Environmental Health Perspectives
Vol. 74, pp. 67-75, 1987

Biological Markers in Reproductive Epidemiology: Prospects and Precautions

by Zena Stein*†‡ and Maureen Hatch†‡

We begin by defining "biological markers" for the purposes of the present review, distinguishing markers from other types of information, such as subject reports or conventional clinical data. We find the distinctions to be hazy. Next, from the standpoint of epidemiologists, we set out circumstances in which exposure markers might be needed, suggesting requirements for useful markers. We give two instances (lead, PCB), drawn from studies of female reproduction, where the use of exposure markers is compared to environmental or anamnestic data. Effect markers are considered in turn. It is argued that their usefulness (if they are to be more informative than exposure markers) depends on their sensitivity and specificity in relation to the disease outcome. Also, their timeliness, and the use that can be made of the gain in time, for individuals and populations is discussed.

In this context, we consider markers of events before and around fertilization; more specifically, we consider those events that precede the clinical marker of the first missed period. In returning to the potential uses of biological markers in discovering or interpreting female reproductive disorders that might be owed to environmental causes, we compare markers of the pre- and peri-implantation phases with markers of the postimplantation phase, drawing on experience with studies of chromosome anomaly in spontaneous abortion. Finally, we suggest other sensitive reproductive processes for which biological markers might usefully be developed.

Introduction

Epidemiology, in recent years, has developed more and more as a science of methods, a compound of statistics and logic applied to the solution of health problems. Two factors have probably contributed to this. First, today, many exposures that are being studied produce smaller increases in risk and less obvious health effects than those examined in the past, so that increasingly sophisticated manipulation of the data is needed in order to demonstrate those associations that are present. As professional training adapts to cope with these demands, the emphasis on a biological understanding of disease declines among epidemiologists. Second, and perhaps as important, is the influential philosophy expressed by some leading epidemiologists, which challenges "... the common perception that understanding of mechanism is more useful than knowledge of associations," on the grounds that "prevention through control of exposure is often feasible in the absence of knowledge of cellular processes" (*1*).

Other epidemiologists have attempted to struggle out of the shackles of this philosophy, arguing that whenever a researcher has useful clues to the likely mode of action, he or she has a decided advantage in designing research to test and quantify associations. As a result of a stronger design, cause-effect connections may be detected that might otherwise be elusive (*2*). The thrust of the discussion that follows is analysis of the circumstances in which biological markers, by bringing us closer to mechanism, may strengthen epidemiological strategies. These are the "prospects" referred to in our title. The "precautions" in the title reflect experience with an epidemiologic study of aneuploidy involving analysis of human abortus material (see below). In a final section, we consider how an epidemiologist might select biomarkers for exploration, keeping an eye not only on what it is possible to measure in specific areas of reproductive function, but also on which outcomes appear likely to show effects of environmental exposures.

What Are Biological Markers?

We begin with definitions since it is already clear that in the case of "biological markers," as with "molecular epidemiology" and even with "genetics," we are at a Mad Hatter's tea party. Since terms will confound our discussions unless we apply them in the same way, we have listed in Table 1 some uses of terms for which we need general agreement. The first category excluded as a biological marker is measures of the environment. It also seems reasonable to exclude subject reports (although these may be subtle and specialized, e.g., an

*New York State Psychiatric Institute, 722 W. 168th Street, New York, NY 10032.
†Sergievsky Center, Columbia University, 630 W. 168th Street, New York, NY 10032.
‡Division of Epidemiology, Columbia University, 600 W. 168th Street, New York, NY 10032.

Table 1. Definitions of biological markers.

Biological markers are not	Biological markers are
Environmental measures in air, soil, water, food	Material measures obtained from the bodies or excretions of individuals
Subject reports	
Physical, anthropometric, and mental examination	Potentially usable to detect: environmental exposures, effects of exposures
Do biological markers include representations of physiological activity or function? e.g., EEG, ECG, ultrasound, NMR, blood pressure, pulse rate	

Table 2. Uses of exposure markers in epidemiology.

When fact of exposure known, but quality of index or source questionable (details not known, biased recall, memory failure, deliberate misinformation)
To validate other information sources
When environmental data are poor approximations of individual dose (uptake, pharmacokinetics)
To document exposure to target tissue
To quantify the biological load from an exposure

Table 3. Maternal blood lead levels by residence and husband's occupation, province of Kossovo, Yugoslavia.[a]

Site	Blood lead level, μg/dL Mean	Range
Mitrovica		
Husbands in lead industry		
Battery, $n = 28$	21.6	5.2–41.3
Refinery, $n = 8$	17.2	11.8–25.5
Smelter, $n = 16$	16.6	6.9–27.3
Other, $n = 79$	17.4	3.4–39.7
Husbands not in lead industry, $n = 324$	17.1	3.1–56.7
Pristina		
All women tested, $n = 648$	5.4	1.3–23.0

[a] Source: J. Graziano et al., personal communication.

