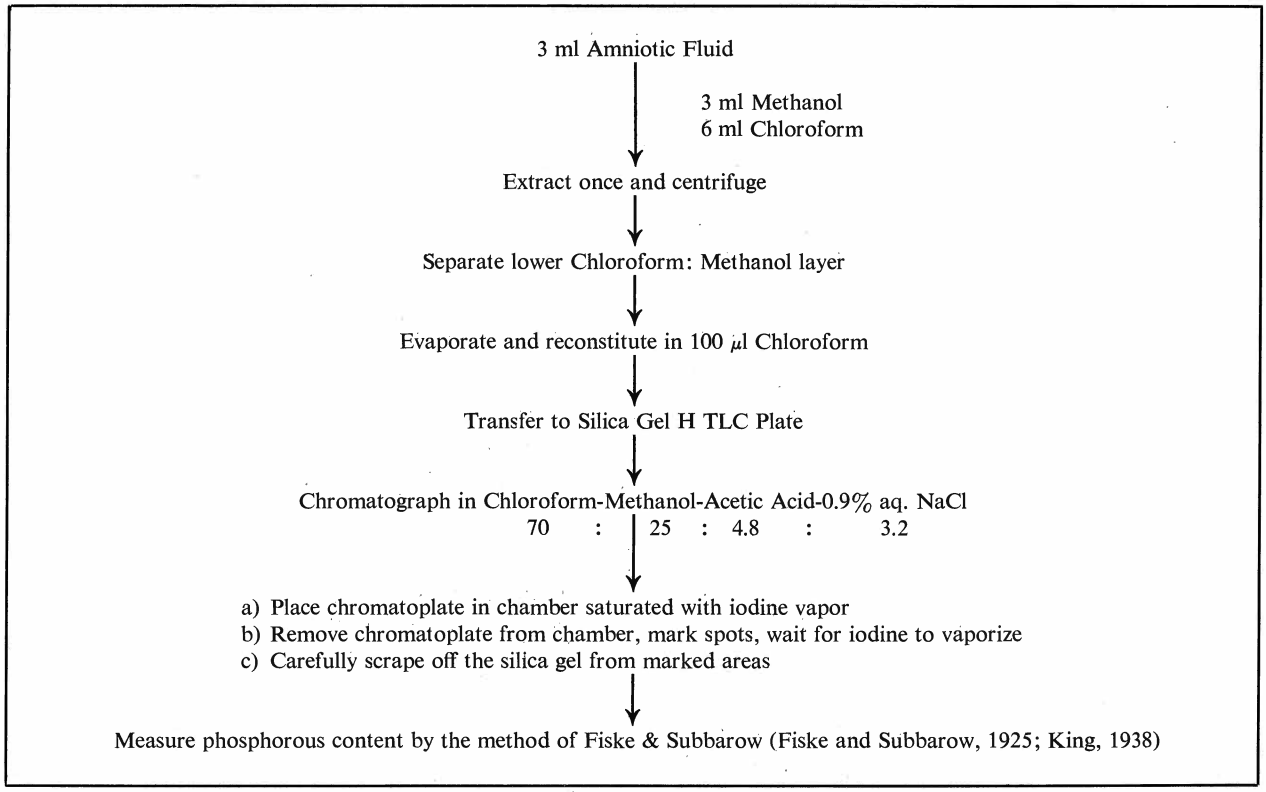

Fig. 4—Methodologic flow sheet.

chloroform methanol layer is removed, evaporated, and reconstituted in 100 µl of chloroform. This is then transferred to a thin-layer plate which is run in the system, chloroform-methanol-acetic acid-0.9% aq. NaCl (70:25:4.8:3.2). We found this system to be better than the old chloroform-methanol-water ones which are normally used for phospholipids. Lecithin and sphingomyelin are visualized by placing the thin-layer plate in a chamber saturated with iodine vapor. The iodine is deposited reversibly on the areas where the lecithin and sphingomyelin are located. The spots are then marked, scraped off, and after the iodine has vaporized, the phosphorus content of the spots is measured by a modification of the Fiske and Subbarow method (Fiske and Subbarow, 1925; King, 1938). As one mole of phosphorus corresponds to one mole of lecithin and sphingomyelin, by measuring the amount of phosphorus, we can calculate the absolute amounts of lecithin and sphingomyelin and hence get the ratio of one to the other. The test we have developed takes an average technician approximately 3 hours for 6 quantitative determinations. For a quick but qualitative result, the test can be shortened by eliminating the quantitative step, spraying the thin-layer plate with a phosphorus specific spray (Dittmer and Lester, 1964) once it comes out of its developing solvent system, and then estimating in gross terms the amounts of lecithin and sphingomyelin by the visual intensities of their spots.

In our laboratory, we take a ratio of 2 or greater to predict fetal lung maturity. Gluck and his group reported a few months ago that based on the results of 302 amniocenteses from 272 pregnancies using their method of monitoring the phospholipids, not one single case of respiratory distress occurred (Gluck, Kulovich, *et al,* 1971). Our experience here at the Medical College of Virginia is of shorter duration. After 49 complicated pregnancies which we have monitored through 63 amniocenteses, we have not encountered any instance of respiratory distress, and among these we have had four notable cases: three of diabetes and one of Rh disease. The Rh disease patient was delivered at 33½ weeks after continuous monitoring of her lecithin-sphingomyelin ratios, and she delivered a healthy viable infant whose weight was compatible with dates. The three diabetics were delivered at 34, 35, and 36 weeks, and all delivered healthy viable infants showing no signs of respiratory distress.

Although the lecithin-sphingomyelin ratio seems to be going a long way in alleviating the frustration

of obstetricians, it should still be used with caution because the volume of data is not sufficient to show what would happen if one were to get a false positive, if one indeed ever gets false positives. The next big step that needs to be taken is a detailed study of the two major biosynthetic pathways for lecithin and a study of the characteristics and properties of the enzymes involved. If a compound could be found to induce one or both of these enzymes, this information would provide a much wanted tool in the hands of an obstetrician or pediatrician for dealing with the fetus or neonate. Such information would also be of great significance in cases of respiratory distress syndrome which are independent of obstetrical management, such as premature labor. In this instance, the physician is confronted with a situation not of his own making and has to deal with it as quickly and as best he can. At present, we are able, with the use of the described test, to predict the possibility of respiratory distress and thus have the necessary facilities such as an incubator or a respirator ready for the neonate immediately after delivery. But of even greater benefit would be the discovery of a compound drug which would induce the choline incorporation pathway. This, as I mentioned earlier, is insensitive to external insult, and once biosynthesis of lecithin has been induced by means of the pathway, the neonate itself can take over the job it is supposed to do. A further goal is the compiling of sufficient data such that a significant statistical analysis can be made concerning the accuracy of predicting the severity of respiratory distress associated with specific ranges of the lecithin/sphingomyelin ratio.

Finally, I would like to express my appreciation to our excellent housestaff who have been very patient in supplying me with samples of amniotic fluid as uncontaminated with blood as possible and also my very deep gratitude to Dr. Dunn for his constant and very enthusiastic encouragement of this work.

REFERENCES

ANDERSON, A. B. M. AND GRIFFITHS, A. D. *J. Obstet. Gynaecol. Brit. Comm.* 75:300, 1968.

AVERY, M. E. AND MEAD, J. *Am. J. Dis. Child.* 97:517, 1959.

BROSENS, I. A. AND GORDON, H. *J. Obstet. Gynaecol. Brit. Comm.* 73:88, 1966.

BRUMLEY, G. W., HODSON, W. A., AND AVERY, M. E. *Pediatrics* 40:13, 1967.

CLEMENTS, J. A. *Proc. Soc. Exp. Biol. Med.* 95:170, 1957.

DITTMER, J. C. AND LESTER, R. L. *J. Lipid Res.* 5:126, 1964.

DROEGEMUELLER, W., JACKSON, C., MAKOWSKI, E. L., AND BATTAGLIA, F. C. *Am. J. Obstet. Gynec.* 104:424, 1969.

FISKE, C. H. AND SUBBAROW, Y. *J. Biol. Chem.* 66:375, 1925.

FUJIWARA, T., ADAMS, F. H., SIPOS, S., AND EL-SALAWY, A. *Am. J. Physiol.* 215:375, 1968.

GLUCK, L. *Hospital Practice* 6:45, 1971.

GLUCK, L., KULOVICH, M. V., BORER, R. C., BRENNER, P. H., ANDERSON, G. C., AND SPELLACY, W. N. *Am. J. Obstet. Gynec.* 109:440, 1971.

GLUCK, L., KULOVIC, M. V., *et al. Pediat. Res.* 1:237 and 247, 1967; Gluck, *et al* 4:352, 1970.

JEFFCOATE, T. N. A. AND SCOTT, J. S. *Canad. M. A. J.* 80: 77, 1959.

KING, E. J. *Biochem. J.* 26:295, 1938.

KLAUS, M. H., CLEMENTS, J. A., AND HAVEL, R. J. *Proc. Natl. Acad. Sci.* (U. S. A.) 47:1858, 1961.

LILEY, A. W. *Am. J. Obstet. Gynec.* 82:1359, 1961.

MANDELBAUM, B., LA CROIX, G. C., AND ROBINSON, A. R. *Obstet. Gynecol.* 29:471, 1967.

MORGAN, T. E., FINLEY, T. N., AND FIALKOW, H. *Biochem. Biophys. Acta* 106:403, 1965.

NEERGAARD, K. VON. *Z. Gesamte Exp. Med.* 66:373, 1929.

PATTLE, R. E. *Nature* (Lond) 175:1125, 1955.

PITKIN, R. N. AND ZWIREK, S. J. *Am. J. Obstet. Gynec.* 98: 1135, 1967.

Hormonal Changes in Pregnancy*

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Because of the time factor involved, it would be an impossible task to present the vast body of biochemical information accumulated during the last decade on the endocrine aspects of pregnancy. However, certain key points can be made concerning the feto-placental unit and the hormone levels and effects in normal gestation. These concepts will be discussed not merely for their academic value, but also as a base to evaluate our present diagnostic and therapeutic endeavors in obstetrical practice. With the audience's indulgence, an occasional personal conjecture will be interjected concerning hormonal mechanisms and clinical practices.

Feto-Placental Unit. The fetus and the placenta are incomplete steroidogenic systems. They lack the capability of synthesizing all necessary steroidal hormones from more simple precursors in contrast to the ovaries, testes, and adult adrenal cortex. To complete the task a well-coordinated interdigitation of the synthetic capabilities of fetus, placenta, and *mother* is required. Since the mother is involved, one wonders whether the term "feto-materno-placental unit" would not be more appropriate.

First the fetal adrenal cortex and then the placenta will be discussed; hopefully, emphasizing the information which may have practical clinical significance.

A. Adrenal Cortex. The fetal zone of the adrenal cortex persists only during gestation, constitutes about 80% of the gland, and is active in steroid metabolism. In the first trimester, it is believed this zone is primarily stimulated by human chorionic gonadotrophin (HCG); ACTH gaining prominence in control thereafter.

The biochemical capabilities and limitations of the fetal adrenal cortex are shown in Fig. 1. Utilizing acetate as a precursor, the fetal zone more readily synthesizes the $\Delta 5$–3β hydroxysteroids pregnenolone and dehydroepiandrosterone (DHEA) than the $\Delta 4$–3 ketosteroids such as cortisol and aldosterone. This is because of a relative block in 3β-hydroxy dehydrogenase and $\Delta 5$ isomerase activity. Thus, the synthesis of large amounts of DHEA and its sulfate is favored, and they are transported to the placenta as such or after 16α-hydroxylation by the fetal adrenal or liver. The placenta can metabolize these androgens to estrogens as will be shown shortly.

However, such a biosynthetic lack would leave the fetus in jeopardy because of a cortisol and aldosterone deficiency. This is rectified by the fact that the fetal zone can utilize the readily available placental progesterone to produce these needed hormones. In fact, after the tenth week of gestation, the increasing placental progesterone secretion may enhance the 17α, 21, and 11β hydroxylase capability of the fetal adrenal cortex to produce the corticoids. Also, the ready passage of maternal cortisol across the placenta is a secondary protective mechanism.

B. Placenta. This endocrine organ is incapable of synthesizing large quantities of progesterone and estrogens from the simple precursor acetate. However, it can efficiently convert maternal plasma cholesterol to progesterone as shown in Fig. 2. This is clinically important for it means that an intact maternal-placental circulation will produce essentially normal progesterone levels even though a fetal demise has occurred. The progesterone produced by the placenta can be metabolized by the mother and fetus to less active progestational metabolites or can be utilized by the fetal adrenal as already described. Since a 17α-hydroxylase deficiency in the placenta precludes the conversion of progesterone to androgens and subsequently estrogens, another mechanism must exist for placental estrogen synthesis.

* Presented at the 43rd Annual McGuire Lecture Series, December 3, 1971, at the Medical College of Virginia, Richmond.

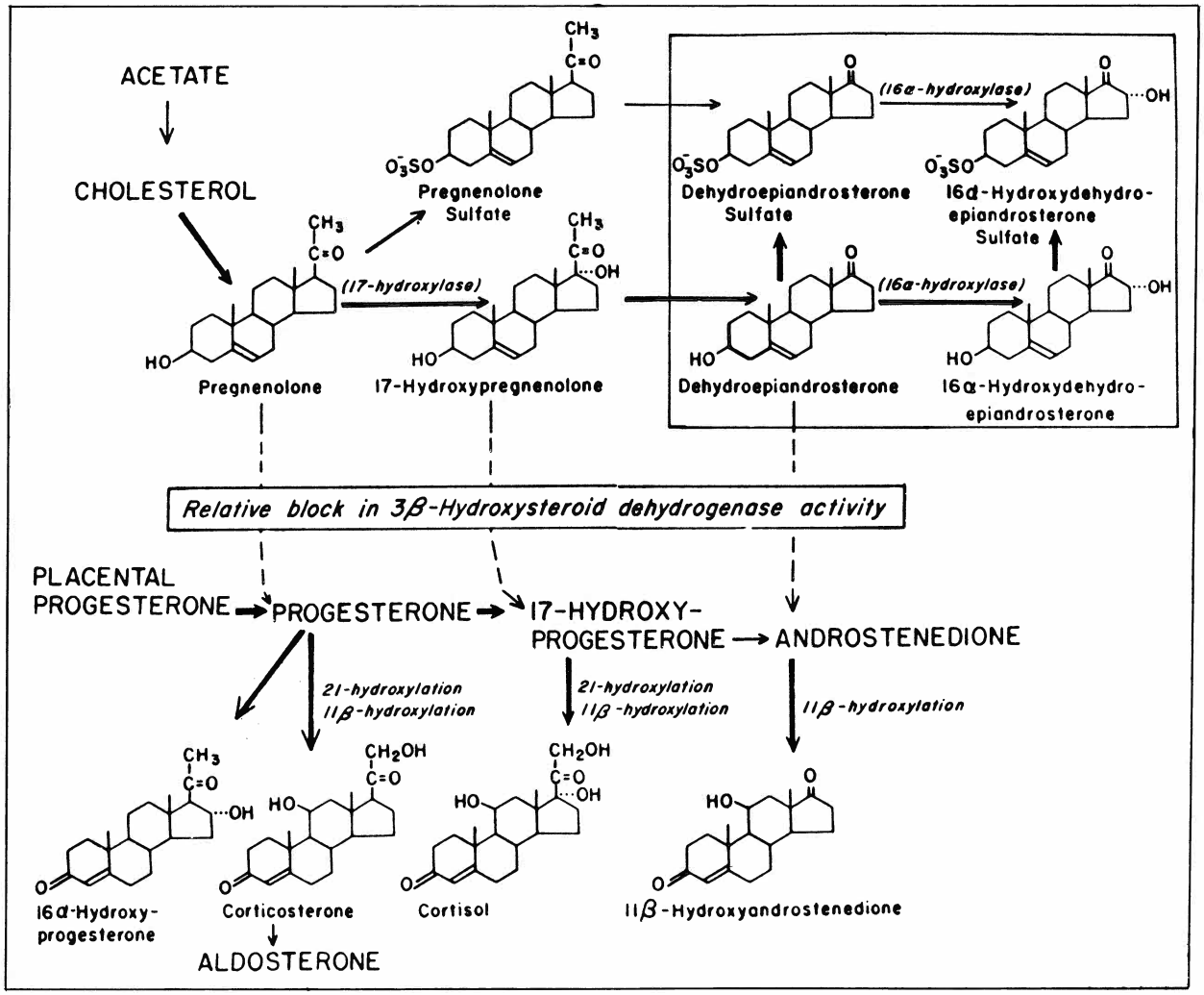

Fig. 1—Pathways of steroid metabolism in the human fetal adrenal cortex. The major secretory products are boxed in. (Reprinted with permission from Villee. *New Eng. J. Med.* 281: 473, 1969.)

As depicted in Fig. 3, the placenta produces estrogens from androgens of maternal and fetal adrenal origin. Androstenedione, DHEA, and testosterone are converted by the placenta to estradiol and estrone. It is estimated that the mother and fetus each contribute about 50% of the androgens which are made into these 2 estrogens. Since the ability to 16α-hydroxylate androgens is essentially limited to the fetal adrenal and liver, the fetus is the major contributor of 16α-hydroxylated androstenedione, DHEA, and testosterone which are converted by the placenta to estriol. The maternal contribution to estriol at term is less than 10% and probably arises from her adrenal androgens which escape conversion to estradiol and estrone on passage through the placenta to fetus. In the fetus, however, her androgens can be hydroxylated at the 16α position before recirculation back to the placenta where synthesis to estriol can occur.

The above information indicates that the clinical assay in pregnancy of plasma progesterone or its major metabolite, pregnanediol, in the urine would mirror placental integrity. On the other hand, estrogen assays, particularly urinary estriol, would reflect fetal integrity.

Hormone Secretion Patterns and Effects in Normal Gestation.

A. Progesterone. The corpus luteum appears to be the initial source of progesterone in pregnancy. Subsequently, it is produced by the placenta in increasing amounts from maternal cholesterol. This transition of secretion from one gland to the other is shown in Fig. 4. Both plasma 17α-hydroxy progesterone and progesterone of corpus luteum

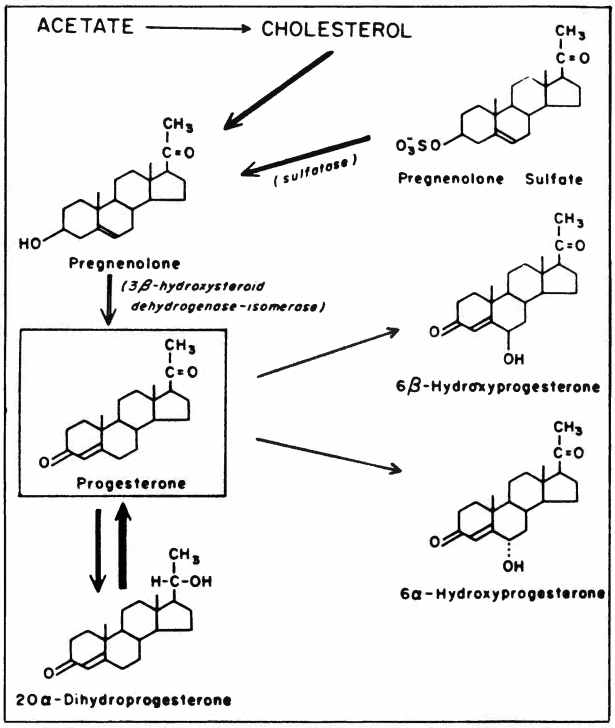

Fig. 2—Principal precursors and metabolites of progesterone in the human placenta. The major steroid secreted by the placenta (progesterone) is boxed in. (Reprinted with permission from Villee. *New Eng. J. Med.* 281:473, 1969.)

origin are seen to peak at 3–4 weeks after ovulation. At 6–8 weeks post-ovulation, the progesterone reaches a nadir while the 17α-hydroxy progesterone continues to decline. Since the placenta lacks significant 17α-hydroxylase capability, it is assumed that the secondary rise in progesterone reflects placental function. It is of interest to note that HCG—believed to maintain the corpus luteum of pregnancy—continues to rise while both hormones of corpus luteum origin are declining.

The secondary rise in placental progesterone continues to increase progressively to a maximum plateau at 36–40 weeks. This is seen from our data in Fig. 5, where the level at 8 weeks of 2 micrograms % increases linearly to about 14 micrograms % at term. The curve seems to parallel that of placental weight and is of similar configuration to that of urinary pregnanediol as depicted in Fig. 6.

It is important to note that no significant drop in progesterone occurs prior to labor. However, during parturition we and others have found a slight downward trend of mean values and this may be a reflection of placental ischemia secondary to the uterine contractions. There is a rapid drop in the plasma progesterone after delivery of the placenta verifying the hormone's short half-life.

It has been estimated that the term placenta produces 200–300 mgms of progesterone daily. The role of this large amount of hormone is not well defined, but possible metabolic functions are:

1. The maintenance of growth and development of the fetus; for example, it is a precursor steroid for the synthesis of corticoids by the fetal adrenal cortex.
2. An immunosuppressive agent to protect the feto-placental homotransplant.
3. A defense mechanism acting on the myometrium to prevent premature expulsion of the fetus.
4. Prepare the breasts for lactation.
5. Mediate a variety of biochemical events; for example, antagonize at the renal tubule the effects of the increased aldosterone concentration found in pregnancy.

B. The Estrogens. Quantitatively, estriol is the major estrogen of human pregnancy. Its biologic potency, however, is significantly less than that of estradiol and estrone. The urinary excretion curve for estriol is presented in Fig. 7; the amount rising from low early levels to high levels at term. An acceleration in the increase is apparent during the last few weeks. There seems to be a good correlation between the estriol curve and that for the fetal weight. Klopper, reviewing the literature, found the average levels to be 10.1, 14.2, 19.8, and 26 mgms at 28, 32, 36, and 40 weeks respectively. Similar curves for urinary estradiol and estrone have been found, but the amounts are in the microgram range. These two estrogens, like estriol, probably reflect the status of the feto-placental unit. Munson in our group has assayed unconjugated estradiol in the plasma of normal pregnancies. A mean value of 200 nanograms % in early pregnancy (9–16 weeks) rose to 1196 nanograms % during the last 8 weeks. A curve configuration similar to urinary estrogen excretion patterns was obtained.