I.Q. assessment is based on questions and responses). Physical and anthropometric measures may be routine or highly specialized, traditional or modern—what excludes them from being "biological markers"? In our categorization scheme, markers include everything the examiner could not hear or see or feel or measure at a routine clinical visit. On the other hand, urinalysis and hemoglobin estimates, widely accepted as biological markers, are simple extensions of the clinician's purview, and what do we do with the noninvasive representations of physiologic activity that we have left undetermined at the bottom of Table 1? Why should an imaging evaluation of function be differentiated from blood or tissue evaluation? For the present, we have excluded only the everyday bedside measures of pulse and blood pressure and included the remaining parameters as biological markers. There is no definitive rationale for these inclusions and exclusions: they are based on convenience and current thinking and are subject to change. Nonetheless, agreement is needed on these usages.

We turn next to a consideration of exposure markers on the one hand, and of effect markers on the other. The distinction between the two seems important, at least in theory, and we have tried to explore the distinction through examples from the field of reproduction (Fig. 1). The differences may become more evident if we take each concept in turn. We begin with exposure markers (that is, biological measurements of internal dose or body burden) and the circumstances under which they serve the needs of epidemiologists (see Table 2).

The first set of circumstances in the table (relating to insufficient details of exposure) is commonly encountered in epidemiology. It should be remembered, however, that while an exposure marker might be useful in this respect, it may not be necessary: careful questioning can often be very informative on the requisite detail. Similarly, although recall bias is an ever-present source of anxiety to the epidemiologist, study design will often handle the problem satisfactorily. Memory failures do occur and increase with lapse of time, but many biological markers fade with time as well. Deliberate misinformation confuses the investigator; however, those who misinform may also be refusers in a testing program that requires informed consent. The precautions may be summarized by warning that one needs to weigh the potential gains carefully before eschewing conventional methods in favor of biological measures.

The second application cited for exposure markers, the need to validate other sources, is a methodological use that is certainly valuable to the epidemiologist, especially one who, for practical reasons, must rely on exposure histories or records in the study population as a whole but could use exposure markers on a small, representative subsample.

The next three uses of exposure markers in epidemiology (the last three entries in Table 2) are of a different order, for they move us toward an understanding of individual exposures that we could not otherwise in all likelihood reach, even from honest, precise, and carefully recalled reports. But what evidence do we actually have that these three 'biological' uses add strength to the arm of the researcher? We illustrate again both the prospects and the precautions.

Table 3 provides data on lead exposure to pregnant women in the province of Kossovo, Yugoslavia, on the Albanian border (Graziano, personal communication). A lead smelter has polluted the area around the town of Mitrovica, affecting not only the workers in the plant but also the resident population. In Pristina, 40 km away, many aspects of life are similar, but there is no pollution by lead. In an investigation headed by Joseph Graziano, we have been studying the effects of intrauterine exposure on the offspring and for this purpose have measured blood lead levels of Mitrovica and Pristina women when they come for prenatal care, usually in midpregnancy. The study includes a series of questions relating to individual exposure, the most useful of

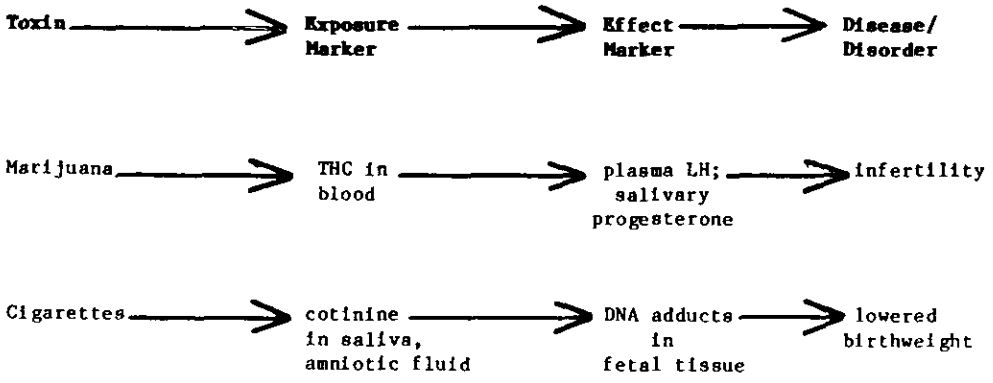


FIGURE 1. Two hypothetical examples of environmental exposure and female reproductive toxicity, showing possible biological markers of exposure and effect.

which are place of residence (Mitrovica or Pristina), and, if Mitrovica, was the husband employed at the lead plant, and, if yes, what was the nature of his work there. (The women seldom worked in the plant.)

Table 3 compares the means and ranges of blood lead levels for women categorized on the basis of the epidemiologic design and the interview data; the table illustrates the added specificity that is gained in this instance from the use of an exposure marker as compared with an exposure gradient constructed from detailed reports. As the data show, while mean blood lead levels for women in Mitrovica, the polluted town, are threefold higher than for Pristina women, there seems to be little additional contribution from the husband's work exposure; in fact, the highest recorded blood level is for a woman whose husband is not employed in the lead industry. The other notable feature of the data is the substantial range in blood levels within exposure categories, indicating that individual variation in absorbed dose is appreciable.

It remains to be seen, as follow-up continues, how useful the biological exposure marker is in identifying adverse effects, but the maternal blood lead has already proven its worth as an index of transplacental exposure. Mothers' serum leads, measured midway through pregnancy and at term, are very highly correlated with umbilical cord blood, so we know the infant's blood reflects the mother's lead burden during her pregnancy, even though the mother's level may have been higher or lower at other times; blood level responds within a few weeks to exposure changes.

We use another example to embody the precautions. Table 4 draws on a very thorough study into the effects on offspring of a mother's ordinary dietary ingestion of PCBs (*3*). Histories of fish consumption (frequency, type, and timing in relation to pregnancy) were taken from Lake Wisconsin women at the time of delivery and scored by the investigators in terms of known PCB content of the species of fish at the time it was eaten. The table compares outcomes among the offspring according to mother's reported total fish consumption, on the one hand, and PCB level in the umbilical cord serum, on the other. Both measures work well, but the edge

Table 4. Birth size and gestational age by cumulative contaminated fish consumption and cord PCB level.[a]

	Fish consumption[b]		Cord serum PCB level[c]	
Parameter	No fish (n = 71)	Fish (n = 242)	< 3 ng/mL (n = 166)	≥ 3 ng/mL (n = 75)
Birth weight, g	3660	3470*	3570	3410*
Head circumference, cm	35.5	35.0*	35.3	34.6*
Gestational age, weeks	39.9	39.2*	39.4	39.5
Maturity score	20.0	18.5*	20.0	19.0

[a] Source: Fein et al. (*3*).
[b] Adjusted for prepregnancy weight, type of delivery, alcohol, caffeine, cold remedies.
[c] Adjusted for infant sex, type of delivery, maternal age, and pregnancy weight gain.
* Significantly different from control value, $p < 0.05$.

is with the reported data, and this we explain on the basis of a finding in the study that it was the mother's consumption before, as well as during pregnancy (that is, the cumulative dose) that precipitated these outcomes; this was a distinction that could not be derived from the biological marker measured only once, in cord blood. We show these data to admonish against a mindless preference for technical measures.

To sum up, for our purposes a useful exposure marker should be superior to a report. It must accurately differentiate exposed from unexposed individuals and should, in general, be noninvasive, acceptable, and applicable on a large scale. Ideally, a marker would provide a memory of cumulative exposure interpretable as time, dose, and duration.

We turn next to effect markers, which for the moment we define as any biologically measurable response to exposure. It is here, perhaps, that the prospects, and the uncertainties, as well, loom larger. If epidemiologists prefer to work one level removed from these uncertainties, then they should leave the matter of effect markers alone. But, if they hold that epidemiology is most effectively used as part of an iterative process to narrow and refine the research questions in collaboration with laboratory and clinical scientists, then they

will try to identify how each group may complement and reinforce the other in an overall program of research (*2*).

We set out once again the desiderata for epidemiologists, in this case for useful effect markers (Table 5). The first requirement, that information on exposure is added to anamnestic data, is the most limited. If this is all an effect marker does, then it is actually functioning like an exposure marker; many of the biological markers in present use (e.g., SCEs, DNA adducts) are of this type. Among the other desirable attributes listed in Table 5, noninvasiveness is again a great advantage (but not an absolute). The effect must be timely, in the sense that it is detectable before the disease supervenes, and it would naturally be highly desirable if with timeliness go prospects for effective interventions, either for the individual or for the community at risk. Stability is quite important. In practice, if a marker does not endure in the individual over some appreciable period of time, it will be less useful than if it does persist, at least until after the exposure is no longer measurable. The requirement that the marker be sensitive to the environment is of course crucial in the present context, and we will have more to say about this shortly.