I suspect that the estrogenic effects of pregnancy are primarily due to estradiol, the most potent of these 3 estrogens. It mediates the growth and function of the maternal reproductive organs and probably is important to fetal growth and development. The reason for the large mass of estriol production is not clear. There is little evidence at the moment that it is involved in the start of human labor.

A biochemical effect of estrogen in pregnancy or when given to a woman exogenously is the increased liver synthesis of certain steroid hormone binding globulins. The increased hormone binding

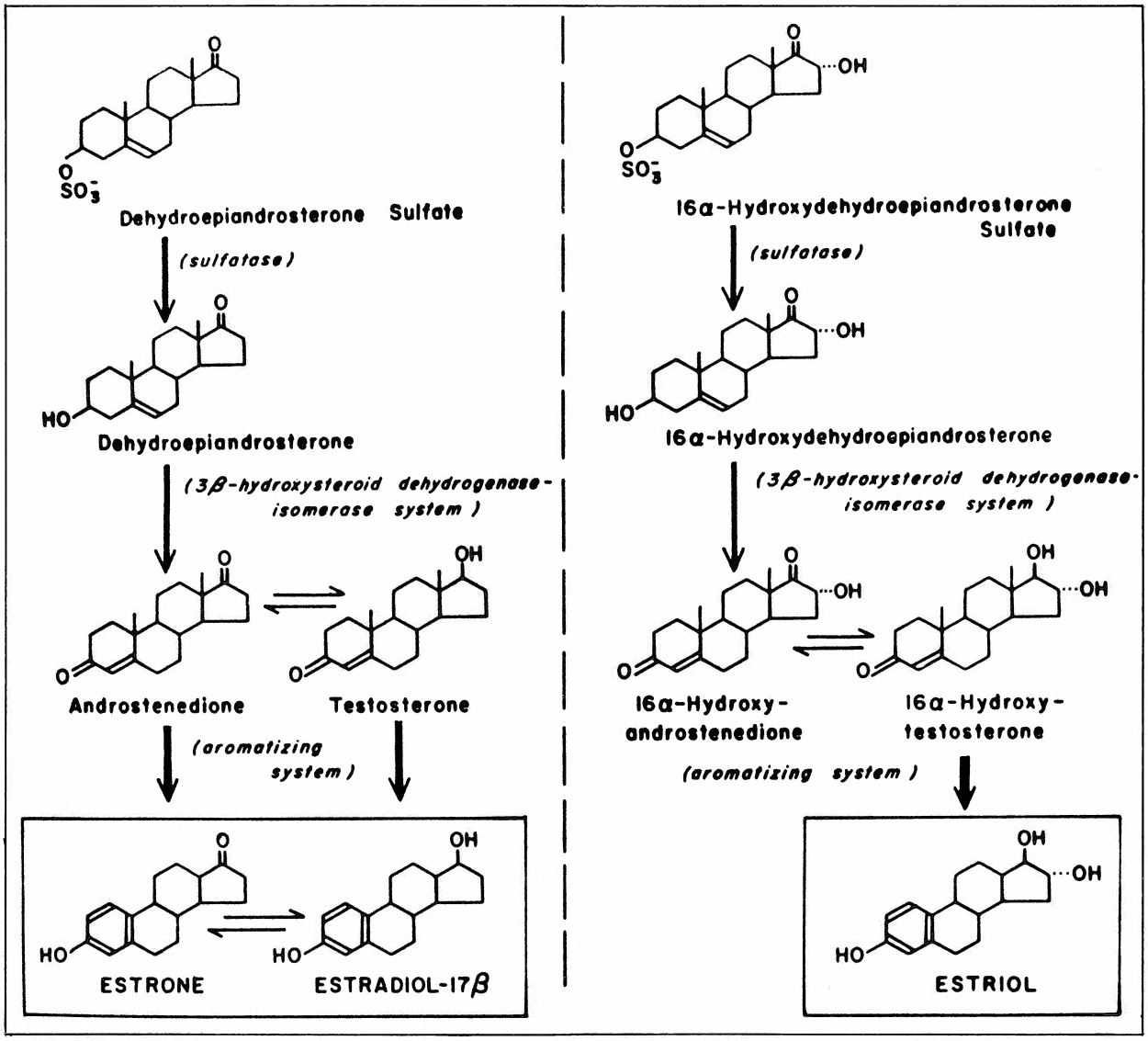

Fig. 3—Synthesis of estrogens by the human placenta. The pathways for the conversion of C19 steroids to estrogens are shown. The major estrogens secreted by the placenta are boxed in. (Reprinted with permission from Villee. *New Eng. J. Med.* 281: 473, 1969.)

results in elevated concentrations of serum thyroxine (also the PBI), plasma cortisol, and testosterone. However, the amount of free or unbound hormone, and presumably the biologically active fraction, is unchanged. Unless this estrogen effect is taken into account, the clinician can be misled by the elevated laboratory result. The only exception to this physiologic change is for plasma cortisol in pregnancy. The large amount of placental progesterone in pregnancy plasma competes with cortisol for binding sites on transcortin and increases the free cortisol fraction. Therefore the pregnant woman is actually in a state of mild adrenal hypercorticism.

C. Human Placental Lactogen (HPL). This polypeptide hormone secreted by the syncitial trophoblast is immunologically similar to but distinct from human growth hormone. Metabolically it appears to be a weaker growth hormone. Its pattern of secretion into the blood is shown in Fig. 8; the increasing concentration throughout pregnancy parallels the curve for placental weight. Like progesterone, its blood concentration reflects placental rather than fetal integrity. Fetal death may not significantly drop the level for a period of time as long as the materno-placental circulation is intact. The placental secretion is essentially unidirectional into the mother; much lower levels being found in

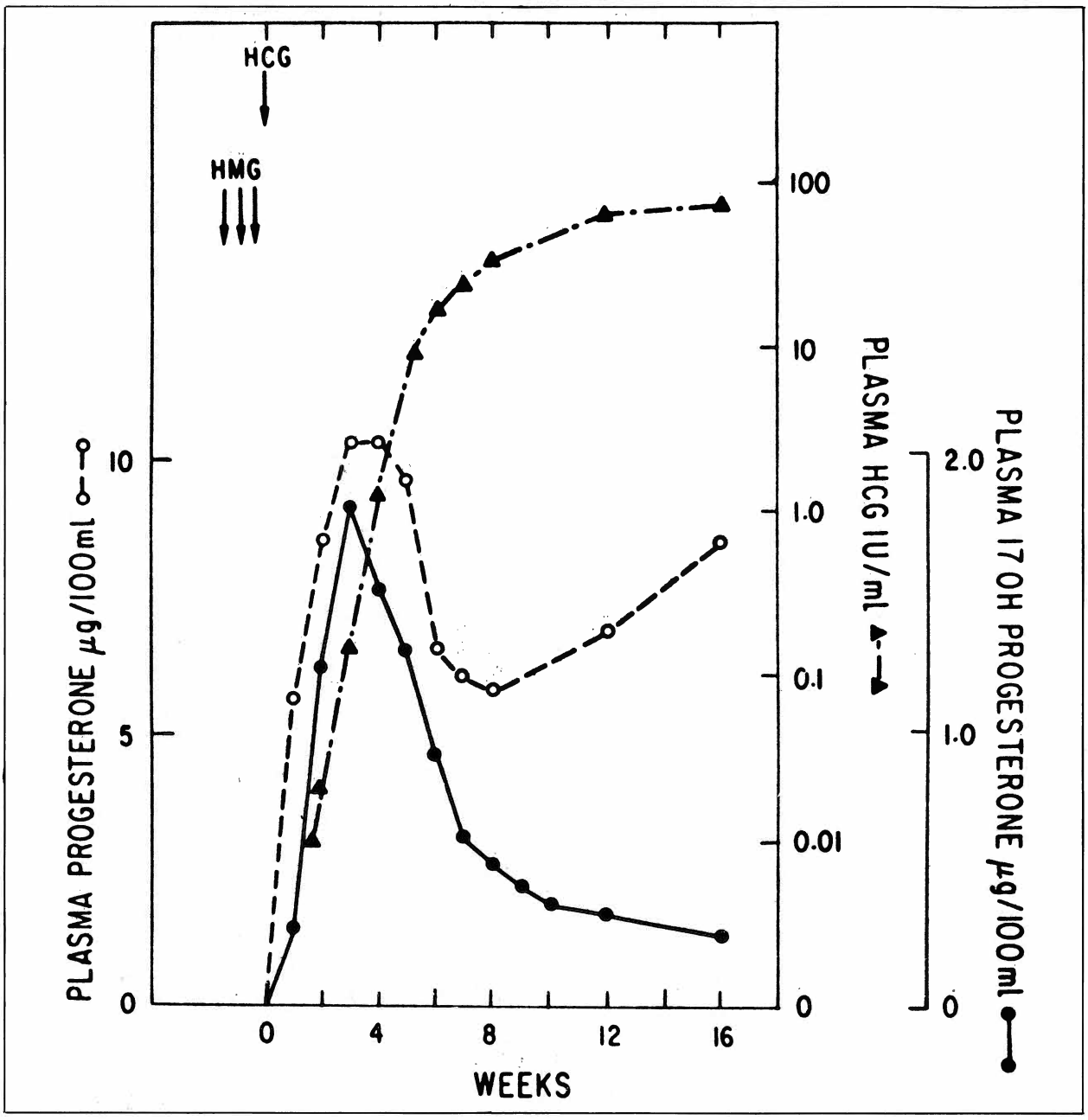

Fig. 4—Mean plasma levels of progesterone, 17-hydroxyprogesterone, and HCG following induction of ovulation with HMG-HCG and subsequent conception. (Reprinted with permission from Yoshimi, *et al. J. Clin. Endocr.* 29: 225, 1969.)

the fetus. The daily production can be as high as 400 mgms.

Possible roles of HPL are preparation of the breasts for successful lactation and indirect growth hormone effects. These include increased nitrogen retention, increased maternal fatty acid utilization sparing glucose for transmission to the fetus. The fetus, like the adult brain, utilizes essentially glucose only for its energy requirements. HPL is antagonistic to insulin and as a consequence may be an important factor in the diabetogenic effect of pregnancy.

D. Human Chorionic Gonadotrophin (HCG). The blood and urine levels of this glycoprotein secreted by the cytotrophoblast differ from the curves seen for the previously discussed horomones and is shown in Fig. 9. The concentration in the blood peaks at about 60 days, then decreases to ¼ to ⅓ of the peak value throughout pregnancy with a lower secondary rise at term.

Its role in pregnancy is not clear, but tradition-

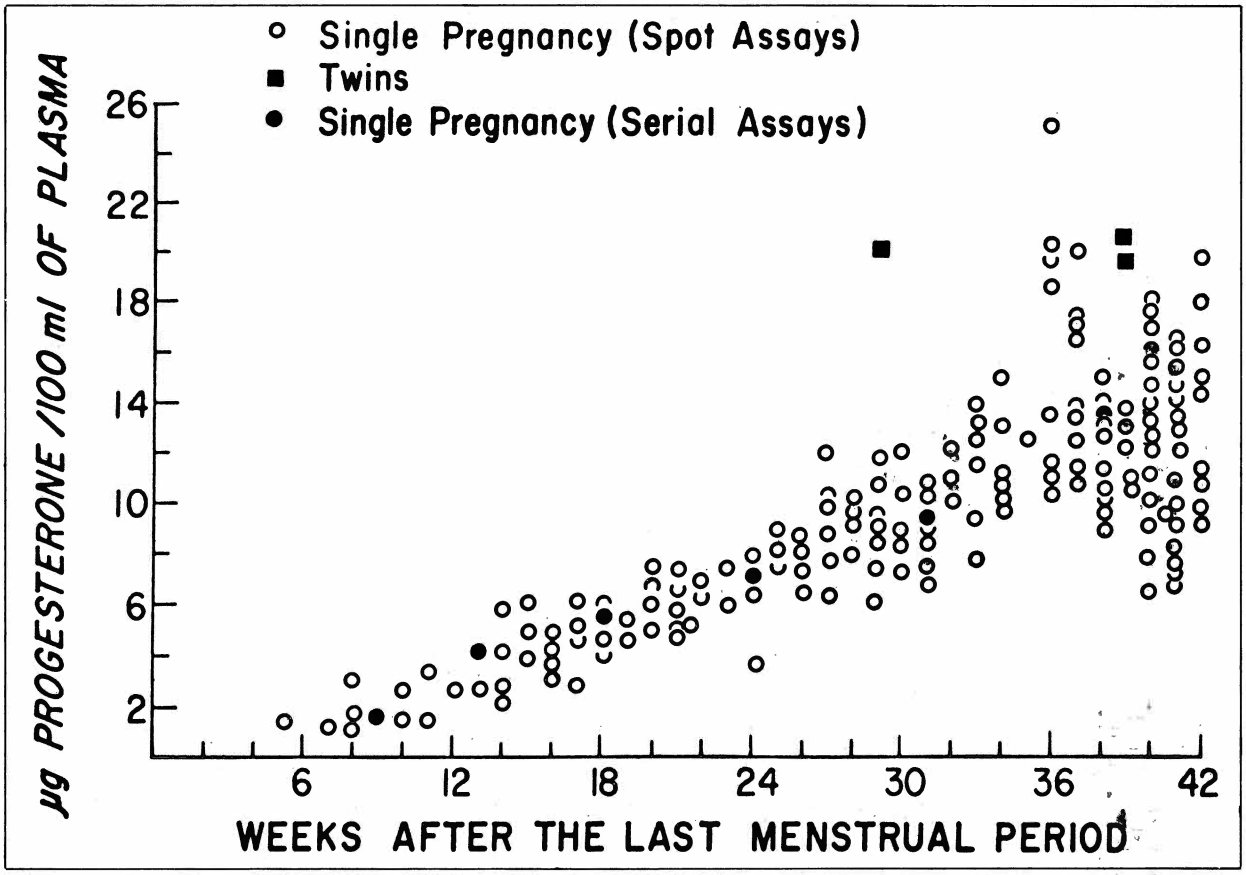

Fig. 5—The plasma progesterone concentration in normal pregnancy expressed as micrograms %. One hundred and twelve of these 179 determinations were reported previously. Note the progressive increase in concentration with advancing gestation. (Reprinted with permission from Yannone. *Steroids* 13: 773, 1969.)

ally is believed to maintain the corpus luteum of pregnancy. It may also regulate steroid secretion by other glands; its possible ACTH effect on the fetal adrenal cortex in early pregnancy has already been mentioned.

Clinical Implications.

A. Progesterone and Pregnancy Status. Plasma progesterone and urinary pregnanediol have been measured in a variety of obstetric disorders to prognosticate dysfunction or to establish its presence. Such diagnostic attempts have been less than satisfactory. Probably the primary reason for this is that the hormone concentration can remain in the normal range even with fetal death as long as the uteroplacental circulation is intact. Other problems are the wide day to day variation in the same individual and the overlap of values in normal and abnormal gestation. Even when the hormone concentration is definitely subnormal, it is usually the effect of an uncorrectable dysfunction rather than the cause. Therefore exogenous progesterone substitution is

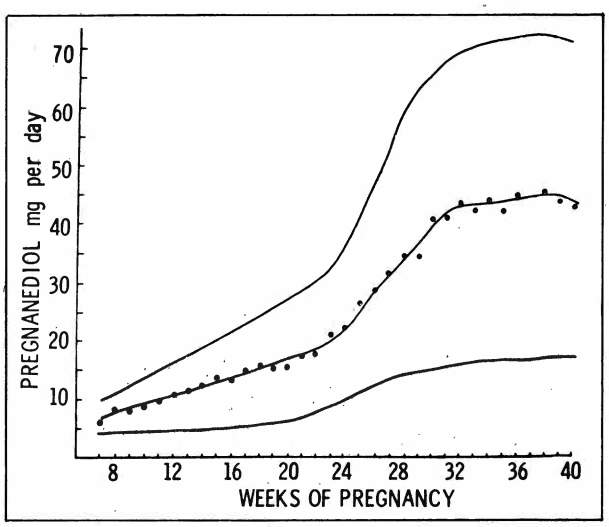

Fig. 6—Pregnanediol excretion in normal pregnancy. The dots represent observed means, the central line fitted means. The upper and lower lines show the 95% probability limits. (Reprinted with permission from Shearman. *J. Obstet. Gynaecol. Brit. Comm.* 66:1, 1959.)

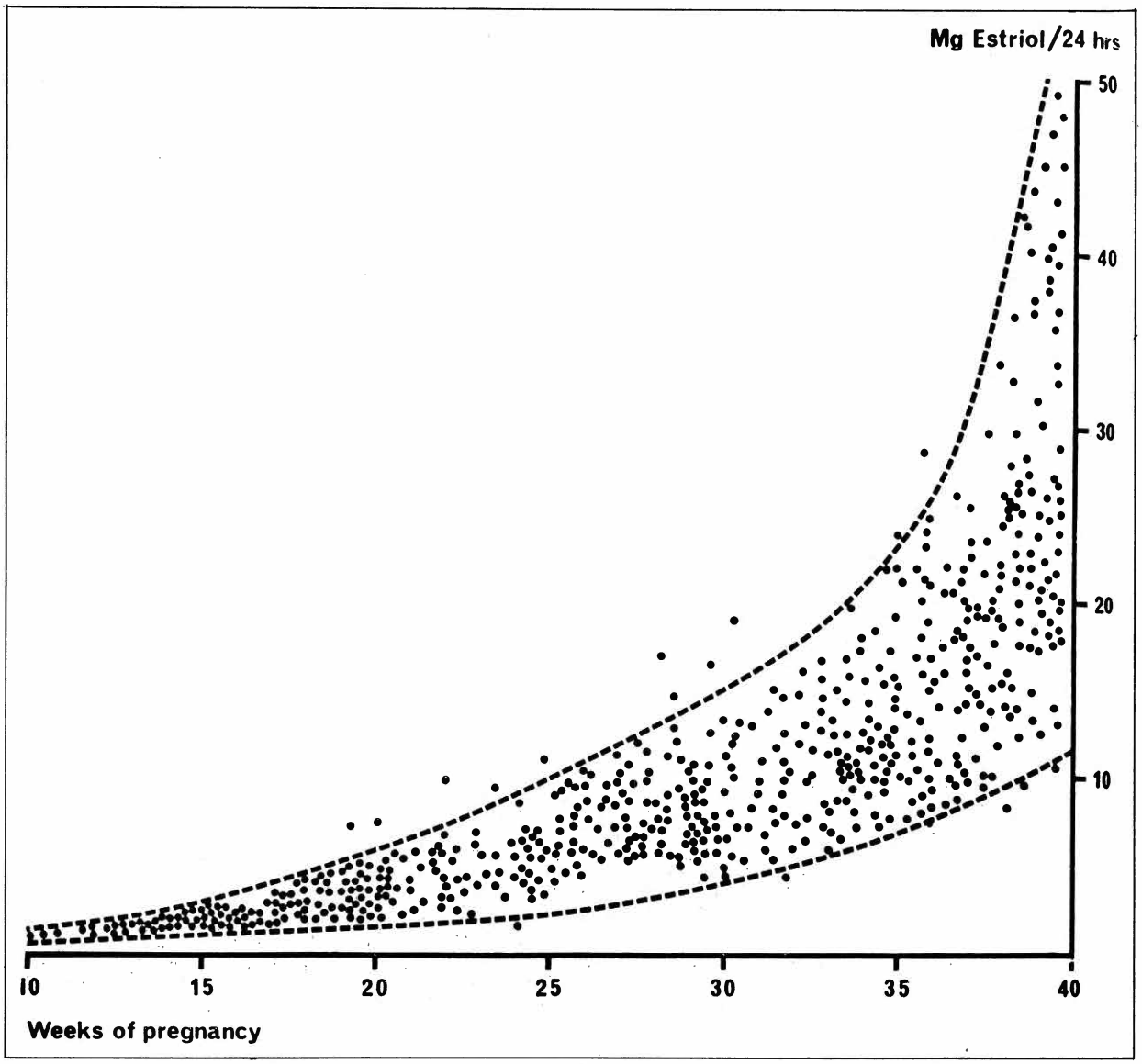

Fig. 7.—Urinary excretion of estriol-16-glucuronide during pregnancy in 31 normal women. The upper and lower dashed lines are 95% confidence limits. (Reprinted with permission from Fuchs and Klopper (eds.). *Endocrinology of Pregnancy, First Edition.* New York: Harper and Row, 1971.)

generally of little value. Similar problems are encountered when serial assays for HPL are used as a diagnostic tool.

B. Progesterone and Adequacy of the Corpus Luteum; Abortion. Of probable clinical value are the use of serial plasma progesterone or urinary pregnanediol assays to evaluate corpus luteum adequacy in certain types of infertility and possibly a few cases of first trimester abortion. Although the majority of such abortions are due to uncorrectable causes such as chromosomal defects, a few could be due to corpus luteum dysfunction with disharmonious transition of steroid hormone production to the placenta. When such corpus luteum inadequacy is incriminated, correction of the infertility or prevention of another abortion may be achieved by insuring an adequate corpus luteum. However, not by the use of exogenous progesterone replacement, but rather by the judicious use of ovulatory inductors such as Clomid® or the gonadotrophins to insure normal follicular maturation which will result in a normal corpus luteum.

C. Progesterone—Term and Premature Labor. Elucidation of the mechanism which institutes labor would enhance our capability to induce labor for medical or convenience reasons and to stop pre-

mature labor. The theory most often advanced to explain the mechanism which institutes parturition is the progesterone-block concept. This maintains that progesterone suppresses myometrial contractility during gestation and labor begins upon withdrawal of the hormone. However, our studies and those of others have not shown a significant and consistent drop in circulating progesterone prior to labor's onset. These findings do not exclude a protective role for progesterone as an effective decrease may occur that is not apparent in the circulating levels. One possibility that we investigated was that the concentration of free and active progesterone decreased with advancing pregnancy because of increased protein binding of the hormone. However, we found the percentage of binding to remain constant throughout pregnancy, which meant that as the total progesterone concentration rose so did the concentration in the bound and unbound fractions. With this negative finding we have turned to studies which may show decreased progesterone levels within the myometrial cells. Our results are too preliminary to draw conclusions.

In spite of our ignorance as to how labor starts, we are not totally devoid of therapeutic agents. Within reason labor can be induced at term with oxytocin. It is much less effective earlier in pregnancy. Prostaglandins such as F2α appear to be effective at term as well as earlier in gestation. However, on parenteral use in the first 2 trimesters, the therapeutic-toxic difference becomes too narrow. This problem may be overcome by using the prostaglandin locally in the vagina to induce an indicated abortion or premature labor. Lastly, with judicious use of intravenous alcohol in premature labor, the expulsion of the fetus can be delayed a few days to a few months in about 65% of the patients.