For now we turn to the issue of prediction. Whether, and how well, a marker predicts disorder in exposed individuals is a measurable characteristic, and it is here that we see room for the inventive epidemiologist to play a part. In Figure 2, we have modelled some of the possible relations among a marker of exposure, an effect marker, and the disease or disorder of concern. First, if exposure and effect markers correspond very closely indeed, there is no benefit in distinguishing one from the other. At the other extreme, if effect markers and signs/symptoms of the disease correspond very closely, then the virtue of using the effect marker resides entirely in its timeliness and the use that can be made of the time. An effect marker that correlates very closely with the disease would not likely improve the chance of detecting cause-effect relationships, although it may add knowledge of process or of precursors. If, however, the effect marker does not correlate closely with the exposure marker and is prognostic of the disease but the correspondence with disease is not absolute, then the gap between exposure, effect, and disease may be fruitfully explored in the hope of identifying individual susceptibility factors or other precipitating circumstances.

AIDS presents us with a paradigm of these relations. Regarding the presence of the virus in the blood as an exposure marker, what use can be made of the time gained by knowledge of infection before the disease becomes manifest? If only a proportion of those infected are destined to get the disease, what are the risk factors for the disease among the infected? In this type of problem, modern, sophisticated epidemiologic models and methods are available.

Table 5. Requirements for useful effect markers.

Adds information to anamnestic data about exposure
Noninvasive
Timely (early)
Points to effective intervention for exposed individuals or groups
Stable
Sensitive to environmental exposures
Predicts disorder in exposed individuals

Areas of Reproductive Function Ripe for Development of Biological Markers

We return now to a consideration of biologic processes that may be sensitive to environmental exposures. In Figure 3, we denote conception, pre-, peri-, and postimplantation of the zygote in the human, marking the number of days from ovulation. On day 15 postovulation, a woman who has a regular menstrual cycle might first suspect a pregnancy. Validated biological markers that fertilization has occurred and that the conceptus has survived at least to implantation have now been provided [for a fuller discussion, see the paper by Canfield and colleagues, (*4*)]. These sensitive new assays permit observations that previously could begin only 1 or 2 weeks later. Thus, the biologic markers of implantation [and perhaps, soon, of conception; see Faulk (*5*)] open up a realm for study virtually unexplored since the classic investigations of Hertig (*6*).

In a similar spirit of excitement and discovery, we began more than a decade ago to explore the distribution and determinants of the aberrant chromosomal forms in clinically recognized miscarriage (*7*). Along with epidemiologic data on parental characteristics and exposures, the products of conception were collected and karyotyped. We draw on that experience now to offer some precautions.

After a decade of research into human aneuploidy, we have come to the view that human chromosomal aberrations, while certainly numerous, varied, and fascinating in their own right, respond rather little, if at all, to the range of environmental influences which we have been able to study (e.g., cigarettes, alcohol, spermicides, occupation) (*8*). It has been possible to document some slight adverse effects on chromosomally normal conceptuses [for instance, cigarette smoking (*9*) and maternal fever (*10*)], but the thousands of karyotyped miscarriages studied have revealed little evidence that the anomalies are sensitive to the environment. The associations that others have reported based on unkaryotyped series of miscarriages are also probably due to a raised risk of chromosomally normal spontaneous abortions since these comprise the majority of miscarriages, and it would take a large increase in chromosome anomaly to raise the overall rate of miscarriage (*11*). Additional support for this view of the relative sensitivity of normal and abnormal conceptions comes from the observation that specific chromosome anomalies vary very little across studies in different populations, whereas chromosomally normal abortions do vary in frequency. The data from our series in New York and