D. Estriol and Complicated Pregnancies. The most important clinical consideration at the moment is the value of serial estriol determinations in the management of high-risk pregnancies. While it can be helpful in certain complications of pregnancy such as diabetes mellitus, toxemia, and dysmaturity to evaluate the fetal status, it is not without pitfalls. First the clinician is at the mercy of the quality control of the laboratory doing his assays. Inaccurate results can lead to false security or ill-timed termination of pregnancy. Even with a good laboratory, the results may not always reflect the true status of the pregnancy. Unfortunately, our knowledge of estrogen metabolism in pregnancy is inadequate to explain such discrepancies any better than it can explain why Rh iso-immunized jeopardized pregnancies do not show subnormal estriol levels. Even more disconcerting is the fact that excellent clinicians using good laboratories are reporting perinatal losses in diabetic pregnancies no better or even

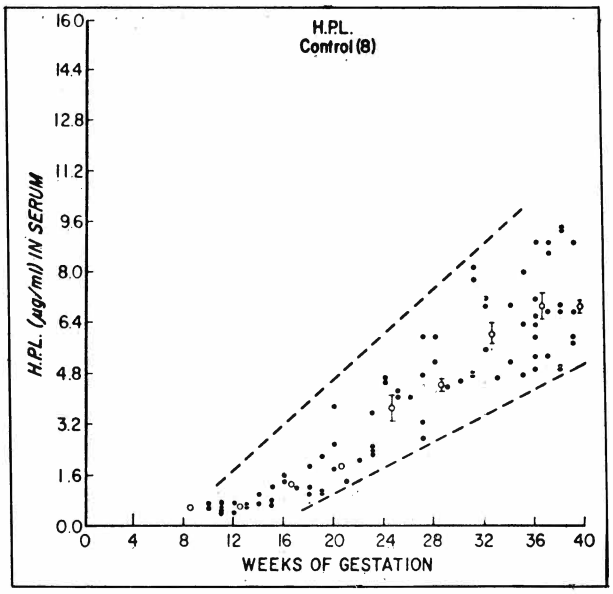

Fig. 8—Human placental lactogen (HPL) in serum. The 80 black dots represent individual values, the open circles represent the means for 4 week intervals and the vertical lines the standard error of the mean. The dash lines show the upper and lower limits of all determinations in the controls. (Reprinted with permission from Samaan, *et al. Am. J. Obstet. Gynec.* 104:781, 1969.)

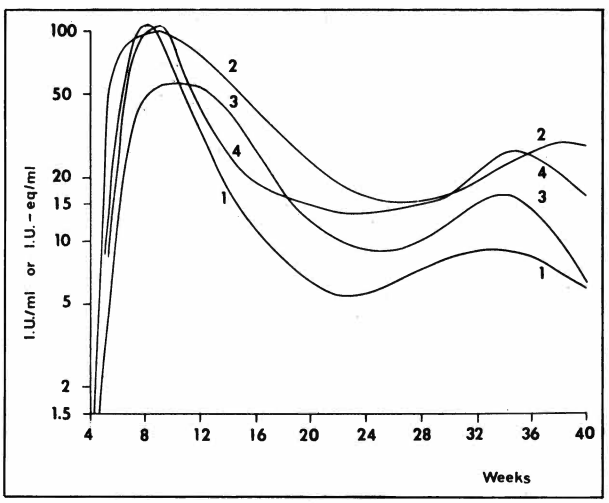

Fig. 9—Mean serum levels of chorionic gonadotrophin in normal pregnancy. The curves are based on results reported by various authors using different bioassay and immunoassay methods. Curve 1: uterine weight increase in rats; Curve 2: ovarian hyperemia in rats; Curve 3: complement fixation; Curve 4: hemagglutination. (Reprinted with permission from Fuchs and Klopper (eds.). *Endocrinology of Pregnancy, First Edition.* New York: Harper and Row, 1971.)

worse than reputable clinics have reported prior to the availability of this assay. So far there has not been any conclusive evidence that a marked reduction in fetal mortality has been obtained because of estriol determination. In this light, the overzealous utilization of estriol assays as a substitute for rather than an adjunct to clinical management is foolhardy.

As an adjunct to clinical judgment, the literature seems to agree on certain helpful points. These are as follows:

1. Significant day to day variation requires serial assays to determine a mean for each patient.
2. In a suspect pregnancy, weekly or biweekly assays should be done from about the 30th week up to the time of reasonable maturity (about 34th week) at which time daily or every other day determinations should be carried out.
3. In any complicated pregnancy which is 34 weeks or beyond, any precipitous fall from normal levels of urinary estriol and which is verified over 2 or more consecutive days dictates immediate delivery.
4. Chronic low estriol levels in preeclamptic toxemia or dysmaturity do not necessitate interruption unless there is a precipitous drop from the low mean. Such babies left *in utero* may not grow very much, but they do achieve greater maturity.
5. Urinary estriol levels can be expected to be low in mothers with anencephalic monsters or on glucocorticoid therapy for medical reasons.

The development of new specific and sensitive hormone assay methods and the increase in sophistication of metabolic studies has given us an impressive array of biochemical information concerning the physiology and endocrinology of pregnancy. However, the application of this knowledge to the improvement of obstetrical care has been difficult. This should not discourage us as medical science must like the infant learn to crawl before it can walk. Eventually we shall have all the facts needed to obtain the obstetrical results that we all desire. We have had a promising start.

REFERENCES

FUCHS, F. AND KLOPPER, A. (eds.). *Endocrinology of Pregnancy, First Edition.* New York: Harper and Row, 1971.

JAFFEE, R. B. Estriol in high-risk pregnancies: Has it kept its promise? *Gynec. Invest.* I. Suppl. 1–6, 1970.

SAMAAN, N. A. *et al.* Serial hormonal studies in normal and abnormal pregnancy. *Am. J. Obstet. Gynec.* 104:781, 1969.

VILLEE, D. B. Development of endocrine function in the human placenta and fetus. *New Eng. J. Med.* 281:473, 1969.

YANNONE, M. E. *et al.* Plasma progesterone levels in normal pregnancy, labor and the puerperium. II Clinical data. *Am. J. Obstet. Gynec.* 101:1058, 1968.

YANNONE, M. E. *et al.* Protein binding of plasma progesterone in pregnancy and labor. *Steroids* 13:773, 1969.

Uterine Blood Flow During Pregnancy*

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Of those parameters pertinent to fetal well-being, the delivery of an adequate amount of blood to the placenta seems to be the most crucial. At our present state of understanding, uterine blood flow (UBF) appears to be that parameter most often affected by physiologic and pathologic conditions, and it is certainly that factor most readily altered, either favorably or unfavorably, by the physician. In this presentation, normal changes in UBF during pregnancy will be reviewed briefly, those factors influencing uterine and placental vascular dynamics will be discussed, and the clinical relevance of these observations will be illustrated on the background of varying levels of uteroplacental adequacy.

Homeostatic Changes in Uterine Blood Flow. While our most comprehensive knowledge of UBF changes during pregnancy has been determined in the ewe, correlative data in subhuman primates and women indicates that results are comparable, at least during the last half of the gestation period (Assali, Douglas, *et al,* 1953; Assali, Rauramo, *et al,* 1960; Lees, *et al,* 1971; Metcalfe, *et al,* 1955; Peterson and Behrman, 1969). These changes are illustrated in Fig. 1. It can be seen that absolute UBF increases most markedly during the period of most rapid absolute fetal growth. Although fetal weight tends to increase more rapidly than UBF, the proportion of UBF perfusing the placenta progressively increases during this same time so that flow per unit weight of fetus, placenta, and uterus remains essentially constant. Similarly, the amount of oxygen extracted from each milliliter of blood also remains essentially constant (Huckabee, *et al,* 1961) indicating that the increase in placental blood flow is matching the increasing demands of the fetoplacental

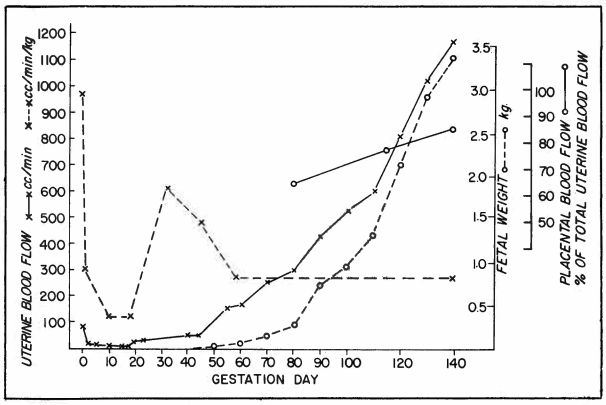

Fig. 1—Derived graph of relative and absolute uterine blood flows, fetal weight and proportionate placental blood flow during ovine pregnancy (Barcroft, 1947; Greiss and Anderson, 1970; Huckabee, *et al,* 1961; Makowski, *et al,* 1968). During the last half of gestation, relative uterine blood flow is constant, and the proportion of uterine blood flow reaching the placenta progressively increases.

unit. After term gestation, however, available information indicates that placental blood flow decreases relative to fetoplacental requirements (Dixon, *et al,* 1963), blood oxygen extraction increases and fetal well-being is progressively compromised. Similarly, placental blood flow is reduced in patients with essential hypertension, chronic renal disease, preeclampsia or eclampsia, twin gestation and premature labors complicated by abnormal bleeding or infection (Browne and Veall, 1953; Dixon, *et al,* 1963; Johnston and Clayton, 1957; Landesman and Knapp, 1960; Morris, *et al,* 1955; Weis, *et al,* 1958). The severity of the reduction in flow with toxemia of pregnancy seems directly related to the severity of the disease process.

Dynamic Control of Placental Blood Flow. A schematic representation of those factors affecting placental and non-placental blood flow in the uterus is shown in Fig. 2. At term gestation in women, the proportion of total UBF perfusing the placenta probably exceeds 85 percent (Lees, *et al,* 1971; Makowski,

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et al, 1968). Therefore, our primary attention should be directed to the second part of the equation accompanying the illustration. This shows that placental blood flow varies directly with the perfusion pressure (UABP-IUP-UVBP) and inversely with the resistance of the spiral arterioles supplying the intervillous space (R_{iPL}) and the resistance imparted to the blood vessels as they course through the myometrium (R_e). The effects of various stimuli on these resistances are summarized in Table I. All vasodilator stimuli exert inconsequential or no effect while all vasoconstrictor stimuli are capable of producing marked persistent reductions in flow. Four observations are of particular pertinence to the clinician: 1) Under normal circumstances, the placental vasculature approaches maximal vasodilatation (Greiss, 1966). This means that we have no effective way of further increasing placental blood flow. It also means that placental perfusion will vary directly with and in proportion to changes in systemic blood pressure† (Fig. 3), that is, a 30% decrease in systemic blood pressure will cause a 30% decrease in placental blood flow. 2) Endogenous or exogenous vasopressor hormones or drugs (Fig. 4) and sympathetic nerve stimulation (Fig. 5) cause significant vasoconstriction (increase R_{iPL}) usually proportionately greater than that induced in other peripheral vascular beds. This means that although

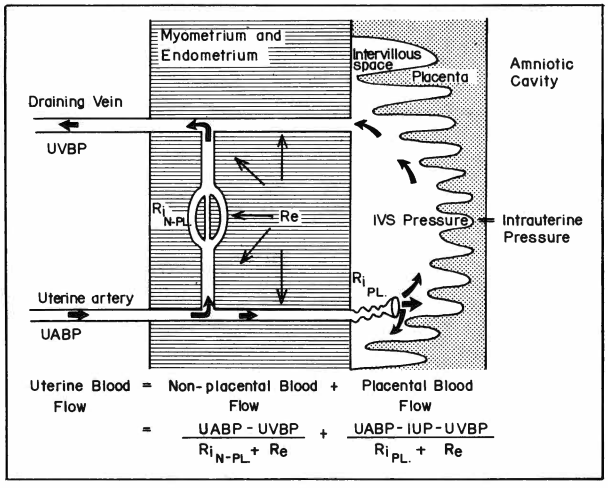

Fig. 2—Schematic representation of those factors determining placental and non-placental uterine blood flow. Note that the reactivity of the non-placental vasculature (R_{iN-PL}) is different from that of the placental vasculature (R_{iPL}), the extrinsic vascular resistance (R_e) imparted by contracting myometrium affects both vasculatures similarly, and that by increasing intrauterine and intervillous space pressures, myometrial contractions decrease placental perfusion pressure. UABP and UVBP indicate uterine arterial and venous pressures, and IUP equals intrauterine (intra-amniotic) pressure.

† For practical purposes in the absence of uterine contractions, perfusion pressure is determined primarily by arterial blood pressure.

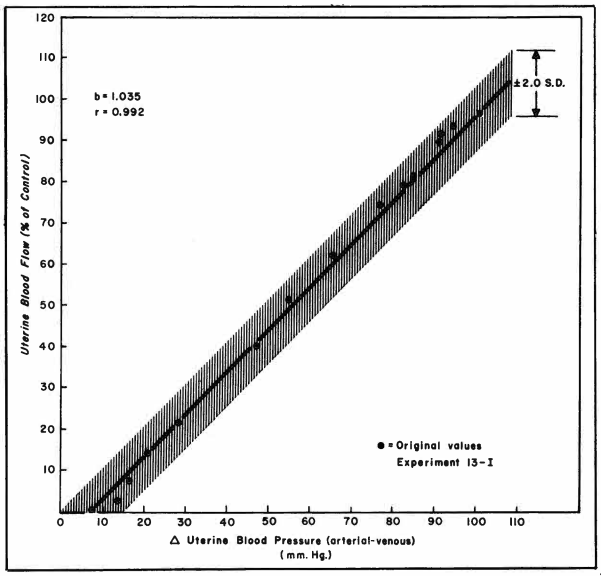

Fig. 3—Graph of the relationship between perfusion pressure and uterine blood flow at term gestation in the ewe indicating that the dominant placental vasculature is almost maximally dilated and that placental perfusion decreases linearly with decreases in the systemic blood pressure. (Reprinted with permission from Greiss. *Am. J. Obstet. Gynec.* 96: 41, 1966.)

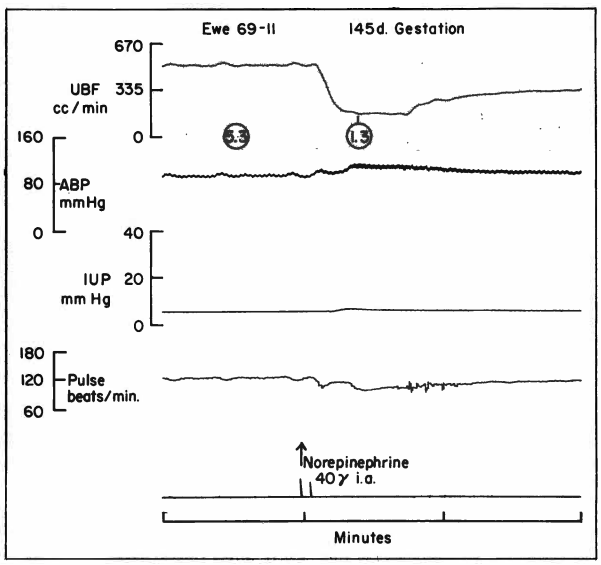

Fig. 4—Original tracing of the effect of norepinephrine on uterine blood flow in the gravid ewe. Circled numbers indicate uterine conductance values. Such profound vasoconstriction and decrease in uterine blood flow is typical of the action of endogenous adrenal medullary hormones and peripherally acting vasopressor agents. (Reprinted with permission from Greiss. *Am. J. Obstet. Gynec.* 112: 20, 1972.)

TABLE 1

Responses of the Uterine Vasculature During Pregnancy

	Vasoconstriction	Vasodilatation
Demonstrated to be Effective	1. Alpha adrenergic stimulation[1] (epinephrine, norepinephrine) 2. Sympathomimetic Agents[2] (vasopressor drugs) 3. Sympathetic nerve stimulation[3] (adrenergic) 4. Myometrial contractions[4] 5. Hypoxemia, severe[5]	1. Acetylcholine and parasympathomimetic agents[6] (minimal, transient) 2. Nitroglycerine (mild, transient)[7] 3. Estrogens (site of dilatation undetermined)[8]
Demonstrated to be Ineffective		1. Ischemia*[9] 2. Hypoxemia, mild*[10] 3. Beta adrenergic stimulation*[11] (isoproterenol, epinephrine) 4. Sympathetic nerve stimulation[12] (cholinergic) 5. Parasympathetic nerve stimulation[13]

* Indicates responses different from those in the non-pregnant uterus.

[1] Ahlquist, 1950; Greiss and Pick, 1964
[2] Ahlquist, 1950; Greiss and Van Wilkes, 1964
[3] Greiss and Gobble, 1967
[4] Ahlquist, 1950; Borrell, *et al*, 1964; Greiss and Anderson, 1968; Lees, *et al*, 1971; Ramsey, *et al*, 1963
[5] Boba, *et al*, 1966
[6] Ahlquist, 1950; Greiss, Gobble, *et al*, 1967
[7] Greiss, 1970
[8] Greiss and Marston, 1965
[9] Greiss, 1966
[10] Greiss, Anderson, and King (in press)
[11] Greiss, 1972; Greiss and Pick, 1964
[12] Greiss and Gobble, 1967
[13] Greiss, Gobble, *et al*, 1967

perfusion pressure will be increased by such stimuli, the degree of placental vasoconstriction will be so great as to cause a net decrease in placental perfusion. 3) Myometrial contractions reduce placental blood flow by constricting arteries as they course through the myometrium (increase R_e) and by increasing intervillous space pressure which in turn decreases the perfusion pressure. Since the pressure in the intervillous space is the same as that in the amniotic cavity (Schwarcz, *et al,* 1967), placental blood flow will vary inversely as the intra-amniotic (intrauterine) pressure (Fig. 6). This means that during an individual contraction, placental perfusion will vary inversely with the intensity and duration of the contraction and over a given time interval, mean flow to the placenta will be determined by the frequency of contractions as well. 4) Since gravid UBF cannot be practically increased by the physician and since almost every stimulus potentially detrimental to placental perfusion is effective, the physician's role becomes one of recognizing and minimizing detrimental effects when they may be critical to fetal survival.

Clinical Applications of Uterine Vascular Dynamics. A hypothetical relationship between the adequacy of placental perfusion and the condition of the fetus is described in Fig. 7. While the percentages of optimal placental blood flow are educated guesses (Greiss, unpublished observations; Hess and Hon, 1960), they cannot be considered actual and serve only as reference points to illustrate the effects of clinical conditions upon placental perfusion and the fetus. Four zones of fetal status are described; two compatible with prolonged but not necessarily optimal fetal growth and development, one permissive of short-term survival during progressive fetal deterioration, and one incompatible with life for more than ten or fifteen minutes. These zones are defined by the mean flow during a fixed period of time, but within an observed time interval, transient reductions or complete cessation of placental blood flow may occur. Such reductions are most frequently related to

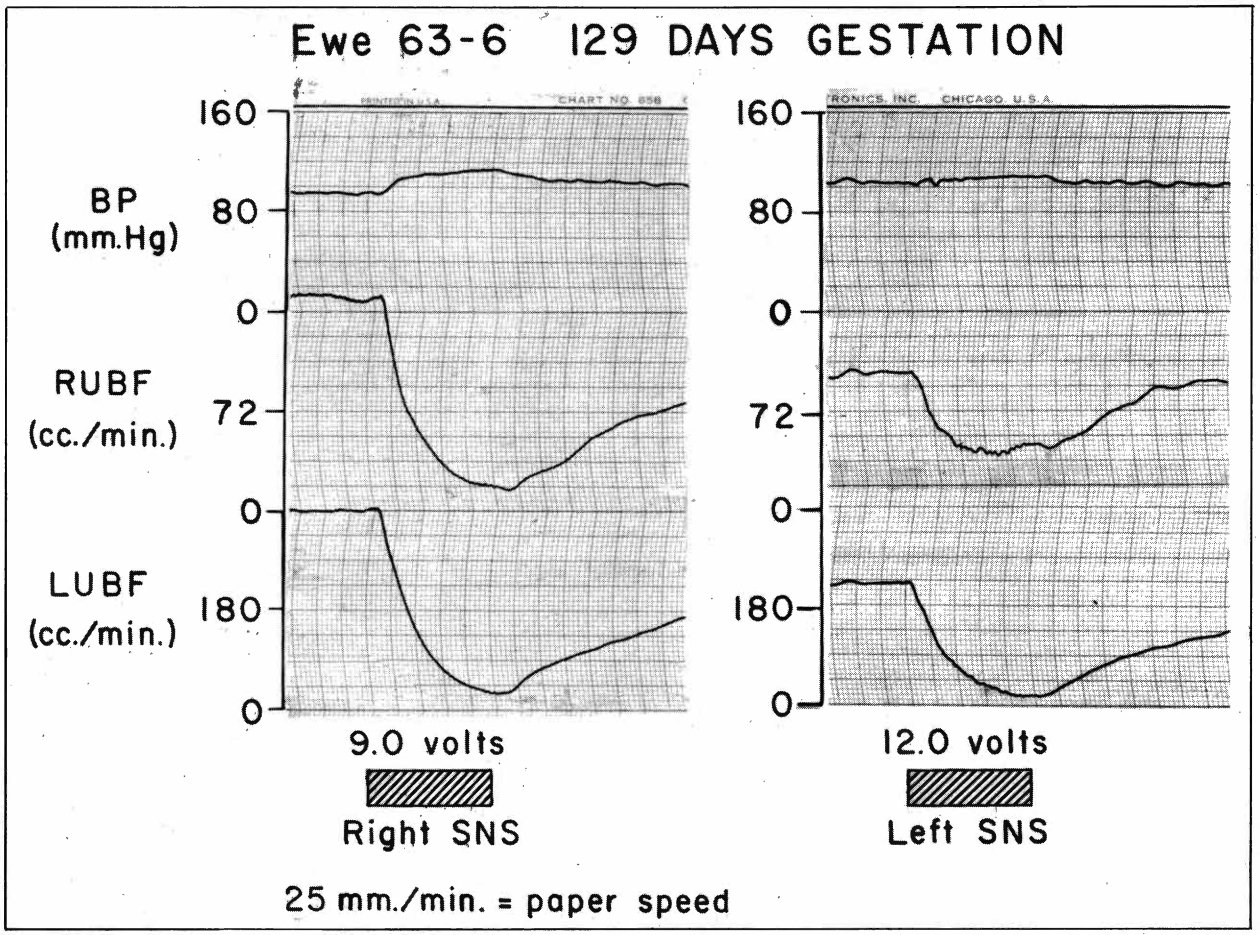

Fig. 5—Original tracings of the effect of lumbar sympathetic nerve stimulation on uterine blood flow in the pregnant ewe. Although sympathetic tonus is low during resting conditions, profound uterine vasoconstriction is evoked by maternal hemorrhage. (Reprinted with permission from Greiss and Gobble. *Am. J. Obstet. Gynec.* 97: 962, 1967.)

uterine contractions (Borrell, *et al,* 1964; Ramsey, *et al,* 1963). Under optimal conditions, brief episodes of intervillous stasis evoke no evidence of fetal distress. However, when such episodes are superimposed upon a fetoplacental unit already compromised with respect to placental perfusion, fetal hypoxia will be manifested initially by late deceleration of the fetal heart rate with respect to uterine contractions, later by progressive fetal acidosis and finally by persistent bradycardia signaling incipient fetal demise (Hon and Quilligan, 1968).