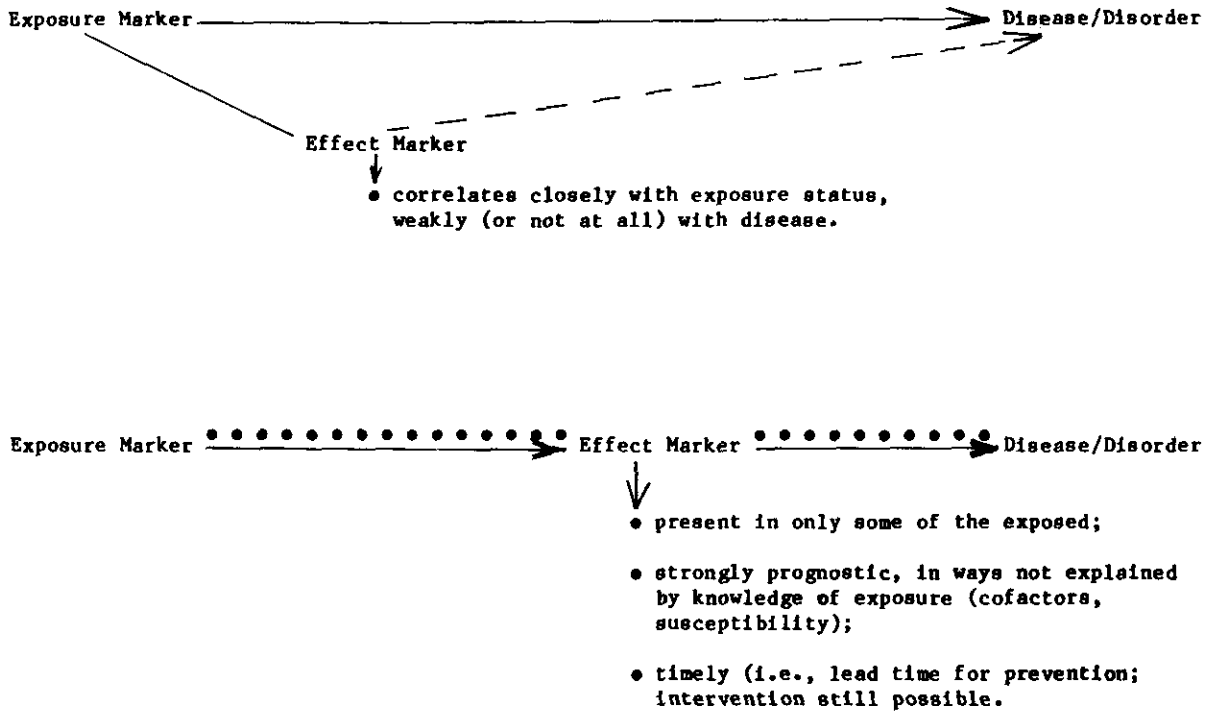


FIGURE 2. Schematic representation of two possible relationships among exposure markers, effect markers, and reproductive disease or disorders. In the first, the effect marker, while a measure of response, is linked closely to exposure but very weakly to disease. In the second, the effect marker is directly in the pathway between exposure and disease.