Labor. During strong myometrial contractions, placental perfusion temporarily ceases. For illustrative purposes, let us assume that intervillous flow stops for 30 seconds of a 45 second contraction and 45 seconds of a 60 second contraction. The effects of such contractions on mean placental blood flow are illustrated in Fig. 8. As the duration and frequency of myometrial contractions increase, mean placental flow progressively decreases. If labor begins when placental flow is optimal, even the most active contractions (solid line) cause no fetal jeopardy. However, when flow is suboptimal initially, active labor will evoke fetal distress (long dashed line) and when the pre-labor flow is borderline, even mild labor will cause distress or fetal death (short dashed line). It is evident, therefore, that *labor is inherently stressful to the fetus.* This fact must be remembered whenever patients with high-risk pregnancies begin spontaneous or induced labor.

Hemorrhage. Maternal hemorrhage is a most potent stimulus of adrenal medullary hormone secretion and sympathetic nerve stimulation. Acute moderate hemorrhage (500–750 ml. within 15 minutes) causes minimal changes in maternal blood pressure and pulse rate. However, these parameters are maintained by peripheral vasoconstriction with an inevitable decrease in placental blood flow (Fig. 9). Acute severe hemorrhage (1500 ml. in 15 minutes) evokes a further increase in peripheral and uterine

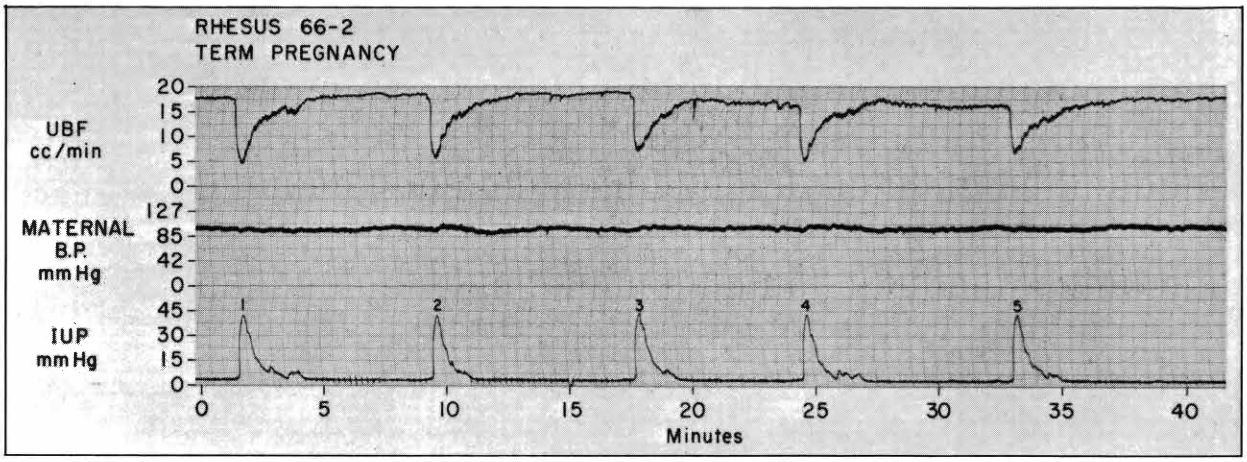

Fig. 6—Original tracing of the effect of spontaneous uterine contractions on uterine blood flow in the term-pregnant Rhesus monkey The uterine blood flow trace is the inverse image of the intrauterine pressure pattern. Uterine blood flow achieves maximum levels during myometrial diastole only. Therefore, mean uterine blood flow will decrease as the frequency of contractions increases and the duration of myometrial diastole decreases. (Reprinted with permission from Greiss and Anderson. *Clin. Obstet. Gynec.* 11: 96, 1968.)

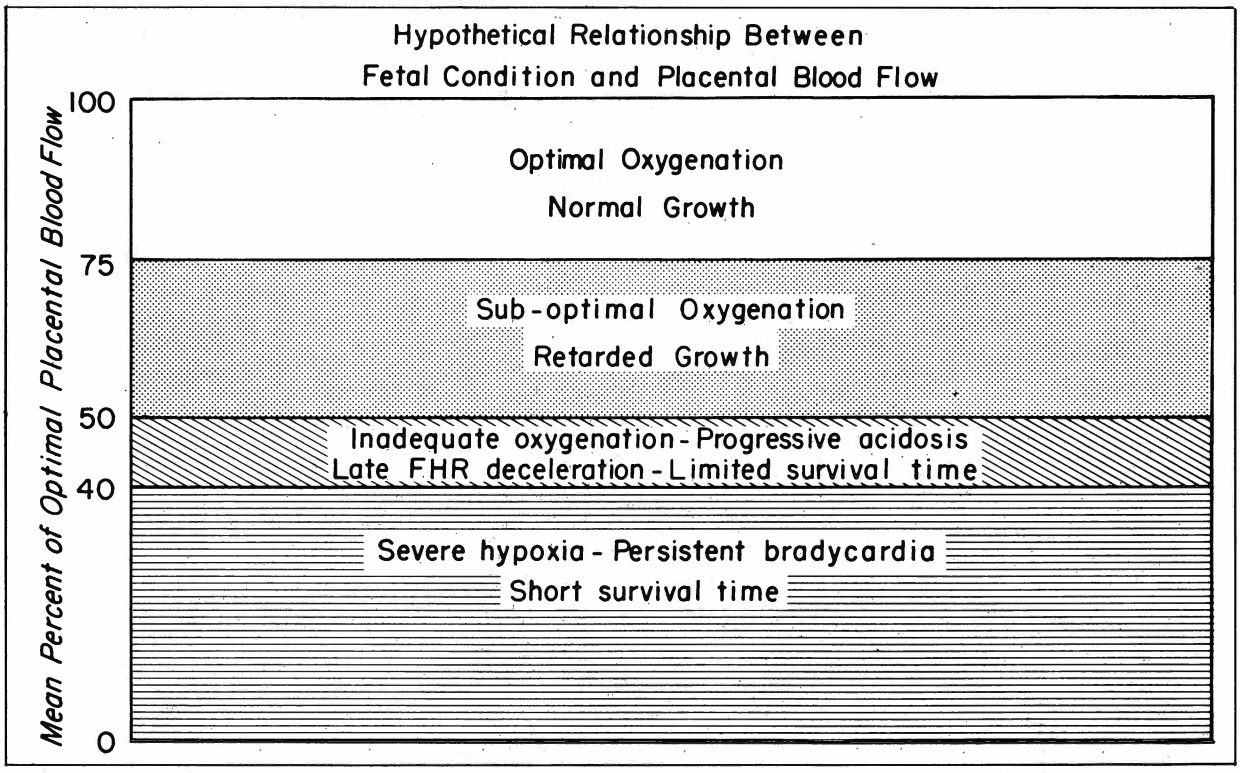

Fig. 7—Hypothetical relationship between placental blood flow and the status of the fetus. Of the many parameters determining fetal well-being, placental blood flow is the most variable and the parameter most subject to pathologic change. The significance of detrimental influences on placental blood flow increases as the pre-stress level of flow decreases.

vasoconstriction with an associated decrease in systemic blood pressure. Together, these two factors cause a marked decrease in placental perfusion (Greiss, 1966). Figure 9 shows that acute severe hemorrhage may rapidly compromise the fetus even when the pre-hemorrhage status of placental perfusion is optimal. At slower rates of blood loss, the degree of peripheral vasoconstriction necessary to maintain maternal integrity will be less because of compensatory shifts of body fluids into the vascular compartment, and placental perfusion will not be reduced so drastically. Therapy for blood loss should

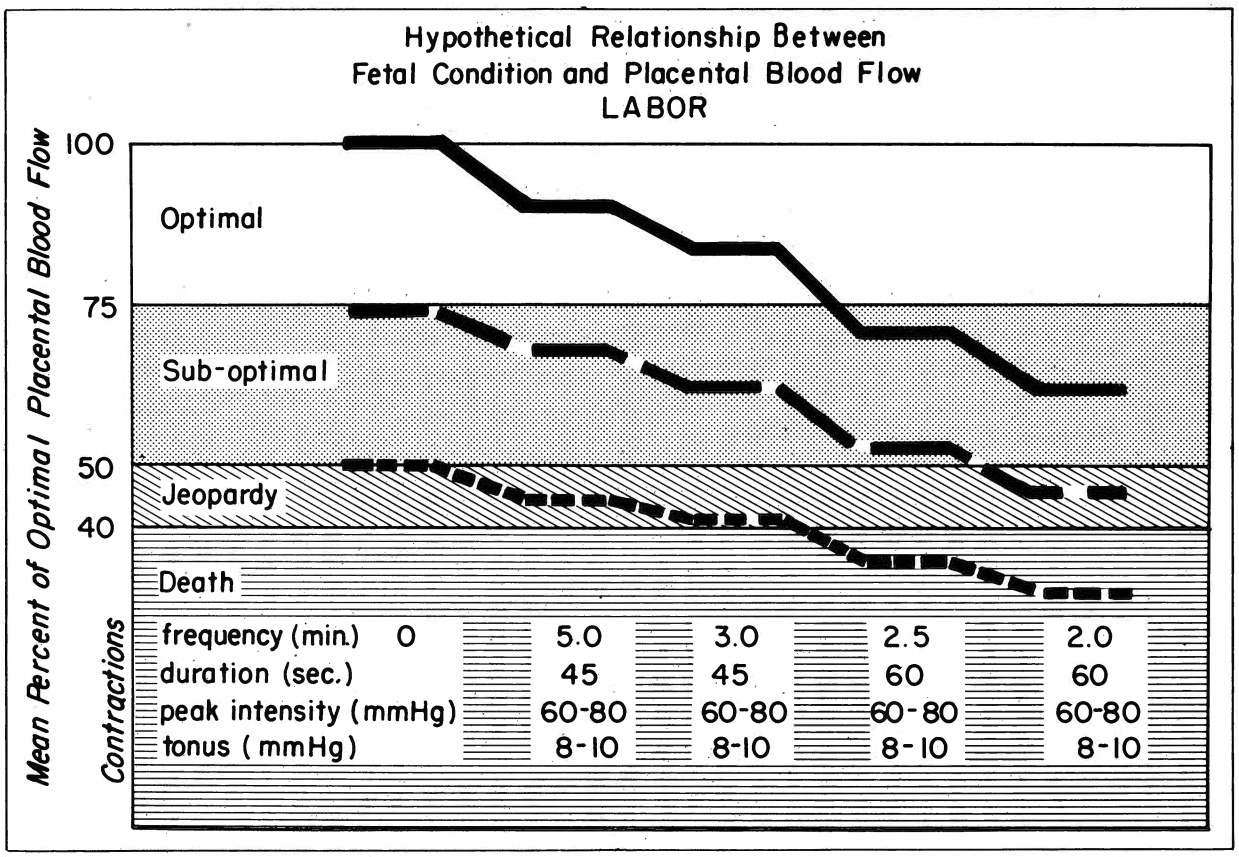

Fig. 8—Effect of uterine contractions on mean placental blood flow. As the duration and frequency of contractions increase, mean flow progressively decreases. If labor begins with optimal blood flow, placental perfusion is more than adequate even during very active contractions (solid line). However, if placental perfusion is borderline before labor such as occurs with toxemia, even mild contractions may cause fetal distress or demise (short dashed line).

be directed toward correcting the vascular volume-capacity discrepancy. Optimally, this should be accomplished with whole blood. However, in emergency situations when blood is not available or is being readied, rapid intravenous infusions of salt solutions or blood substitutes will still improve placental perfusion significantly (Boba, *et al,* 1966).

Non-hemorrhagic Hypotension. Except for hemorrhagic and septic shock, most circumstances that decrease maternal blood pressure do not change placental vascular resistance. Therefore, placental blood flow will decrease in proportion to the degree of hypotension. Such circumstances occur most frequently following spinal anesthesia for cesarean section. The effects of a typical clinical situation on placental blood flow are illustrated in Fig. 10. It should be evident that only those fetuses in the suboptimal environment prior to anesthesia will be in jeopardy and only those in the lower suboptimal range may die. This is especially true since hypotension of this severity is usually corrected promptly. Generally, hypotension will respond to a combination of left-uterine displacement and a rapid intravenous infusion of 500–1000 ml. of salt or dextrose solution (Greiss and Crandell, 1965). The former relieves pressure on the inferior vena cava, thus increasing venous return to the heart and cardiac output. The latter reduces the vascular volume-capacity disparity induced by sympathetic nerve paralysis and peripheral vasodilatation. However, some patients do not respond to these techniques, and vasopressor therapy becomes necessary. At this point, a knowledge of the vasomotor control of the placental blood vessels is requisite for the selection of that vasopressor agent

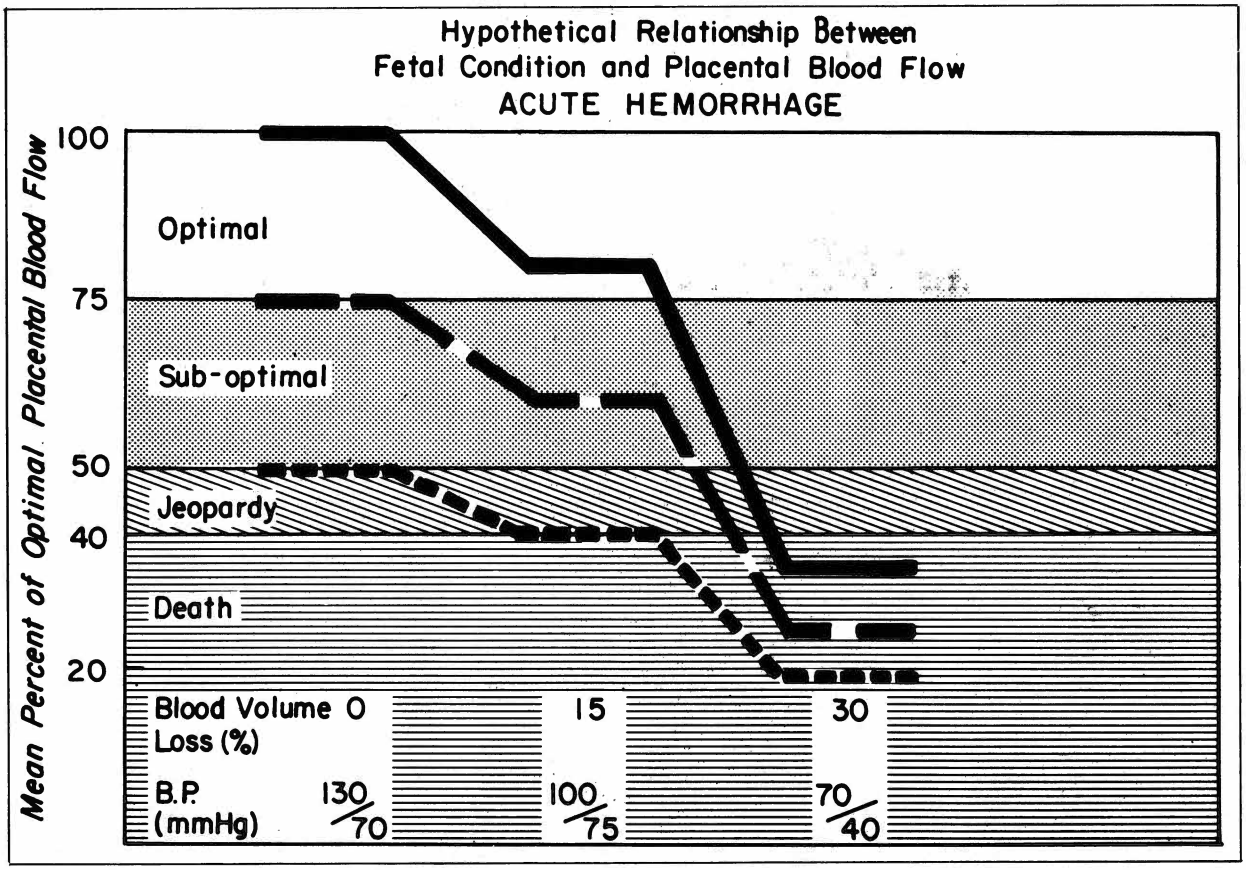

Fig. 9—Effect of acute sudden hemorrhage on placental blood flow in the ewe. Prompt therapy with whole blood or blood volume expanders is necessary to protect the fetus even if pre-hemorrhage conditions were optimal.

most beneficial to placental perfusion. The effects of two agents, ephedrine and metaraminol‡, on placental blood flow are depicted in Fig. 10 (James, *et al,* 1970). By acting primarily on the heart with minimal uterine and peripheral vasoconstriction, ephedrine accomplishes the greatest improvement in placental blood flow. Metaraminol improves maternal blood pressure by acting in roughly equal amounts on the heart and peripheral blood vessels. Therefore, the beneficial effect of improved blood pressure is partially offset by placental vasoconstriction so that the fetal environment is improved less effectively. The difference between the two agents becomes significant when one considers flow responses in a fetus with initial borderline placental perfusion (short dashed line, Fig. 10). Ephedrine therapy improved perfusion sufficiently to produce a live although probably depressed infant while metaraminol therapy was inadequate to accomplish even this outcome. Not shown in Fig. 10 are the effects of primarily peripherally acting pressor agents such as phenylephrine.* Such agents cause so much placental vasoconstriction that despite restoration of normal blood pressure, placental perfusion is not improved and may even decrease further (Greiss and Crandell, 1965).

The above illustrations depict the effects of individual conditions upon placental blood flow. In clinical practice, it should be evident that such conditions may occur concomitantly, and actually there is a positive tendency for this to happen. Therefore, placental perfusion is often reduced by multiple factors. Our ability to minimize the effect of these factors on the fetal environment may make the difference between a healthy child and a stillborn or retarded infant.

‡ Aramine®, Merck Sharp & Dohme

* Neo-Synephrine®, Winthrop Labs.

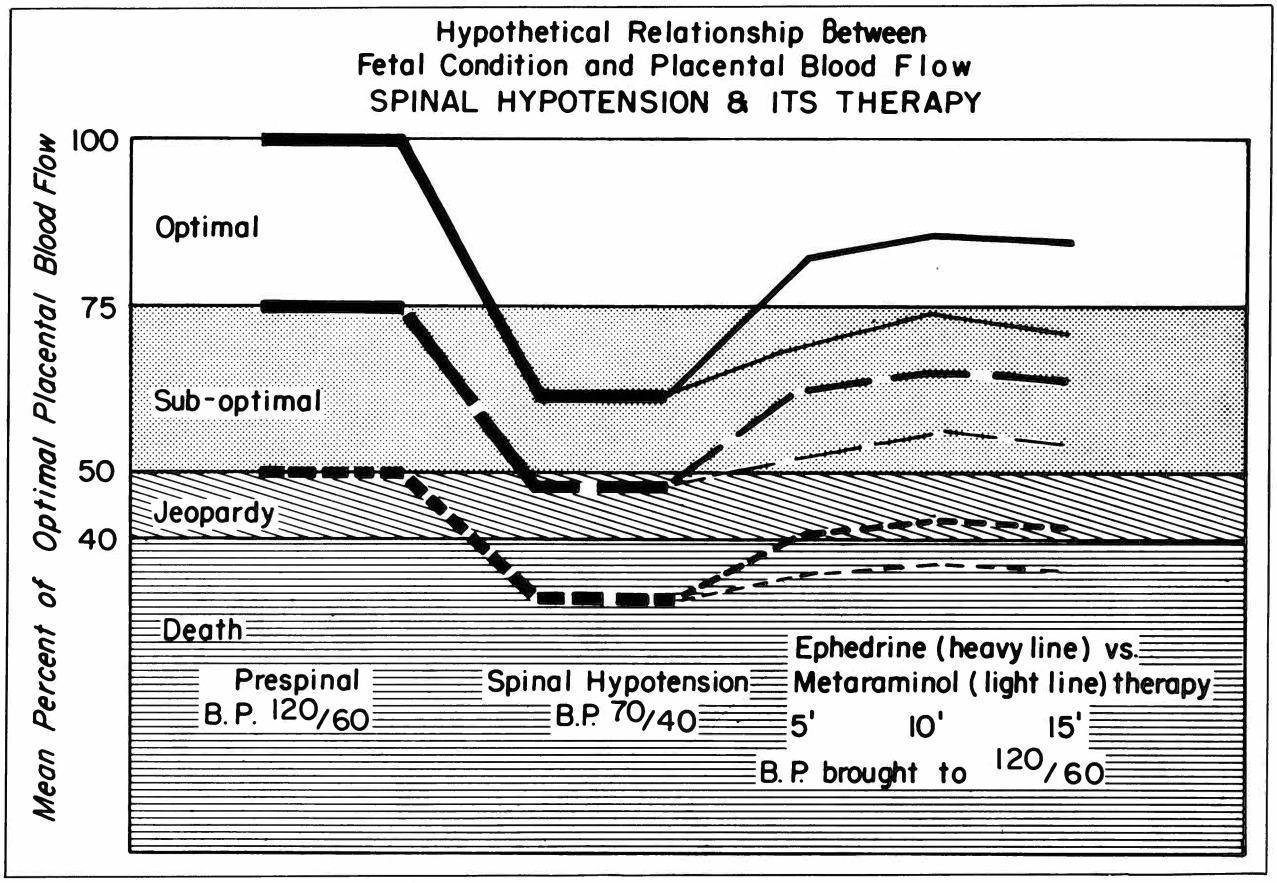

Fig. 10—Effect of hypotension induced by spinal anesthesia on placental perfusion. Only those fetuses with initial suboptimal placental blood flow will be affected by marked hypotension (dashed lines). If vasopressor therapy is required, appropriate selection of the drug according to its mode of action may be crucial to fetal survival.