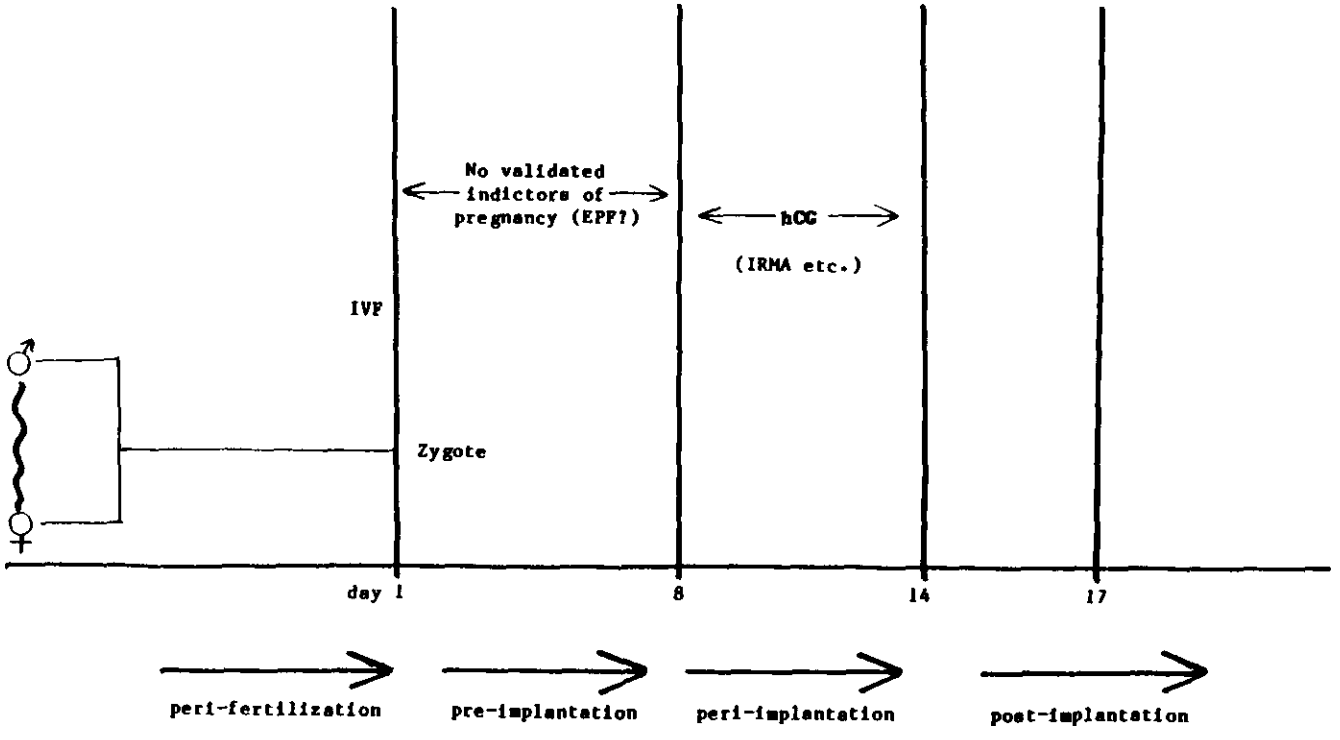
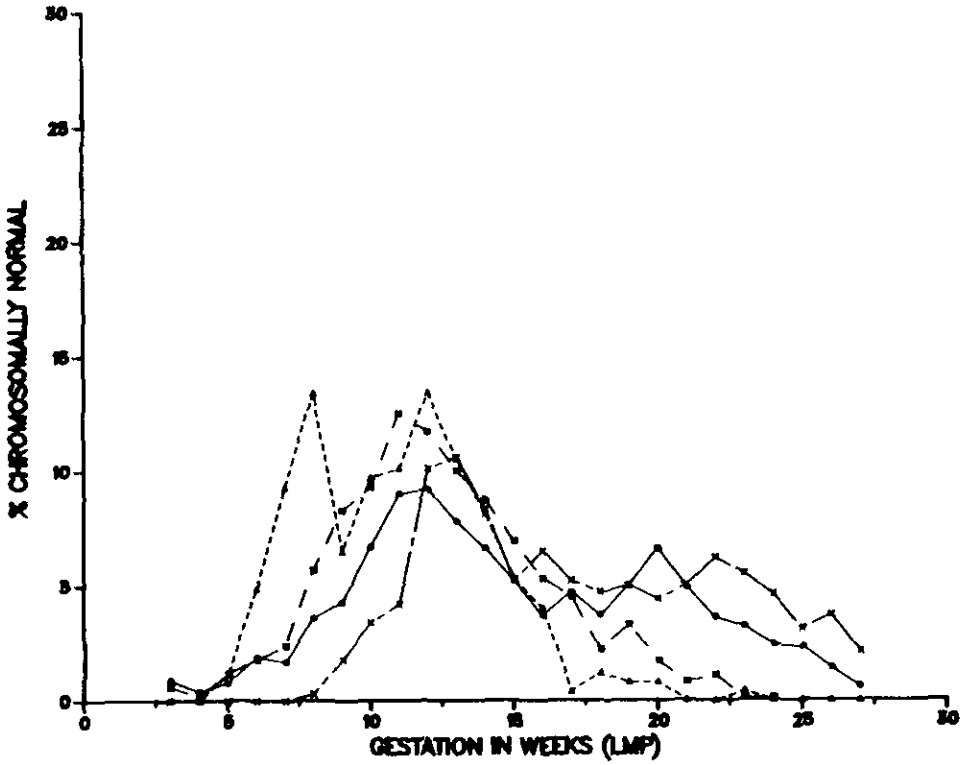
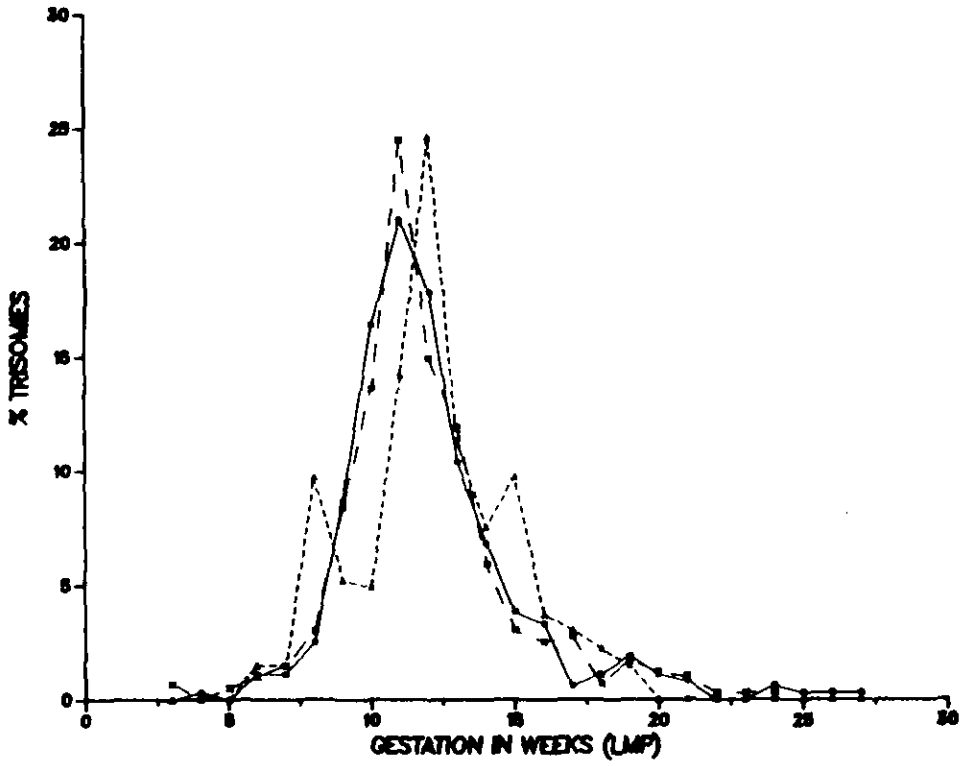


FIGURE 3. Sources of material and biological markers relating to events from the time of fertilization up to the first missed period.