REFERENCES

AHLQUIST, R. P. The action of various drugs on the arterial blood flow of the pregnant canine uterus. *J. Am. Pharm. Assoc.* (Scient. Ed.) 39:370, 1950.

ASSALI, N. S., DOUGLAS, A., JR., BAIRD, W. W., NICHOLSON, D. B., AND SUYEMOTO, R. Measurement of uterine blood flow and uterine metabolism IV. Results in normal pregnancy. *Am. J. Obstet. Gynec.* 66:248, 1953.

ASSALI, N. S., RAURAMO, L., AND PELTOMEN, T. Measurement of uterine blood flow and uterine metabolism VIII. Uterine and fetal blood flow and oxygen consumption in early human pregnancy. *Am. J. Obstet. Gynec.* 79:86, 1960.

BARCROFT, J. *Researches on Prenatal Life.* Springfield: C. C. Thomas, 1947, p. 1.

BOBA, A., LINKIE, D. M., AND PLOTZ, E. J. Effects of vasopressor administration and fluid replacement on fetal bradycardia and hypoxia induced by maternal hemorrhage. *Obstet. Gynecol.* 27:408, 1966.

BORRELL, U., INGMAN, F., OHLSON, L., AND WIQVIST, N. Effect of uterine contractions on the human uteroplacental blood circulation. *Am. J. Obstet. Gynec.* 89:881, 1964.

BROWNE, J. C. MCC., AND VEALL, N. Maternal placental blood flow. *J. Obstet. Gynaecol. Brit. Comm.* 60:141, 1953.

DIXON, H. G., BROWNE, J. C., AND DAVEY, D. A. Chorio-decidual and myometrial blood flow. *Lancet* 2:369, 1963.

GREISS, F. C., JR. Pressure-flow relationship in the gravid uterine vascular bed. *Am. J. Obstet. Gynec.* 96:41, 1966.

GREISS, F. C., JR. Uterine vascular response to hemorrhage during pregnancy. *Obstet. Gynecol.* 27:549, 1966.

GREISS, F. C., JR. Differential reactivity of the myoendometrial and placental vasculatures: Vasodilatation. *Am. J. Obstet. Gynec.* 111:611, 1971.

GREISS, F. C., JR. Differential reactivity of the myoendometrial and placental vasculatures: Adrenergic responses. *Am. J. Obstet. Gynec.* 112:20–30, 1972.

GREISS, F. C., JR. Unpublished observations.

GREISS, F. C., JR. AND ANDERSON, S. G. Uterine blood flow during labor. *Clin. Obstet. Gynecol.* 11:96, 1968.

GREISS, F. C., JR. AND ANDERSON, S. G. Uterine blood flow during early ovine pregnancy. *Am. J. Obstet. Gynec.* 106: 30, 1970.

GREISS, F. C., JR., ANDERSON, S. G., AND KING, L. C. Uterine vascular bed: Effects of acute hypoxia. *Am. J. Obstet. Gynec.* In press.

GREISS, F. C., JR. AND CRANDELL, D. L. Therapy for hypotension induced by spinal anesthesia during pregnancy. *JAMA* 191:793, 1965.

GREISS, F. C., JR. AND GOBBLE, F. L., JR. Effect of sympathetic nerve stimulation on the uterine vascular bed. *Am. J. Obstet. Gynec.* 97:962, 1967.

GREISS, F. C., JR., GOBBLE, F. L., JR., ANDERSON, S. G., AND MCGUIRT, W. F. Effect of parasympathetic nerve stimulation on the uterine vascular bed. *Am. J. Obstet. Gynec.* 99:1067, 1967.

GREISS, F. C., JR., GOBBLE, F. L., JR., ANDERSON, S. G., AND MCGUIRT, W. F. Effect of acetylcholine on the uterine vascular bed. *Am. J. Obstet. Gynec.* 99:1073, 1967.

GREISS, F. C., JR. AND MARSTON, E. L. The uterine vascular bed: Effect of estrogens during ovine pregnancy. *Am. J. Obstet. Gynec.* 93:720, 1965.

GREISS, F. C., JR. AND PICK, J. R. JR. The uterine vascular bed: Adrenergic receptors. *Obstet. Gynecol.* 23:209, 1964.

GREISS, F. C., JR. AND VAN WILKES, D. Effects of sympathomimetic drugs and angiotensin on the uterine vascular bed. *Obstet. Gynecol.* 23:925, 1964.

HESS, O. W. AND HON, E. H. The electronic evaluation of fetal heart rate: III. The effect of an oxytocic agent for induction of labor. *Am. J. Obstet. Gynec.* 80:558, 1960.

HON, E. E. AND QUILLIGAN, E. J. Electronic evaluation of fetal heart rate. IX. Further observations on "pathologic" fetal bradycardia. *Clin. Obstet. Gynecol.* 11:145, 1968.

HUCKABEE, W. E., METCALFE, J., PRYSTOWSKY, H., AND BARRON, D. H. Blood flow and oxygen consumption of the pregnant uterus. *Am. J. Physiol.* 200:274, 1961.

JAMES, F. M., III, GREISS, F. C., JR., AND KEMP, R. A. An evaluation of vasopressor therapy for maternal hypotension during spinal anesthesia. *Anesthesiology* 33:25, 1970.

JOHNSTON, T. AND CLAYTON, C. G. Diffusion of radioactive sodium in normotensive and preeclamptic pregnancies. *Br. Med. J.* 5014:312, 1957.

LANDESMAN, R. AND KNAPP, R. C. Na^{24} uterine muscle clearance in late pregnancy. *Am. J. Obstet. Gynec.* 80:92, 1960.

LEES, M. H., HILL, J. D., OCHSNER, A. J., III, THOMAS, C. L., AND NOVY, M. J. Maternal placental and myometrial blood flow of the rhesus monkey during uterine contractions. *Am. J. Obstet. Gynec.* 110:68, 1971.

MAKOWSKI, E. L., MESCHIA, G., DROEGEMUELLER, W., AND BATTAGLIA, F. C. Distribution of uterine blood flow in the pregnant sheep. *Am. J. Obstet. Gynec.* 101:409, 1968.

METCALFE, J., ROMNEY, S. L., RAMSEY, L. H., REID, D. E., AND BURWELL, C. S. Estimation of uterine blood flow in normal human pregnancy at term. *J. Clin. Invest.* 34:1632, 1955.

MORRIS, N., OSBORN, S. B., AND WRIGHT, H. P. Effective circulation of the uterine wall in late pregnancy measured with $^{24}NaCl$. *Lancet* 268:323, 1955.

PETERSON, E. N. AND BEHRMAN, R. E. Changes in the cardiac output and uterine blood flow of the pregnant Macaca Mulatta. *Am. J. Obstet. Gynec.* 104:988, 1969.

RAMSEY, E. M., CORNER, G. W., JR., AND DONNER, M. W. Serial and cineradioangiographic visualization of maternal circulation in the primate (hemochorial) placenta. *Am. J. Obstet. Gynec.* 86:213, 1963.

SCHWARCZ, R., JR., ALTHABE, O., JR., FISCH, L., AND PINTO, R. M. Relationships between intervillous space amniotic fluid and maternal arterial blood pressures. *Am. J. Obstet. Gynec.* 98:924, 1967.

WEIS, E. B., JR., BRUNS, P. D., AND TAYLOR, E. S. A comparative study of the disappearance of radioactive sodium from human uterine muscle in normal and abnormal pregnancy. *Am. J. Obstet. Gynec.* 76:340, 1958.

Placental Circulation*

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One of the most important developments of recent years in the field of uterine physiology has been the recognition that the endometrial changes occurring during the menstrual cycle and those associated with pregnancy are interlocking, sequential events in an ordered progression from the first day of the cycle to parturition—and not separate phenomena as was formerly believed. No component of the endometrium illustrates this progression more strikingly than does the vasculature.

Much of the story of uterine vascular pattern and circulatory mechanism is based upon studies in the rhesus monkey, employing *in vivo* techniques inapplicable to clinical patients. (These studies were carried out in the Department of Embryology, Carnegie Institution of Washington at Baltimore.) Subsequent checking of the monkey findings against their human counterparts, in operative and necropsy specimens, etc., has shown the monkey to be a valid experimental model with reproductive system anatomy and physiology closely similar to the human (Ramsey and Harris, 1966).

Following the menstrual slough the vasculature regenerates *pari passu* with the endometrial stroma and glands (Fig. 1). Initially a long capillary network forms between the stumps of spiral arteries in the basalis and the epithelial surface. Subsequently, muscular and elastic layers forming around the capillaries transform them into true arteries. It may be noted parenthetically that this is a more accurate description than the familiar statement that "spiral arteries grow toward the endometrial surface." A rich capillary bed remains in the immediately subepithelial layer and connects the arteries with veins which run perpendicularly toward the myometrium.

Although the follicular phase of the cycle is frequently referred to as the "growth phase," growth of spiral arteries continues unabated during the corpus luteum phase and even further on, as we will see. Indeed, vascular growth during the lutein phase outstrips stromal growth, so that the increasing length of the arteries must be accomodated within the endometrium by ever increasing coiling (Fig. 1).

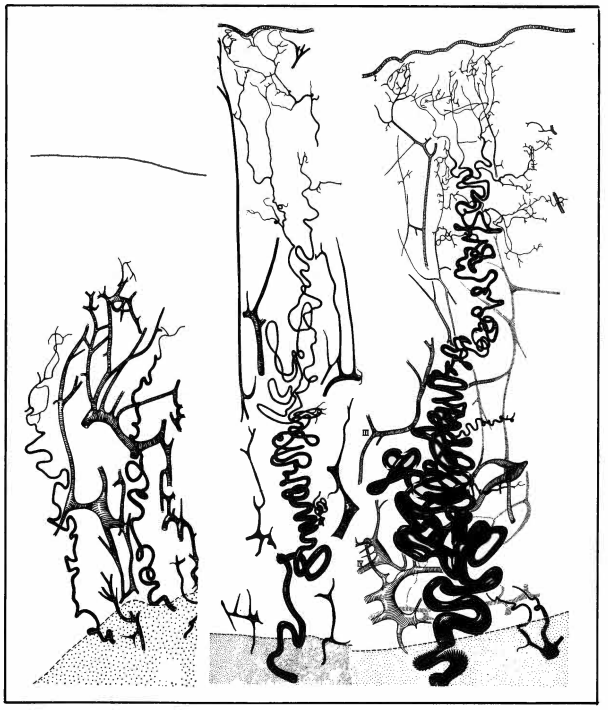

Fig. 1—Camera lucida drawings of the vascular bed at three stages of the menstrual cycle in the rhesus monkey. *Left.* postmenstrual; *Center.* postovulatory; *Right.* late secretory. Myometrium stippled. (Reprinted with permission from Bartelmez. *Contrib. Embryol.* 36:153–182, 1957.)

The implanting ovum achieves its first contact with the maternal blood supply when the penetrating trophoblast both taps and engulfs capillaries of the subepithelial network (Fig. 2), permitting maternal blood to seep, under very low pressure, into the

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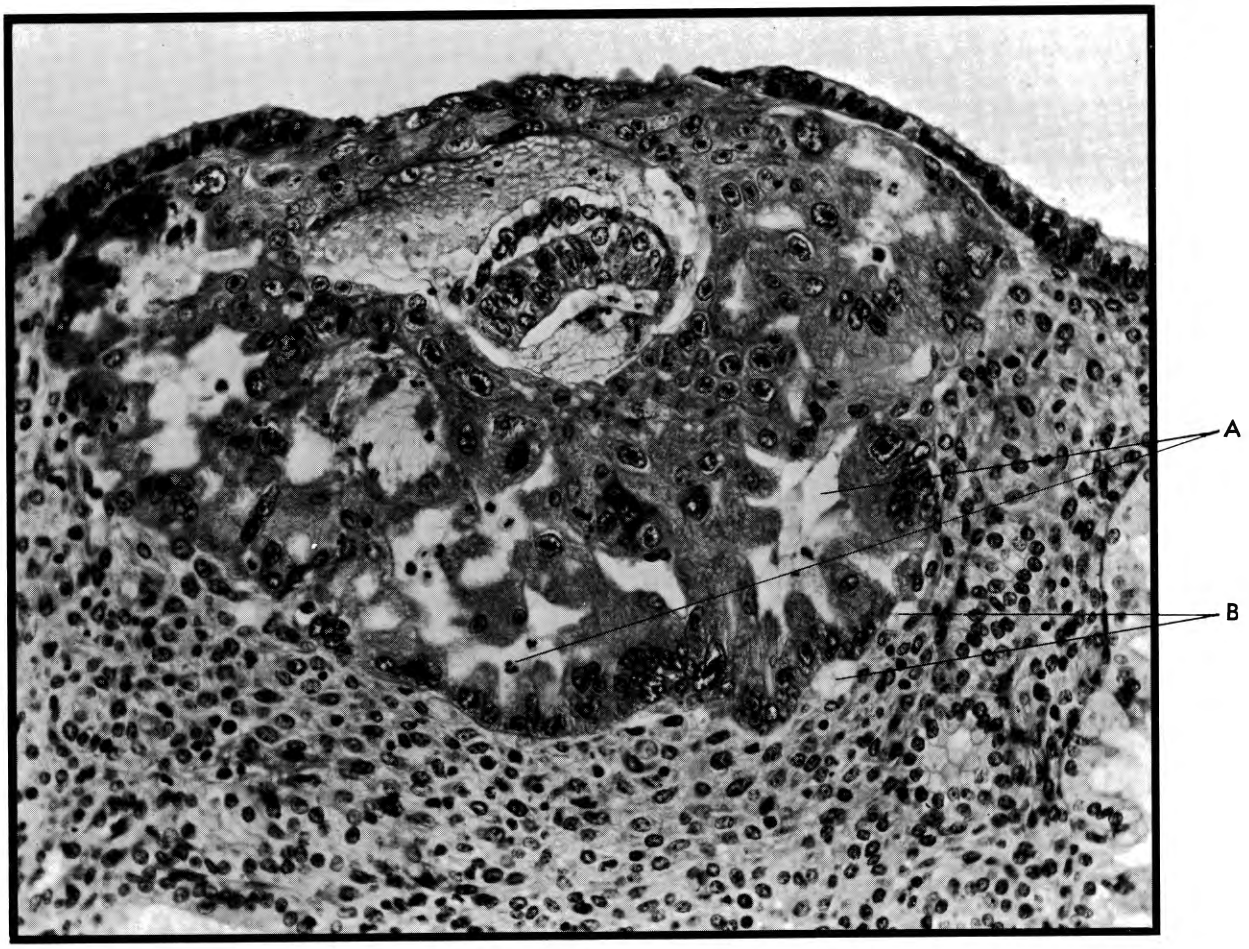

Fig. 2—Photomicrograph of an early human implantation. (a) trophoblastic lacunae; (b) maternal capillaries. Carnegie Collection 8004, 7th day of pregnancy, section 11-4-4. (Reprinted with permission from Hertig and Rock. *Contrib. Embryol.* 31: 65-84, 1945.)

lacunae of the trophoblastic shell. With progressive penetration by trophoblast, the terminal tips of spiral arteries are opened up and maternal arterial blood flows into the shell. This meanwhile has itself been enlarged and transformed by the development of chorionic villi (Fig. 3). It is around the villi, in the inter-villous space, that the maternal blood now flows and from now on we may speak of a placenta and placental circulation.

Reconstructions of representative uteroplacental arteries, both human and monkey, at comparable stages of gestation (Fig. 4), show that there is very little qualitative change in growth pattern during the first weeks after implantation (Harris and Ramsey, 1966; Ramsey, 1949). The coiling of the arteries continues and there is just a slight indication of a new process at the arterial tips where trophoblast is beginning to replace normal wall structure. Soon however a change does become manifest. Arterial elongation (as determined by careful micromeasurements) is continuing, but the thickness of the endometrium is being diminished as the result of trophoblastic erosion combined with pressure of the overlying conceptus. Thus, the previously vertical arterial stems are diverted toward the margins of the implantation site, an increasingly sharp angulation developing. With the continuation of these processes in succeeding weeks, the increased coiling of the artery is no longer sufficient to effect its accomodation in the thinned endometrium, so back and forth and lateral looping is added. A terminal dilatation of the artery develops proximal to its point of entry into the intervillous space.

At about midpregnancy when, as Reynolds has shown (Reynolds, 1947), the enlargement of the uterus by growth of its parts gives place to enlargement by stretching, the coils of the arteries are "paid out," as coils of rope on the deck of a ship are paid out when the space between ship

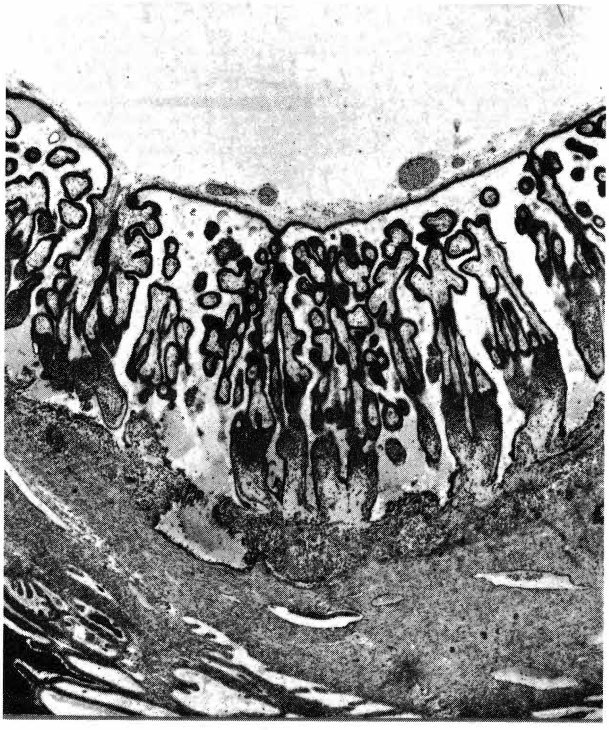

Fig. 3—Photomicrograph of a portion of a monkey placenta *in situ*. Chorionic plate above; entrance of an endometrial spiral artery into the intervillous space at the left. Carnegie Collection C-477, 29th day of pregnancy, section 47b.

and anchorage is increased. The coils are more fully smoothed away in the monkey than in man, probably because monkey endometrium undergoes the greater stretching.

The terminal dilatations of arteries communicating with the intervillous space appear to be the result of the weakening of the vessel wall brought about by replacement of muscle and elastic tissue by trophoblast. Appearing first as an intraluminal accumulation (Fig. 5a), the trophoblastic cells gradually invade and replace the vessel wall (Fig. 5b). The invasion is earlier in the monkey and baboon than in the human, but it is deeper and more extensive in the latter. Human cytotrophoblast penetrates the endometrial stroma as well as entering the arterial lumen and invasion of the wall proceeds from without as well as from within (Fig. 6). The more drastic elimination of normal vascular wall resistance in man doubtless occasions the larger and more persistent terminal dilatations of human uteroplacental arteries. A further result of greater trophoblastic activity in the human is the erosion of arteries all the way to the midendometrium where branches arise from the main spiral stems. These branches then communicate with the intervillous space which explains why there is a proportionately greater number of arterial entries in humans than in monkeys. Upon occasion the trophoblastic action, in contrary fashion, may cause occlusion of branches or even main arterial stems.

Venous drainage, at all stages of the reproductive cycle, is a less dynamic affair than arterial inflow. The basic venous pattern in the endometrium is a grid with dilatations into venous lakes at the junction of vertical and lateral limbs. These relationships continue into pregnancy with certain of the vertical channels increasingly distended as they are required to accommodate the ever increasing volume of placental blood. Other channels are passively obliterated by external compression.

On the physiological side there is again continuity between prepregnant and pregnant states. From the standpoint of circulation, this is most apparent in the persistence of an intrinsic contractile potential in the spiral arteries. This is manifested during the menstrual cycle by isolated contractions at the myoendometrial junction which produce ischemia leading to foci of endometrial necrosis and slough (Bartelmez, 1957), and in pregnancy by intermittency of flow through individual spiral arteries into the intervillous space (Martin, McGaughey, *et al,* 1964).

The opposite number to uteroplacental circulation is of course fetoplacental circulation. Propelled by the *vis a tergo* of fetal blood pressure, fetal blood courses through the umbilical arteries into the subdivisions which run laterally through the chorionic plate. Finally, the vessels dip into the substance of the placenta and travel through the arborizations of the fetal villous tree. They proceed in comparable subdivisions to the terminal villi. There the fetal capillary bed, coming into its closest proximity to maternal blood in the intervillous space, forms the ultimate area of maternal-fetal exchange. Oxygenated blood returns via vessels running through the same villous stems to the umbilical vein and thence to the fetal body (Martin and Ramsey, 1970).

The mechanism of circulation within the placenta, first hypothesized upon the basis of anatomical data, has been established by radioangiographic studies (Donner, *et al,* 1963). Especially with cineradioangiography, it is possible to visualize directly the inflow of arterial blood to the intervillous space (Fig. 7), its circulation through the space, and finally its drainage back to uterine veins.

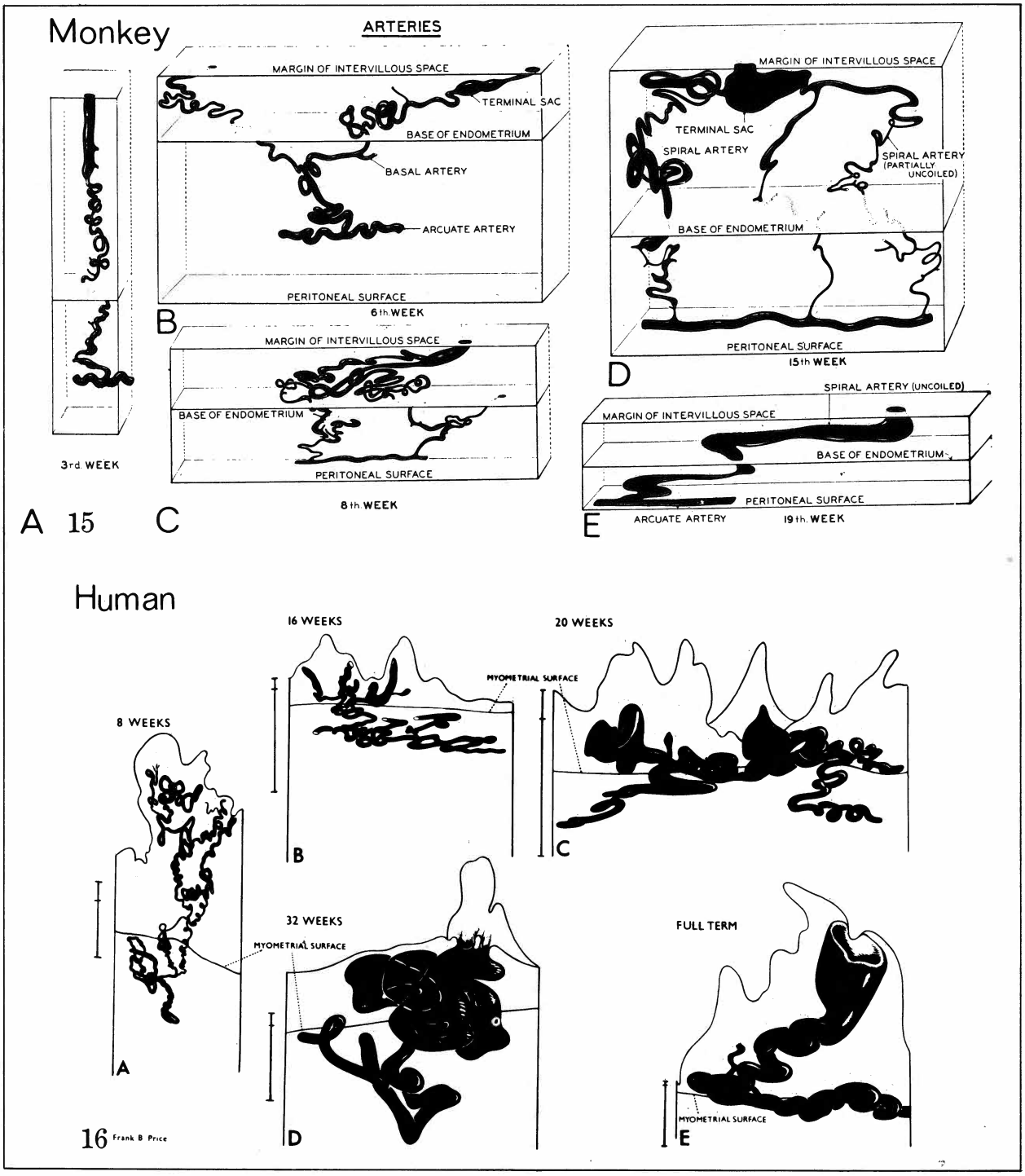

Fig. 4—Diagrammatic representations of the course and configuration of the uteroplacental arteries in the rhesus monkey and man, at comparable stages of gestation. (Reprinted with permission from Harris and Ramsey. *Contrib. Embryol.* 38: 43-58, 1966.)

The propulsive force throughout is the head of maternal blood pressure which drives blood into the intervillous space in discreet, fountainlike "spurts." The incoming blood wafts aside the villi surrounding the orifices of entry, but once the propulsive force is reduced, in part by the baffle action of the villi, the blood disperses laterally crowding the existing content of blood through the venous orifices in the basal plate into the uterine drainage channels (Fig. 8). During uterine con-

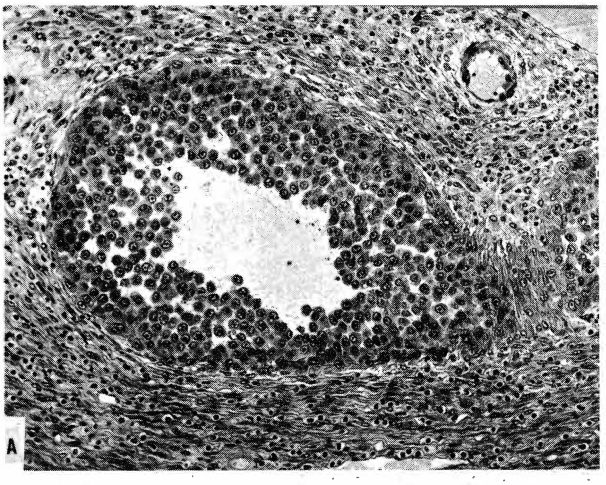

Fig. 5a—Photomicrograph of uteroplacental arteries in the monkey illustrating early accumulation of trophoblast within the lumen of the artery. Carnegie Collection C-477, 29th day of pregnancy. (Reprinted with permission from Wislocki and Streeter. *Contrib. Embryol.* 27:1–66, 1938.)

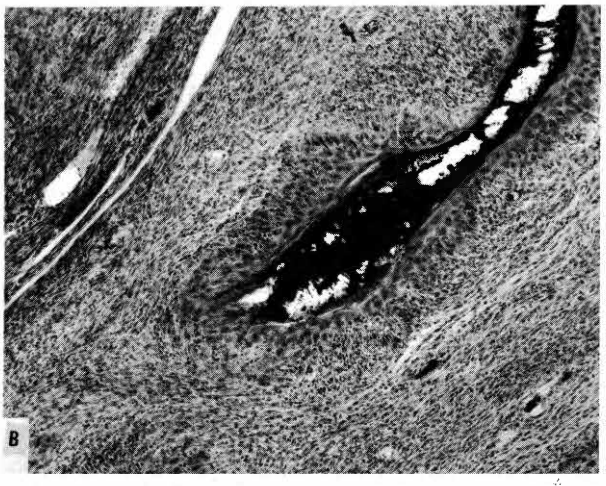

Fig. 5b—Photomicrograph of uteroplacental arteries in monkey illustrating subsequent replacement of the arterial wall without trophoblastic penetration of stroma. Carnegie Collection C-629, 53rd day of pregnancy. (Reprinted with permission from Ramsey. *Contrib. Embryol.* 33:113–147, 1949.)

tractions both inflow and outflow cease, in whole or in part, depending upon the strength of the contraction (Ramsey, Martin, McGaughey, *et al,* 1966). The volume of the placental pool, however, is maintained. That is to say, the old concept that "contractions squeeze the placenta like a sponge" is incorrect; rather blood is trapped in the placenta during contractions.

Radioangiography of the fetal side of placental circulation (Martin, Ramsey, and Donner, 1966)

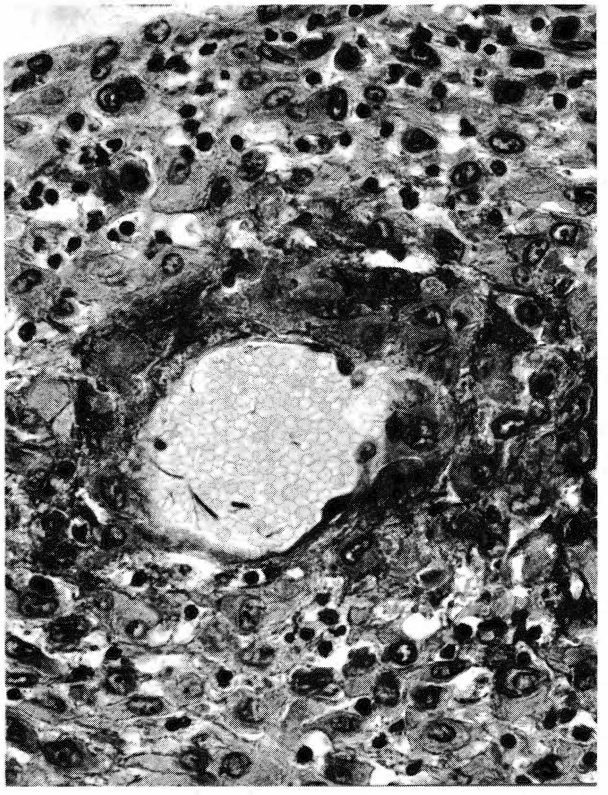

Fig. 6—Photomicrograph of a human uteroplacental artery showing replacement of wall and penetration of stroma by trophoblast. Carnegie Collection 10117, 85th day of pregnancy. (Reprinted with permission from Ramsey. *Prenatal Life.* Wayne State University Press, 1970, pp. 37–53.)

shows the progress of blood from the fetal body into the capillary network of the fetal cotyledons and back via the umbilical vein. Double injection of a radiopaque medium (Ramsey, Martin, and Donner, 1967), that is, into fetal and maternal circulations in rapid succession, permits visualization of the 1:1 relationship between maternal spiral arteries and fetal cotyledons (Fig. 9).

Two points of clinical interest emerge from the foregoing. The first is that placental circulation ceases during strong contractions. That this may present the fetus with periods of anoxia is clear and should contractions be unduly prolonged, as the result of pathology or medication, it could indeed be critical. Second, and somewhat mitigating the implied threat of the cessation of flow, is the fact that the pool of placental blood is preserved throughout. Thus, under normal conditions, continued maternal-fetal exchange is made possible.

And that exchange, of course, is the whole purpose of the long and elaborate procession of vascular changes from Day 1 of the menstrual cycle to parturition.

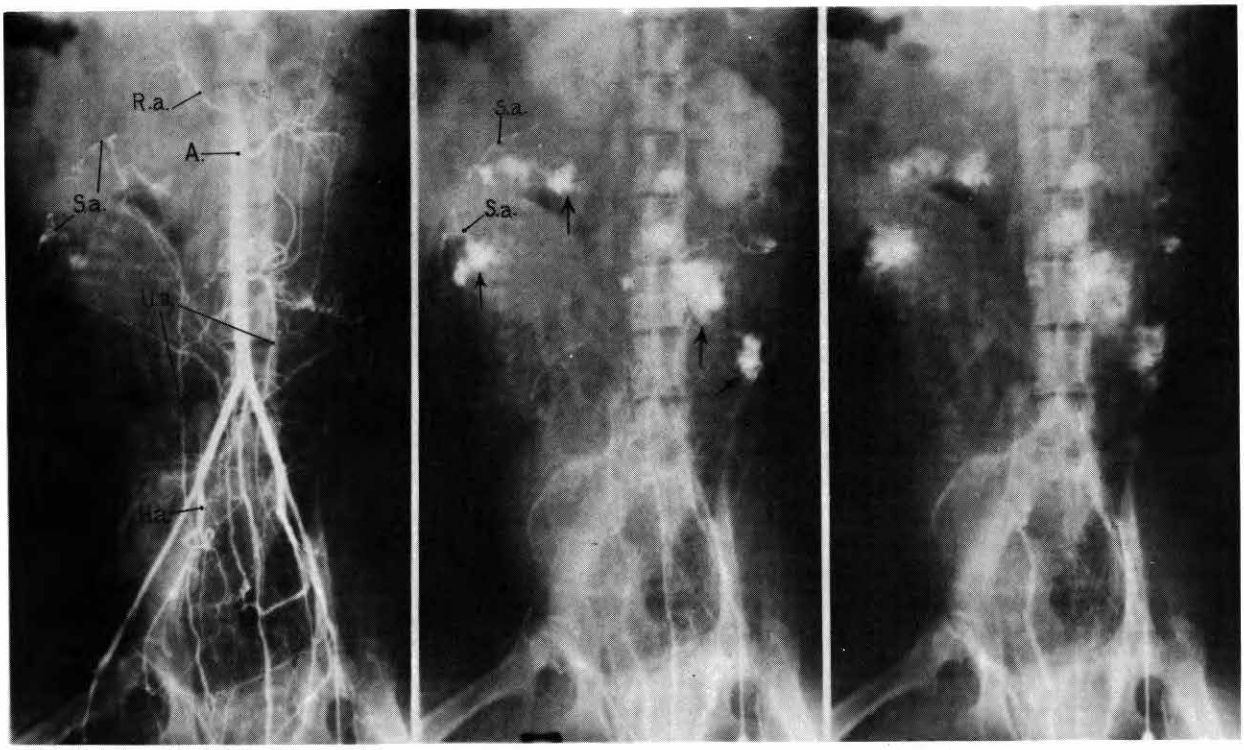

Fig. 7—Photographs of X rays made at 2, 3, and 7½ seconds respectively after injection of contrast material into a femoral artery of a monkey. (R.a.) renal artery; (S.a.) endometrial spiral artery;→"spurts" into intervillous space. Carnegie Collection Monkey 60/14, 100th day of pregnancy. (Reprinted with permission from Ramsey, *et al.* Montanino Editore, Napoli. II: 1779-1784, 1962.)

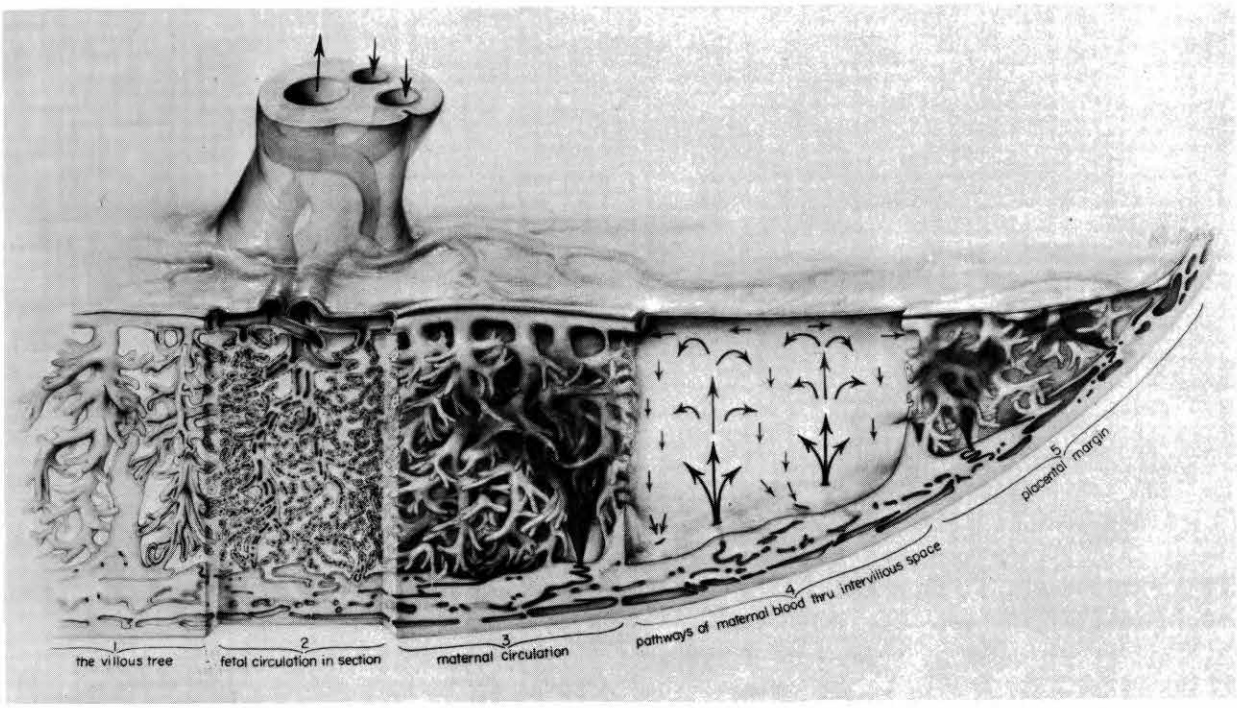

Fig. 8—Composite drawing of the primate placenta to show its structure and circulation. (Drawing by Ranice Davis Crosby for E. M. Ramsey. Courtesy of the Carnegie Institution of Washington.)

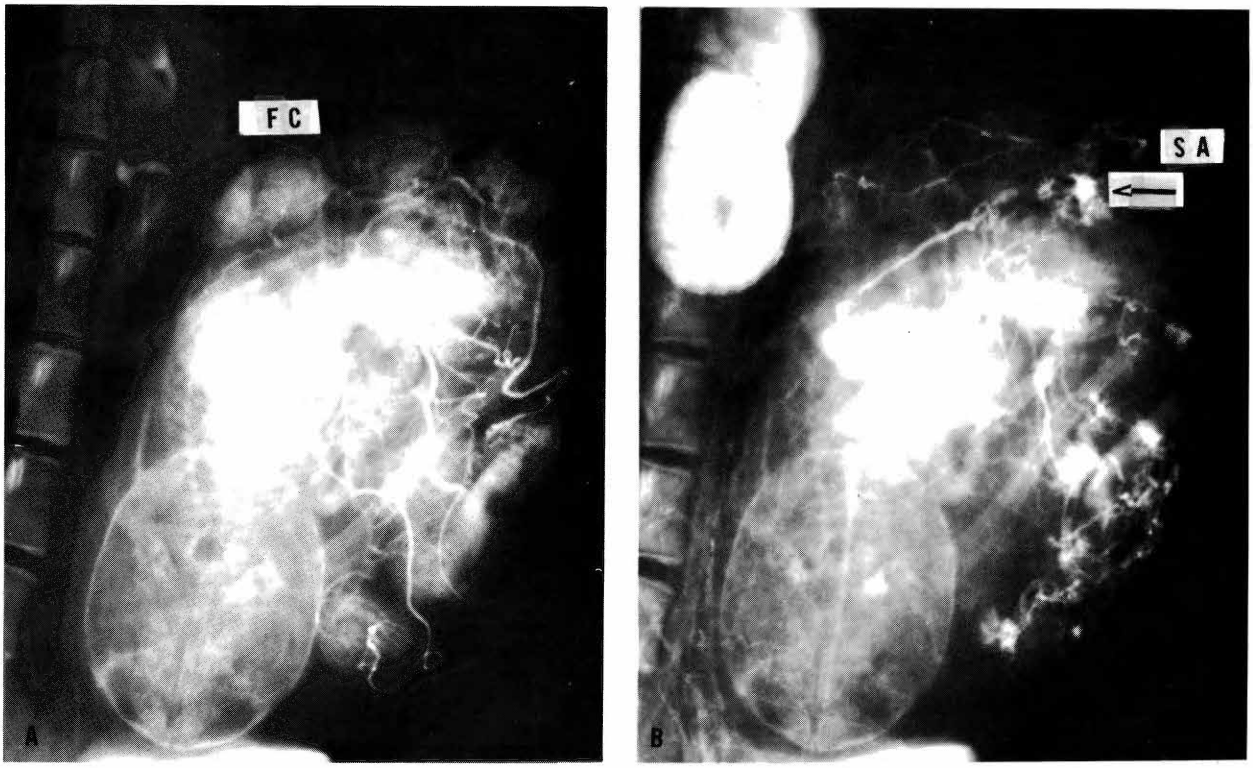

Fig. 9—Spot films made during a combined fetal and maternal injection study. (A) taken 3 seconds after injection of contrast material into the fetal circulation; (B) taken 2 seconds after immediately subsequent maternal injection. (FC) fetal cotyledon; (SA) endometrial spiral artery;→"spurts" into the intervillous space. Carnegie Collection Monkey 65/80, 152nd day of pregnancy. (Reprinted with permission from Ramsey, *et al. Am. J. Obstet. Gynec.* 98: 419-423, 1967.)

REFERENCES

BARTELMEZ, G. W. The form and the functions of the uterine blood vessels in the rhesus monkey. Carnegie Inst. Wash., *Contrib. Embryol.* 36:153–182, 1957.

DONNER, M. W., RAMSEY, E. M., AND CORNER, G. W., JR. Maternal circulation in the placenta of the rhesus monkey; A radioangiographic study. *Amer. J. Radiol. and Roentgen. Therapy.* 90:638–649, 1963.

HARRIS, J. W. S. AND RAMSEY, E. M. The morphology of human uteroplacental vasculature. Carnegie Inst. Wash., *Contrib. Embryol.* 38:43–58, 1966.

HERTIG, A. T. AND ROCK, J. Two human ova of the previllous stage, having a developmental age of about seven and nine days respectively. Carnegie Inst. Wash., *Contrib. Embryol.* 31:65–84, 1945.

MARTIN, C. B., JR., MCGAUGHEY, H. S., JR., KAISER, I. H., DONNER, M. W., AND RAMSEY, E. M. Intermittent functioning of the uteroplacental arteries. *Am. J. Obstet, Gynec.* 90:819–823, 1964.

MARTIN, C. B., JR. AND RAMSEY, E. M. Gross anatomy of the placenta of rhesus monkeys. *Obstet. Gynecol* 36:167–177, 1970.

MARTIN, C. B., JR., RAMSEY, E. M., AND DONNER, M. W. The fetal placental circulation in rhesus monkeys demonstrated by radioangiography. *Am. J. Obstet. Gynec.* 95:943–947, 1966.

RAMSEY, E. M. The vascular pattern of the endometrium of the pregnant rhesus monkey (*Macaca mulatta*). Carnegie Inst. Wash., *Contrib. Embryol.* 33:113–147, 1949.

RAMSEY, E. M. Placental circulation in rhesus and man. *Prenatal Life.* Proceedings of the Third Annual Symposium on the Physiology and Pathology of Human Reproduction. Harold C. Mack (ed.). Detroit: Wayne State University Press, 1970, pp. 37–53.

RAMSEY, E. M., CORNER, G. W., JR., DONNER, M. W., AND STRAN, H. M. Visualization of maternal circulation in the monkey placenta by radioangiography. Scritti in onore del Prof. Giuseppe Tesauro nel XXV anno del Suo insegnamento. Montanino Editore, Napoli. II:1779–1784, 1962.

RAMSEY, E. M. AND HARRIS, J. W. S. Comparison of uteroplacental vasculature and circulation in the rhesus monkey and man. Carnegie Inst. Wash., *Contrib. Embryol.* 38:59–70, 1966.

RAMSEY, E. M., MARTIN, C. B., JR., AND DONNER, M. W. Fetal and maternal placental circulations. *Am. J. Obstet. Gynec.* 98:419–423, 1967.

RAMSEY, E. M., MARTIN, C. B., JR., MCGAUGHEY, H. S., JR., KAISER, I. H., AND DONNER, M. W. Venous drainage of the placenta in rhesus monkeys: radiographic studies. *Am. J. Obstet. Gynec.* 95:948–955, 1966.

REYNOLDS, S. R. M. Uterine accommodation of the products of conception: physiologic considerations. *Am. J. Obstet. Gynec.* 53:901–913, 1947.