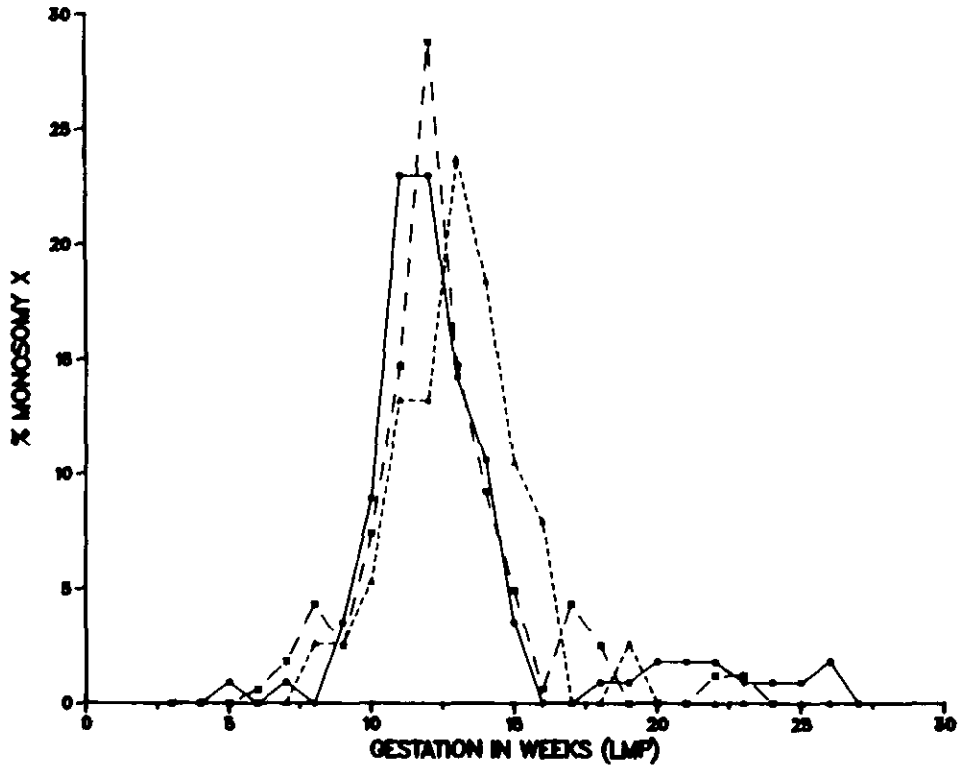


A

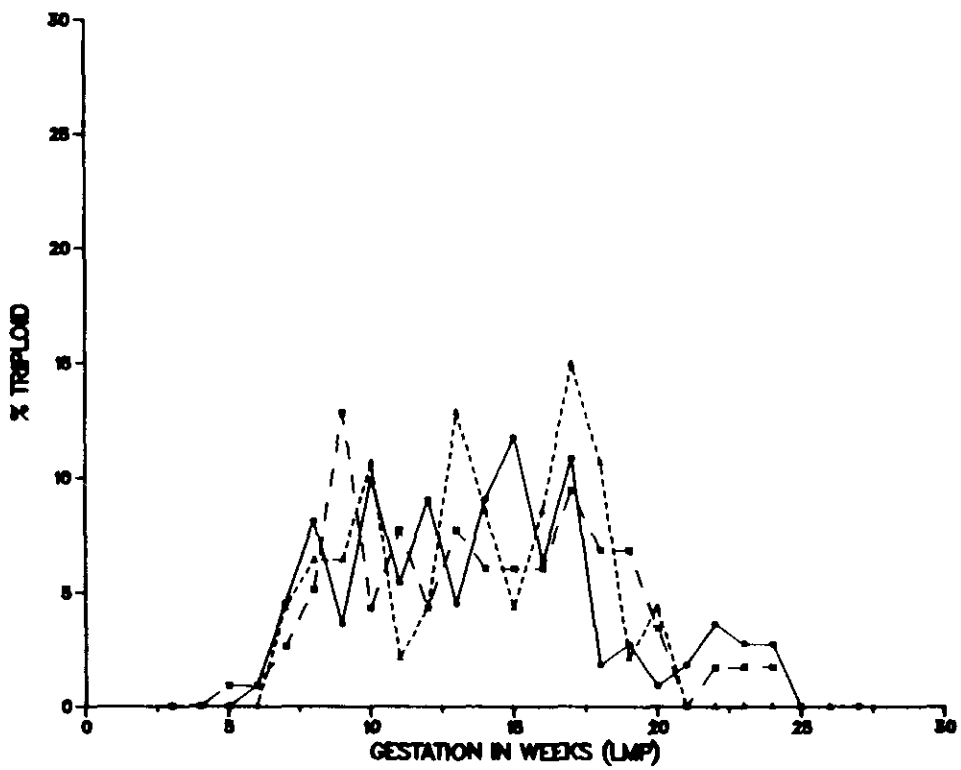


B

FIGURE 4. Gestational age distributions of (*A*) euploid; (*B*) trisomic; (*C*) monsomic X; and (*D*) triploid spontaneous abortions from four series. The series are represented in the graphs as follows: *Hiroshima* (▲); *Hawaii* (■); *New York* (●); *London* (*X*). *Source: Kline and Stein* (*8*).
Figure continued on next page.



C



D

IGURE 4. *Continued.*

for series in London, Honolulu, and Hiroshima are shown in Figure 4.

How do these inferences about recognized miscarriage bear on observations of very early losses (peri- and early postimplantation losses that would seldom enter a series of hospitalized miscarriages)? It is entirely too soon to say with any certainty, but we may speculate, first, that the majority of very early losses are chromosomal anomalies, and second, if this is so, that environmental influences on this distribution will probably not be marked.

There are several lines of evidence in support of the first point (that the majority of very early losses are chromosomally abnormal). First is the observation that in recognized miscarriage (in spite of some irregularities, possibly artifactual), the prevalence of chromosome anomaly generally increases as gestation time of the abortus decreases (*12*). Second, there is the theoretical argument that many chromosome anomalies which are believed to occur are presently unaccounted for and so are presumed to be lost very early on. For example, monosomy might be as frequent as trisomy (that is, there would be a missing chromosome for every supernumerary one), but monosomies are observed only half as often trisomies in recognized pregnancies; ergo, the missing monosomies could have been lost in the preclinical period. There is evidence in the mouse to suggest that this is so (*13*). In addition, there are trisomies of specific chromosomes that are underrepresented in recognized pregnancies and so are presumptive early losses (*14*). Chromosomal studies would be required to establish this with certainty, but these studies are at present technically infeasible.

On the second point (that the likelihood of environmental influences will be less, the greater the concentration of chromosomal anomalies) there are indications that the experience in very early loss will parallel that of clinical miscarriage. For example, a review by Baird and Wilcox of the toxicologic literature (*15*) found that preimplantation loss was induced by much the same exposures as caused postimplantation loss. It seems unlikely that in the human a new and distinct set of causes of environmental origin will specifically, and only, cause peri-implantation loss. Of course, this can only be confirmed empirically. Moreover, it will be interesting and important to establish the natural history of very early loss, even apart from the question of environmental influences.