WISLOCKI, G. B. AND STREETER, G. L. On the placentation of the macaque (*Macaca mulatta*), from the time of implantation until the formation of the definitive placenta. Carnegie Inst. Wash., *Contrib. Embryol.* 27:1–66, 1938.

Electronic Monitoring of the Fetus*

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Although the fetal electrocardiograph (FECG), was recorded earlier in this century, utilization of the electrical activity of the heart for fetal assessment is really a procedure of approximately the last two decades. This has come about primarily through the work of Dr. Ed Hon in Southern California and Dr. Roberto Caldeyro-Barcia in Montevideo. Early attempts to obtain signals were made from the maternal abdomen, but they were frustrated by problems of noise either from the maternal heart or from the muscles of the abdominal wall. The advantages of working in the vagina—it strikes me as strange that this should take so long to occur to an obstetrician—is an insight of the last decade, resulting in the placement of an electrode on the fetal scalp, rather than on the maternal abdomen, with a vast improvement in the signal-to-noise ratio and reliability. At the present time, the main use of the electrical activity of the heart obtained through such an electrode is to drive a heart rate-sensing device to give the fetal heart rate which is then used to indicate fetal status. Much speculation has been made as to whether or not the FECG is a more sensitive indicator of fetal status than the heart rate, but that question is unresolved, and there is certainly a good deal of evidence to indicate that such is not the case. The fetal electrocardiograph obtained from the maternal abdomen can be used to detect fetal life, multiple pregnancies, and it can be extracted from the background noise by means of computer averaging techniques in order to study the electrical signal itself.

The concept utilized in electronic monitoring of the fetus is that the application of a stress to the organism in question (feto-placental unit) will produce a response which allows assessment of status prior to the production of permanent damage. Fortunately, there is a physiologic situation in which there is stress to the organism—namely labor. The steady-state of exchange across the placenta, which characterizes pregnancy and is adequate to allow not only survival of the fetus but also growth, is normally terminated by labor, a process which deletes part of the time available for exchange. Through the work of Dr. Hon, studies of the implication of fetal heart rate changes observed during labor became much clearer.

It has been pointed out that much of the methodology used in medicine at present is not fully understood. The results are, therefore, not always satisfactory; but the alternative to applying incompletely understood methodology is to deny a patient a modality of diagnosis or therapy which may be of substantial benefit. In the case of electronic monitoring of the fetus, an attempt is made to intercept a pathological process before the more clearly defined end points of irreversible damage occur. That fact in itself raises the need for much sharper criteria of jeopardy at a point of reversibility.

Labor imposes a period of temporary hazard, the main component of which is related to a loss or diminution of exchange across the placenta. (One must also be mindful of the hazard to the fetus which can be imposed by antenatal medication.) Exchange across the placenta is adversely affected by a variety of conditions:

1. *Obstetrical catastrophies* such as abruptio placentae, placenta previa, and uterine rupture which usually cause disruption of the intervillous space and sheering off of a part or all of the placenta.
2. *Hypotension and hypovolemia* whether due to compression of the vena cava, hemorrhage, or epidural anesthesia, reduce the uterine blood flow.
3. *Uterine hypertonus* whether due to oxytocin stimulation, toxemia, or idiopathic

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results in a decreased net perfusion pressure of the placenta and a decreased uterine diastole.

4. *Mechanical interference* with the umbilical cord may occur either as a total event with prolapse or, as is more commonly found, with contractions. Surprisingly, intermittant cord compression may be well tolerated, although it requires close vigilance and preparation for possible operative delivery.
5. *Maternal vascular insufficiency* such as may be encountered with chronic hypertension, long standing diabetes, or toxemia, lowers the uterine blood flow and hence, the availability of oxygen and nutrients to the fetus.

The methodology is as follows. Stress is measured by means of a pressure transducer connected to the amniotic sac by a fluid-filled catheter. The response of the fetus is measured by means of a heart rate meter which is driven by the FECG signal obtained through an electrode attached to the fetal scalp. These two parameters are measured and displayed by a Corometrics† fetal monitor which was designed by Dr. Hon. The experience at the Medical College of Virginia has demonstrated this unit's reliability, which is a prime factor in our choice of it. The electronic circuitry is digital so that if the machine is working, it can be expected to give correct answers. Heart rate and intra-amniotic pressure are simultaneously displayed, and the chart patterns are used to predict compromise.

The development of more precise instrumentation for evaluating fetal heart rate patterns has brought with it the necessity of inventing a more precise vocabulary to describe the various aspects demonstrated by the new methodology.

1. *Bradycardia* is a decrease in the baseline of the heart rate.
2. *Tachycardia* is an increase in the baseline heart rate.
3. *Acceleration* is a periodic increase in the fetal heart rate.
4. *Deceleration* is a periodic depression of the heart rate.
5. *Irregularity* is a variation from the baseline heart rate and is characteristic of the fetus who retains homeostatic mechanisms.
6. *Period changes* are the ones which are of interest in predicting fetal jeopardy. The periodicity is related to the uterine contraction.

Three patterns emerge from the extensive work of Dr. Hon which could be correlated with physiologic events. The first of these is called *early deceleration* and is characterized by a deceleration in the fetal heart rate which occurs with the onset of uterine contraction. The change tends to mirror the uterine pressure curve and may be abolished by the use of atropine. This type of change implies no alteration in the acid-base status and has a good prognosis. It may, however, be quite marked as will be demonstrated in Fig. 1. The second pattern is that of *late deceleration,* and it is associated with utero-placental insufficiency. This type of deceleration has a late onset with respect to the onset of the uterine contraction; in fact, the onset of the deceleration is often at or beyond the acme of the uterine contraction. It is frequently associated with an elevated fetal heart rate and may not fall below 100 beats per minute. This type of deceleration is related to anoxia and is connected with markedly depressed infants in significantly high statistics. The ability to pick up this type of deceleration is one of the distinctive advantages of continuous electronic monitoring. The third type of deceleration pattern is labeled *variable deceleration,* and it is believed to be due to compression of the umbilical cord. It is variable with respect to the onset of the uterine contraction and tends to be variable in shape. It may have either a "fractured" configuration or a deep U-shape. This pattern is the typical one leading to cesarean section for fetal distress. Cord compression, while it may have dire implications, is usually well tolerated, at least in the initial phases. The cord compression pattern appears also to be vagally mediated and is susceptible to marked modification by the use of atropine. At this point I would like to say that when the cord is clamped at birth, physiologic adjustments are required to compensate for that part of the cardiac output which would normally go to the placenta (about 50–60% of the fetal cardiac output). These adjustments which take place about the time of the first breath result in the alteration of the fetal heart from the parallel arrangement to a series configuration. This alteration cuts the cardiac output in half, and incorporates the lung as an organ through which all blood must pass.

Now let us look at some specific illustrations of the various cord patterns and indicated therapy. Figure 1 illustrates some of the problems of moni-

† Corometrics Medical Systems, Inc.

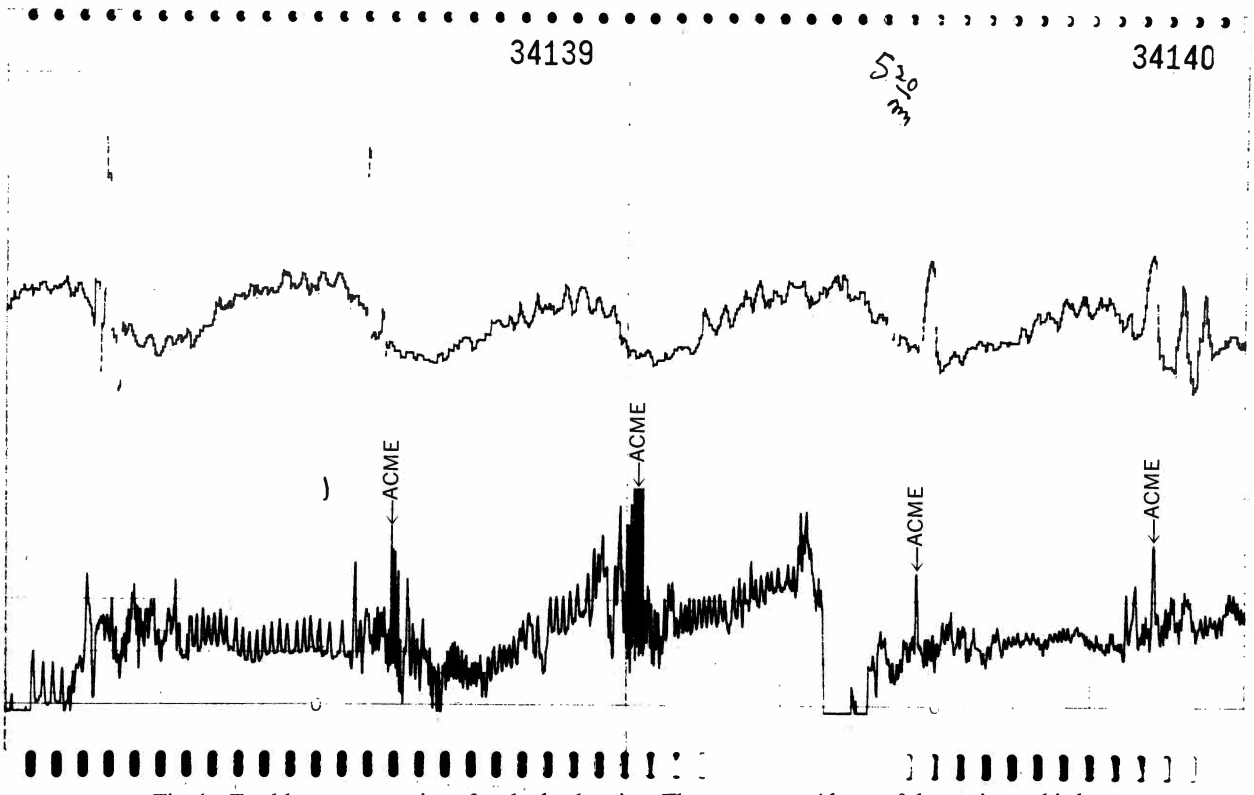

Fig. 1—Fetal heart rate tracing of early deceleration. There was no evidence of depression at birth.

toring the pressure from the abdomen as well as demonstrating marked early deceleration due to compression of the fetal heart. This tracing was recorded with the father present in the labor room. He had expressed a strong interest in having this monitoring equipment used on his wife when she was in labor. After application of the scalp electrode it was noted that there were falls to levels of 80 beats per minute or less with every contraction. However, the acmes of both the deceleration and the uterine contraction occurred approximately simultaneously. The prediction was that the baby would show no evidence of depression, and such was the case.

Figure 2 is that of a patient with Rh disease who went into spontaneous labor at 35 weeks after an amniocentesis. The fetal heart rate tracings show marked deceleration with contractions which are interpreted as the variable type due to cord compression. Since this was a high-risk baby, I would have had to deliver this patient by cesarean section if I had not had the instrumentation to precisely monitor the fetus during her labor. In spite of the fetal heart rate tracings, I was required to watch the patient during a labor lasting several hours, following which she delivered a baby without evidence of depression. The normal delivery was best for the mother and for the baby, but it was certainly harder on the physician.

Figure 3 shows an instance in which the physicians managing the patient did not understand the significance of fetal heart rate tracing. This episode occurred in our early experience at M.C.V. In addition to marked late deceleration, frequent premature contractions are also noted. This infant died while preparations were being made for cesarean section after the physicians had been watching this pattern for a prolonged period of time.

It should be pointed out that not every fetus will observe the same degree of reserve, so that some infants will exhibit utero-placental insufficiency with relatively mild uterine contractions, whereas other fetuses will exhibit very little response to even marked hypertonus.

Figure 4 is included to show a tracing which was presented in one of our obstetrical conferences. It is typical of the longer tracing which should always be consulted for evidence of progression with passage of time. The pattern is that of variable de-

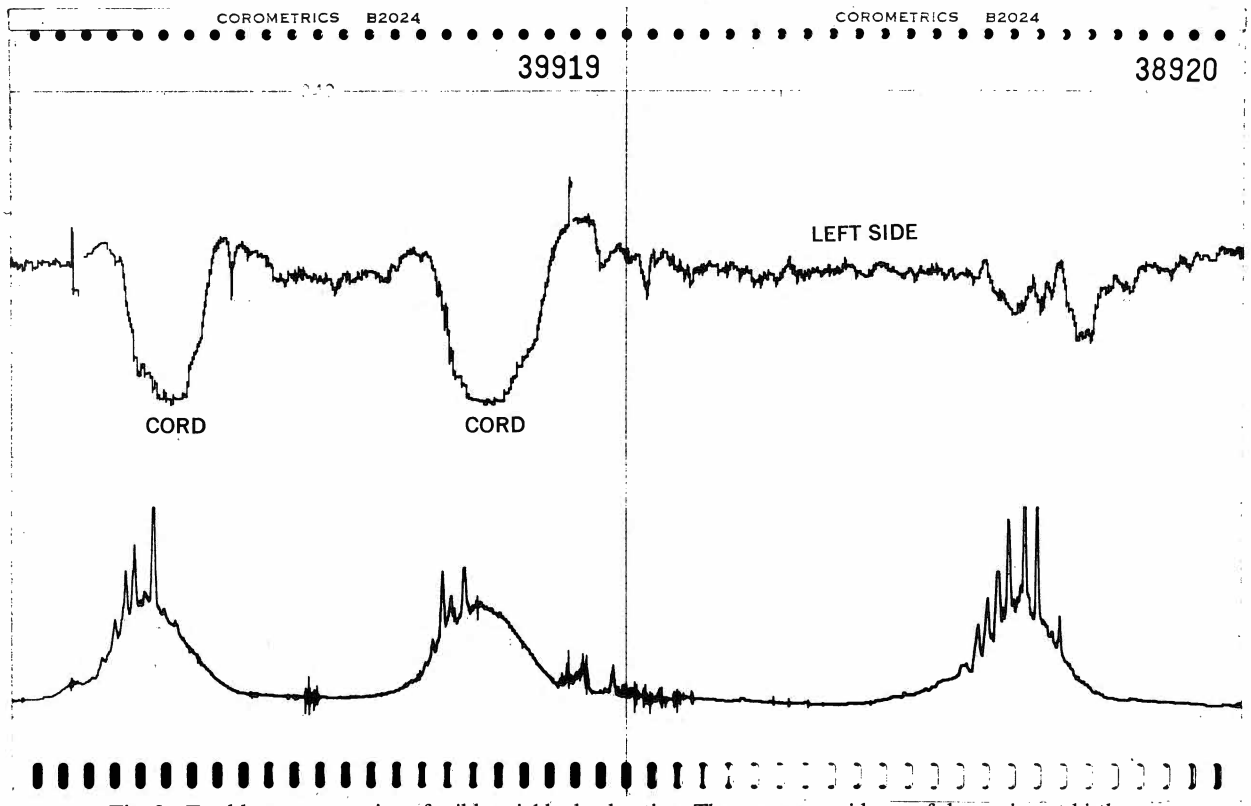

Fig. 2—Fetal heart rate tracing of mild variable deceleration. There was no evidence of depression at birth.

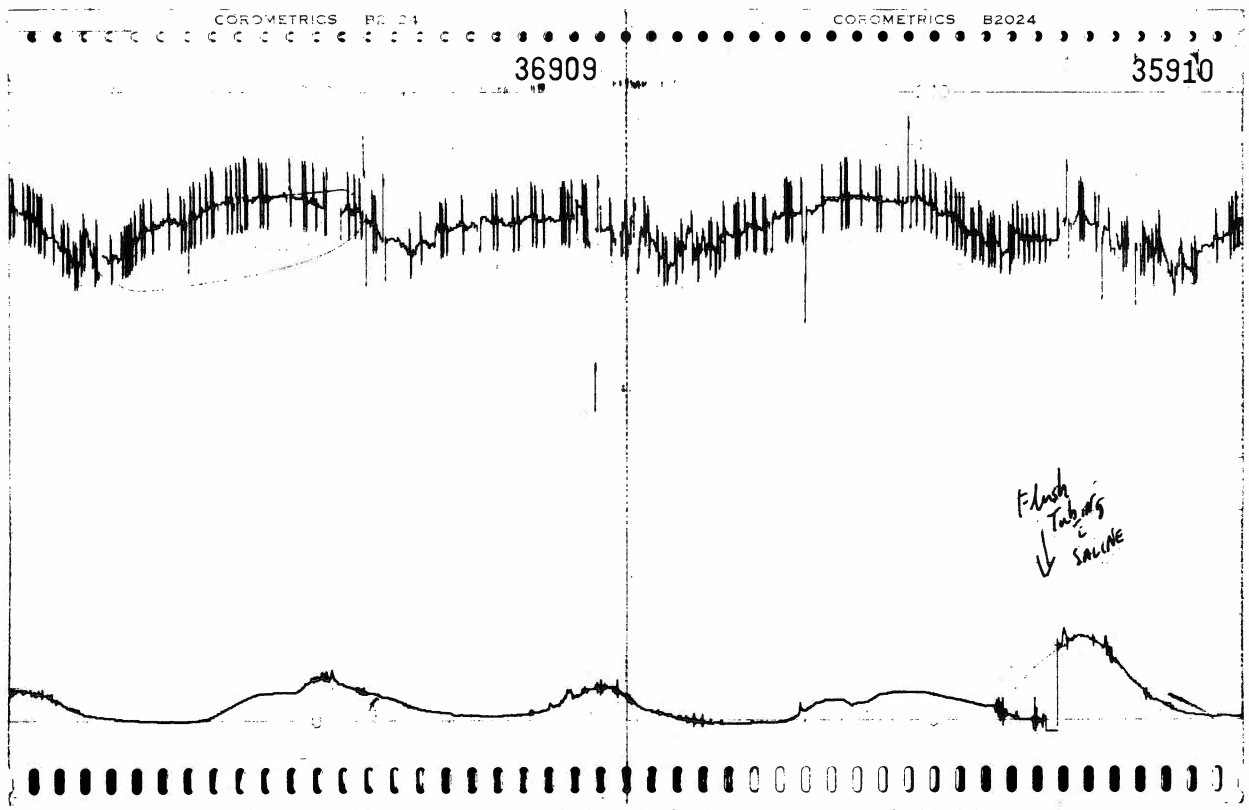

Fig. 3—Fetal heart rate tracing of severe late deceleration. The fetus died in labor.

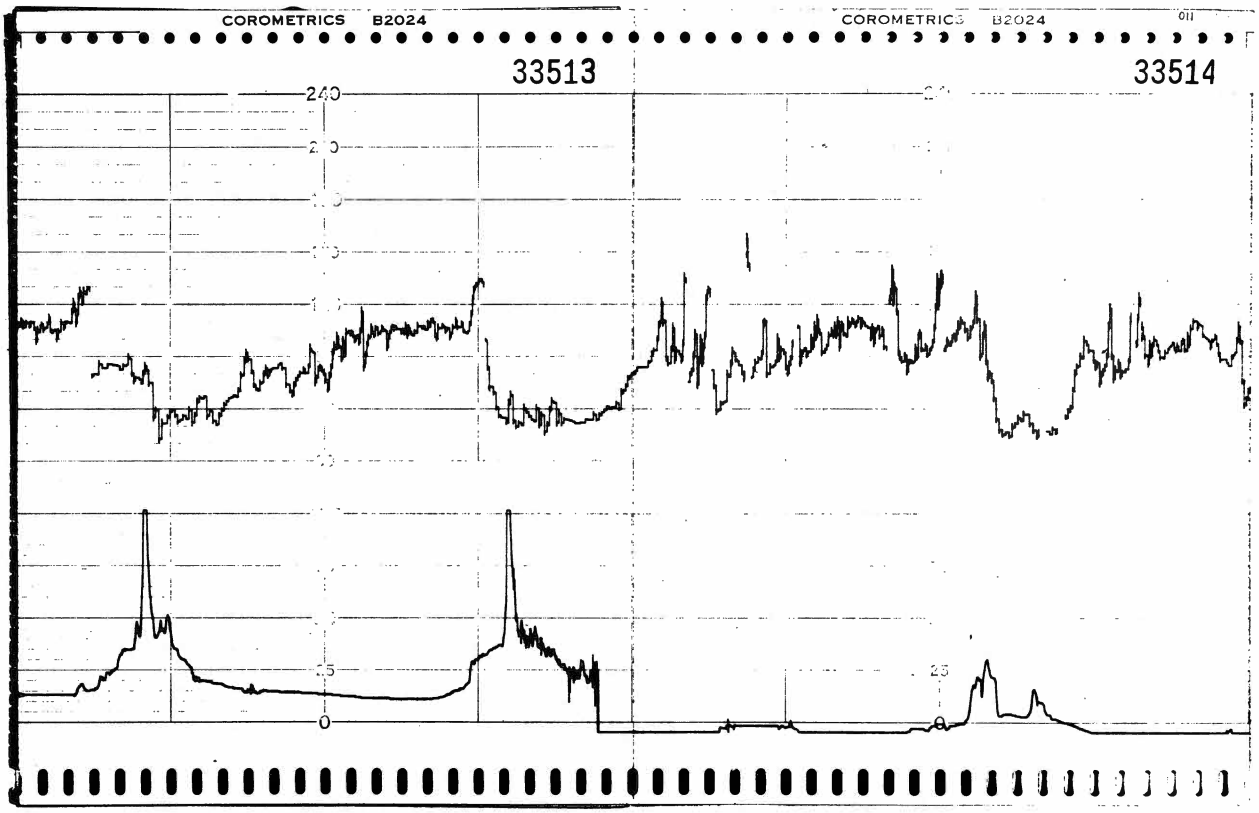

Fig. 4—Fetal heart rate tracing of variable deceleration from tight umbilical cord. There was no evidence of depression at birth.

celeration with a rounding off of the shoulder of the recovery phase. By following this pattern, we saw that there was a prolongation of recovery most consistent with a progressive cord problem. The infant delivered before evidence of anoxia appeared in the chart, without evidence of depression. There was as predicted, a tight cord around the neck.

While I feel that the technology required for electronic monitoring of the fetus has reached a degree of development adequate for clinical use, a few words of caution seem to be in order.

1. The emotions of the obstetrician are treated much more roughly than previously since he is committed to a vigilance which may last several hours in contrast to simply performing a cesarean section which might or might not be of benefit to the mother and fetus.
2. The instruments are only slightly more fool-proof than the obstetricians using them and do have weak links; for example, an integral part of the units utilized at M.C.V. is a Statham® strain gauge. Over a period of 6 weeks, about twelve hundred dollars' worth of these strain gauges were rendered inoperable by improper technique—an occurrence that has not been unique to M.C.V.
3. Reading the tracings requires an active interest and practice which appears to make it difficult to establish the technique in a new hospital unless someone is there to instruct the obstetricians in the technique and to act as a back-up consultant.
4. External monitors and accessories to convert present monitoring units to external monitoring are beginning to appear which will increase acceptability of electronic monitoring. These instruments generally work on the Doppler principle which is utilized in most well-known instruments such as the Doptone® to detect the fetal heart. Though this type of instrumentation has been demonstrated here, we have not seen any which we

consider sufficiently reliable to consider abandoning the use of the scalp electrode. Probably, reliable external monitoring equipment will become generally available in the next two years.

5. The insertion of the amniotic catheter is troublesome and requires some practice. The catheter usually can be inserted and does give accurate measurements of the labor. Monitoring the labor from the abdomen is not as satisfactory. It gives only a rough estimate of intensity and no indications of changes in baseline pressure.
6. The present reliable instrumentation requires rupture of the membranes, which may not always be acceptable. Dilatation of 3–4 cm is also required for easy application and insertion.

What are the benefits?

1. Treatment is made on a more exact diagnosis. It is very true that at this stage the information extracted is relatively crude and that the mechanisms of the changes and their implications for long-term survival and function are only incompletely understood. But the presence of compromise in labor is more accurately predicted than by other methods of assessment. Most of us will see this method of evaluating the fetus become routine for both high-risk and routine labors.
2. The use of electronic monitoring encourages the obstetrician to consider the implications of labor to both the mother and the fetus, rather than just as a period of time which must be passed before an attempt is made to deliver a baby, at whose condition we can only guess.

REFERENCES

CALDEYRO-BARCIA, R., ESCARCENA, L., GULIN, L., ARNT, I. C., AND MENDEZ-BAUER, C. Relationship between blood pH and heart rate in the human fetus during labor. *Am. J. Obstet. Gynec.* 97:4, 1967.

HON, EDWARD H. The classification of fetal heart rate. I. A working classification. *Obstet. Gynecol.* 22:137, 1963.

HON, E. H. AND QUILLIGAN, E. J. The classification of fetal heart rate. II. A revised working classification. *Conn. Med.* 31:799, 1967.

HON, EDWARD H. AND KHAZIN, AIDA F. Observations on fetal heart rate and fetal biochemistry. *Am. J. Obstet. Gynec.* 105:5, 1969.

Femininity and Sexuality*

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In the second of the two McGuire Lectures, Dr. Barnes discussed the nature of femininity and sexuality. He began by pointing out that people usually turn first to the medical profession when seeking help in solving their sexual problems. Though some of these individuals may require psychiatric care, most of them are simply misinformed and need to be reeducated. "We [physicians] need to consider their problems non-judgmentally and not let our own particular attitudes and views intrude too much," said Dr. Barnes.

A contributing factor to this general misinformation about sexuality is the reluctance to acknowledge that sexual intercourse is the most pleasurable physical act one can perform. Mothers rarely convey this to their daughters when discussing sex with them, and teenagers find it embarrassing. Recognizing this, Dr. Barnes declared that he would devote most of his talk to outlining a number of "true things" about the enjoyability of sexual intercourse. Among these are the facts that (1) it is the most pleasurable act one can perform; (2) it is an act of bilateral enjoyability; and (3) this enjoyability is basic to heterosexual relationships. It is also true that the enjoyability of intercourse can be analyzed by dividing it into sexual arousal and orgasm. Doctor Barnes then went on to discuss the process of being aroused, its physiological aspects in both the human male and female, and the physiological nature of sexual orgasm.

Another important "true thing" about the enjoyability of sex is that the pleasure is different in the male and female. This difference in the mental component versus the physical, tactile component in each gender interests Dr. Barnes most. For the male, the process of becoming aroused is overwhelmingly mental. Once he is aroused, it becomes physical. With the female, almost the exact opposite is the case. "Becoming sexually aroused is a matter of being embraced, . . . stroked, . . . kissed, . . . caressed, finding erogenous zones. . . . As soon as [arousal] is achieved, the process becomes predominantly mental.

"But," continued Dr. Barnes, "there is another phenomenon that takes place [which] we . . . lump as appetite. . . . Appetite is [the] desire for sexual contacts . . . that might arouse . . . or that might lead on to orgasm. For the male . . . it is probably correct to use the word 'hunger' because hunger actually has associated with it physical and chemical changes, and in the male . . . the urge to [have] intercourse in the absence of being aroused is based upon the fullness and tension in the seminal vesicles." In the female, this urge is true appetite, defined as being the memory of something that makes one feel pleasant or happy. Through the years the female's appetite for sex increases while the male's decreases, and such divergence in needs requires compromise and adjustment in both partners.

One of the most important "true things" about the enjoyability of sexual intercourse, and the one which usually brings the patient into the physician's office, is that it may be lost or it may never have been achieved. Doctor Barnes feels that there are a variety of ways in which this could occur. The stimulus that is applied is either wrong or inappropriate. For example, too often young people who are going to school while they are trying to hold down a job, or keep up the housework, relegate an act that is basic to their marriage to that part of the day when they are too tired to do anything else. Loss of sexual pleasure may be due to "poor male technique." This is related to the assumption in our society that the male is automatically an expert in lovemaking and that any instruction from the female lowers his status. Or there is the behavior factor. "Behavior as we think we are expected to behave . . . accounts for much of what passes as husband and wife love," says Dr. Barnes. It also accounts for the reason why a

* Summary of the second McGuire Lecture presented on December 3, 1971, at the Medical College of Virginia, Richmond. Doctor Barnes did not wish to have his speech published in its entirety.

woman may lose her enjoyment of sex at the time she has a baby or after a hysterectomy.

Or sexual enjoyment may never be achieved at all. In our society we seem to worship simultaneous orgasm, but Dr. Barnes believes that our real obligation is ". . . for each partner to make sure the other is satisfied." He also feels that, contrary to the popular theory put forth in the manuals on sexual technique, the man should "go slow," ". . . the . . . fundamental woman's obligation in sexuality is to go fast."

Doctor Barnes then addressed himself to those people under thirty as potential parents: "The loss of the enjoyability of [sexual] intercourse is less common today than it ever was before. . . . But in your children we can take another great step forward if we can, in truth, raise them to accept their sexuality without difficulty, without embarressment, without hangups. But I make four suggestions: First . . . will you be not too fixed or rigid in the definition of femininity you hold out before [your daughter]. . . . this is a society that puts a woman somewhat at a disadvantage, and she is adjusting to this role, she is feeling insecure. . . . Second . . . remember that you owe us the obligation of pushing [sex education] on back into the grade schools and into kindergarten. . . . Third . . . find your reasons for the standards you maintain and announce them. But don't leave the implication that [sex] is an unpleasant or a bad act. . . . Fourth . . . raise your daughters with a positive pride in their femininity. It is a wonderful thing to be a woman. Make sure your daughter knows that."

MCV/Q STAFF

Venereal Disease*

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Venereal disease, as we define it, is any disease that is propagated or transmitted by sexual intercourse. Traditionally we have taught medical students that this included chancroid, lymphogranuloma venereum, granuloma inguinale, gonorrhea, and syphilis. Then we taught them enough to be able to match up Donovan bodies, Frei test, Thayer Martin media, and at that point we felt that we had pretty much done our job. If we are to stick to the criteria of diagnosis, I suggest we should also consider as venereal diseases, Trichomonas, incomplete abortions, septic abortions, ectopic pregnancy, unwanted pregnancy, crab lice, herpes simplex virus (type 2), and carcinoma of the cervix. There is considerable disparity among these conditions, but they all have one thing in common: they reflect sexual activity. Trichomonas, on the one hand, may represent sexual activity several years in the past. The trichomonad may lie asymptomatic in the female vagina or the male urethra only to become apparent weeks, months, or even years later. Squamous cell carcinoma, on the other hand, is more like a scoreboard of total sexual activity. Early intercourse, multiple partners, multiple pregnancies are all contributing factors in the development of carcinoma of the cervix. Cervicitis may lie somewhere in between these two extremes and indeed be somewhat related to both.

Currently, gonorrhea is enjoying the most popularity, both in the literature and in the population. We are unquestionably in the midst of an epidemic. Last year in the United States, there were probably two million cases of acute gonorrhea. We developed forty thousand cases a week. One person gets gonorrhea every 15 seconds; every week sixty-five boys returning from Viet Nam have the disease. Most of these are under treatment. More than 25% of patients with gonorrhea are less than 20 years of age; more than 50% of patients with gonorrhea are less than 29 years of age. We are finding that the incidence in the general population is much higher than previously supposed. Screening in OB and GYN clinics across the country have consistently come up with figures of between 5 and 10 percent incidence of gonorrhea in those screened. It can be safely said that one out of every ten girls between the ages of 15 and 25 years of age harbors the gonococcus.

The fact that we have had asymptomatic female carriers has been known for sometime. As a matter of fact, probably 75% to 80% of women with gonorrhea are totally asymptomatic. But they are still infectious. They can still spread the disease. We are just lately finding, much to our consternation, that there are asymptomatic male carriers as well.

In the city of Norfolk, the Public Health Department made a series of studies on male contacts of known female patients with gonorrhea. The result was a raw incidence of 12% positive cultures in asymptomatic men. If you corrected this by ruling out the patients from whom cultures had been taken during the incubation period, you would still have a figure of 6%. In one uncontrolled and unpublished series that was performed here by our Health Department, they found 22 positive cultures in asymptomatic men out of a group of 144 male contacts. I hasten to add that these 22 were not separated out as to whether or not they were in the incubation period. With this large number of asymptomatic patients in both the male and female patient population, the obviously pressing problems are to discover an adequate method of diagnosis and to treat these people.

The first culture technique for the diagnosis of gonorrhea was the use of chocolate agar. This is plated with pus from the urethra, or the cervix, or the anus, and then incubated under 10% of CO_2 at 37°C. With this classic type of culturing, sub-

* Presented at the 43rd Annual McGuire Lecture Series, December 3, 1971, at the Medical College of Virginia, Richmond.

culturing must be carried out as well as sugar fermentations. It is frequently ten days before the diagnosis can be made. Overgrowth is a problem, particularly with proteus; since it may obliterate the gonococcal culture.

The next improvement in diagnostic method was the development of Thayer Martin media. This media initially was chocolate agar with the addition of antibiotics, ristocetin, and polymixin. Lately it is referred to as the VCN plate which contains Vancomycin®, colistin, and nystatin for the suppression of the non-pathogenic bacteria and other contaminates such as staph, strep, and the gram negatives. Thayer Martin media obviously has its advantages, but its chief disadvantage is that in order to be effective it should be plated directly from the patient, and as a result, the specimen frequently loses viability on the way to the lab. Thayer Martin media also has one small drawback due to its inhibitive factor, so that in terms of speed, the gonococcus may take up to three days to grow out, whereas on chocolate agar it will frequently grow out in 24 hours.

Carrying media prepared by Stuart is a liquid in which the specimen can be immersed and shipped to the lab. It is a non-nutrient media, and some cultures are lost because of this, but it has the advantage of protecting the gonococcus from oxidation.

Transgro has recently been developed. This is a combination of a selective gonococcal media that is also a transport media. It is a modified Thayer Martin type media. It differs from the others in that it is put up in a one-ounce medicine bottle with a narrow neck. The media is layered on the side that it forms a flat plate on the inside of the bottle much like a blood culture bottle. Transgro contains an increased amount of agar to make the culture media more rigid for mailing, and it contains a higher concentration of sugar. The bottle has an atmosphere of 10% CO_2 under a rubber screw tip. It can be plated directly from the patient at the time of examination and held at room temperature until ready for mailing. It appears to be a very good media for office practice; however, I would use with caution any new technique, as laboratories will need time to get used to it.

In addition to culturing methods for diagnosing gonorrhea, the fluorescent antibody techniques have been developed. The direct method is done by plating the specimen on a glass slide, overlaying it with fluorescent antibody, and then looking at it under an ultraviolet microscope. The gonococcus will fluoresce. Unfortunately, this will not reproduce the results obtainable with ordinary culture. It is fast but its effectiveness is only in the range of 60%. The *delayed* fluorescent antibody technique is much better. It is done by first plating a chocolate agar slant, allowing the specimen to grow on the slant for 18 hours, and then removing the specimen to a slide. During this time, the gonococcus will multiply in such numbers that when it is visualized under an ultraviolet light microscope one has a much higher incidence of positive results. Thayer Martin media, Transgro, chocolate agar, and the delayed fluorescent antibody technique all have fairly comparable results of being positive in 75% to 80% of known cases.

Who should be cultured? I think that we should culture all prenatal patients because the incidence of gonorrheal ophthalmia in newborns is on the rise. In addition to prenatal patients, I am beginning to believe that we should also culture for gonorrhea everyone who is given a pack of birth control pills; the reason being that anyone needing contraception is obviously sexually active. And we should focus particular attention on the 15- to 25-year-olds seeking contraceptives, as they are the most susceptible group.

Gonorrhea is a serious medical problem. Too frequently we think of it as being a nuisance, but it is more than that. Eighty percent of women are asymptomatic, but we must remember that 10% will develop salpingitis, and 3% will be forever sterile from the disease. The largest cause of infertility in our patient population is unquestionably gonorrhea.

The treatment of choice remains penicillin, for the time being anyway. The minimum treatment for the female is 4.8 million units of procaine penicillin. I might interject here that in our practice, we have hospitalized all patients suspected of having salpingitis or those with systemic symptoms and treated them with high levels of aqueous penicillin, in addition to procaine penicillin. For years, much more modest doses of the drug gave good cure rates. I think the initial recommended dose of penicillin for gonorrhea by the Public Health Department was something like a single shot of 40 thousand units. Now, I am advocating 4.8 million units because of the biologic change in the gonococcus. In 1947, the minimum lethal concentration of penicillin was 0.004 units/ml; in 1957, 0.1 units/ml; in 1967, 1.0 units/ml. This indicates a two hundred and fifty-fold decrease in the sensitivity of the gonococcus to penicillin. You can see by this trend that the time is coming when we can no longer use penicillin, because the capacity of the female

buttock to receive procaine penicillin has been reached. Much of the current emphasis in research is on developing antibiotic alternatives for penicillin. Tetracycline is usually considered a second-line drug. Good results have also been achieved with kanamycin, chloramphenicol, and erythromycin. The combination of ampicillin and probenecid has been used with success, and I might add that probenecid, which decreases excretion of penicillin, may well be advised with the use of procaine penicillin. There is one study in Greenland using a combination of 5 million units of procaine penicillin and one gram of probenecid. With this they have shown that they are able to arrest the decreasing sensitivity of the gonococcus to penicillin.

Benzathine penicillin should never be used to treat gonorrhea. A series of patients given 1.2 million units of benzathine penicillin were checked 24 hours later for circulating penicillin. More than 50% had less than 0.1 unit/ml which is ten times less than the amount necessary to treat gonorrhea, and this may in fact have lead to the desensitization of the disease to the drug.

If gonorrhea is the prima donna of venereal diseases, the spectre lurking in the shadows is syphilis. We are not currently seeing a surge in the incidence of syphilis; however, last year the total number of cases did rise slightly although the rate of new cases did not. Three hundred babies born last year had congenital syphilis. Consider three hundred babies with smallpox, syphilis being the great pox. Two thousand people died last year of syphilis. There are five hundred thousand patients in this country with latent syphilis untreated or undertreated today. That syphilis is not showing the same current increase as gonorrhea is attributed by many to the fact that syphilis may be aborted by the treatment of gonorrhea. One series has attempted to prove that the current recommended dosage of procaine penicillin will abort an incubating syphilis infection. This series, begun in 1957, utilized as its criteria for the cure of syphilis, the fact that the patients were sero-negative and had no lesion of syphilis after three months. I think that we should be a bit cautious about predicting whether or not these patients are going to develop latent syphilis, and there are physiologic reasons for having doubts. We know that we can recover the spirochete from the anterior chamber of the eye and from the cerebrospinal fluid, and we also know that it is extremely difficult to get therapeutic levels of penicillin into these body fluids. A second physiologic fact that makes me question this evidence is that penicillin will kill a spirochete only when the spirochete is replicating. It replicates every 32 hours, but we can assume that every spirochete is not killed the first time. Procaine lasts 72 hours with good levels, so the use of relatively short-acting penicillin like procaine as opposed to the long-acting benzathine leaves one to wonder if all these syphilis infections are cured or if the presenting symptoms are merely delayed or aborted altogether with the possibility of latent syphilis developing.

There has been much speculation, but little has been proven, about the increase in the incidence of venereal disease. It is very easy to say, "Well, more people are doing more things more often than they have been in the past." But it is extremely difficult to prove. There is data to support this. Kinsey, in the 1940's, found that 20% of 20-year-old college women had had premarital intercourse. Vance Packard in the 1960's found that 43% of 20-year-old college females had had premarital intercourse. This increase shows, if it continues along these lines, that 60% of women born this year will have had premarital intercourse by the time they reach 20 years of age. And these are college women. If we add all women, we may well say 90% of women 20 years old will have had premarital intercourse. Now it is quite true that people from higher socio-economic and educational levels have more varied and imaginative sexual lives; however, people from lower socio-economic levels invariably start sooner. The incidence of gonorrhea in the younger age group is high; but not higher now than six years ago. I think that we continue to fool ourselves. We look at the incidence of gonorrhea as we say, "My goodness, twenty-five percent of the patients with gonorrhea are under twenty years of age," and then we forget about it. We come back five years later and say, "My goodness, what are these girls doing!"

Two days ago, the Richmond city newspapers reported that 8% of the girls at a local high school are pregnant, and everyone looked at this with amazement. Well, 8% of those girls have been pregnant for a long time. Seniors in high school are sexually mature girls. I think we must accept these facts. Once a girl has had intercourse for the first time, be it at 14, 15, 16, or 18, she is not going to revert.

Some people blame the current surge in the incidence of gonorrhea on birth control pills. They say that when the condom was used there was a lesser incidence. This is extremely difficult to prove; I haven't seen any condom companies going out

of business lately. It amazes me that a fair number of adolescent women get birth control pills primarily because they don't want to get pregnant. They would not, however, even consider carrying condoms for the same protection. Condoms have some effect in decreasing the spread of gonorrhea, but would you be happy to rest back on condoms for birth control? No one has been, so why should you be satisfied with a condom as a means of controlling the spread of gonorrhea.

There is a current interest in antibiotic vaginal cream. I have a great deal of trepidation about any medication that is antibiotic and therefore antigenic in nature that is persistently rubbed on a mucous membrane. I think this would be an excellent way to sensitize half the population of the country.

The limitations of public education must be realized. The basic idea of alerting the public to the inherent dangers of sexual contact is of academic interest but hardly practical as policy. The increasing sales of tobacco should reinforce that point. It may be of value to disseminate information about the signs and symptoms of venereal disease because it is likely to bring infected patients to medical attention, hopefully before they spread the disease. This is particularly applicable to syphilis because it has a long incubation period during which the patient is noninfectious and may be cured before spreading the disease. Gonorrhea's short incubation period, 4-10 days, makes it difficult for the physician to get to the patient prior to the further spread of the disease even under the best of circumstances.

At this point in time, the most intensive efforts toward education should be made in the medical schools. Very shortly these students will be faced with large numbers of patients with venereal disease, and we must prepare them. Unfortunately, this area in the medical school curriculum has been progressively crowded out by what seemed more important. It is now apparent that even though the prevalence of venereal diseases may wax and wane, they will always represent a sizable medical problem. We must gear medical education and medical reeducation to meet these needs.

REFERENCES

CORNELIUS, C. E. AND DOMESCIK, G. Spectinomycin hydrochloride in treatment of uncomplicated gonorrhea. *Brit. J. Vener. Dis.* 46:212–213, 1970.

MARTIN, J. E., JR., B. A. AND LESTER, A., B. A. Transgro, a medium for transport and growth of Neisseria gonorrhoeae and Neisseria meningitidis. *HSMHA Health Reports* 86: #1, 1971.

MACFAUL, P. A., *et al.* Presence of spiral organisms in the aqueous humor. *Brit. J. Vener. Dis.* 47:159–161, 1971.

MCLONE, D. C., BILLINGS, P. E., AND HARDEGREE, W. E. Gonorrhea in females treated with one oral dose of Tetracycline. *Brit. J. Vener. Dis.* 44:218, 1968.

NICOL, C. S. The sexually transmitted diseases. *Practitioner* 206:277–279, 1971.

NICOL, C. S. Other sexually transmitted diseases. *Brit. Med. J.* 2:448–449, 1971.

PEDERSEN, A. H. B., AND BONIN, P. Screening females for asymptomatic gonorrhea infection. *Northwest Med.* 70:255–261, 1971.

SCHMALE, J. D., MARTIN, J. E., JR. AND DOMESCIK, G. Observations on the culture diagnosis of gonorrhea in women. *JAMA* 210:312, 1969.

SHAPIRO, L. H. AND LENTZ, J. W. Kanamycin as treatment of acute gonorrhea in female. *Obstet. Gynec.* 35:794, 1970.

SHAPIRO, L. H. One day oral ampicillin in young adults. *Obstet. Gynec.* 37:414–418, 1971.

SPARLING, P. F. Diagnosis and treatment of syphilis. *N. Eng. J. Med.* 284:642–653, 1971.

STUART, R. D., TOSHACH, S. R. AND PATSULA, T. M. The problem of transport of specimens for culture of gonococci. *Canad. J. Public Health* 45:73–83, 1954.

THAYER, J. D. AND MARTIN, J. E., JR. Improved medium selective for the cultivation of Neisseria gonorrhoeae and Neisseria meningitidis. *Public Health Report* 81:559–562, 1966.

THIN, R. N., WILLIAMS, I. A., AND NICOL, C. S. (St. Thomas's Hospital, London, England). Direct and delayed methods of immunofluorescent diagnosis of gonorrhea in women. *Brit. J. Vener. Dis.* 47:27–30, 1971.

The VD Crisis. From the International Venereal Disease Symposium, St. Louis, Mo. 1971.

Today's VD Control Problem. Published by American Social Health Association.

Syphilis and Other Venereal Diseases. The Medical Clinics of North America, 48: #3, 1964.

Bacterial and Mycotic Infections of Man. Edited by Dubos and Hirsch, Fourth Edition.