By contrast, ovarian function is an aspect of female reproduction that does apparently reflect environmental factors. There are several epidemiological pointers to suggest this is so. We know, for instance, that there are environmental effects on menarche related to nutritional factors (*16*). Extreme physical exertion (*17*), starvation (*18*), and psychic strain (*19*) have been shown to alter ovulation in women with established cycles, and there are data suggesting that exposure to solvents (*20*), metals (*21*), estrogens (*22*), and chemotherapeutic agents (*23*) induces menstrual disorders.

Contemporaneous cigarette smoking is associated with an earlier menopause (*24*). Is this finding connected with the observation that contemporaneous cigarette smoking delays conception in couples attempting a pregnancy? We do not know if cigarette smoking impedes ovulation or induces very early (preclinical) loss. However, a recent study documents in biological detail the antiestrogenic effect of women's smoking (*25*), and a similar pathway for estrogen metabolism has been shown for malnutrition and weight loss (*26,27*), factors which do disturb ovulation.

We might be able to establish the underlying cause of smoking-induced conception delay and perhaps discern new associations if the epidemiology of ovulatory failure could be studied using biological markers. For several reasons (e.g., applicability to nonconceptive women) ovulation would be easier to study in populations than early pregnancy if an acceptable noninvasive marker of ovulation were available. While irregular menses is a usual accompaniment of ovulatory failure, this is not always the case, and it would be a great advantage to have a marker. Measures of progesterone in urine (*28*) and saliva (*29*) have been bruited. A heat-sensitive device, more sophisticated and acceptable than a rectal thermometer, would be a useful tool.

Anovular cycles indicate a hormonal cause, a somatic change, rather than one that is genetic or chromosomal, within the genome. Here we venture a generalization: there is more evidence that the environment induces damage to the somatic cells—in the woman, mediated through the endocrinal system, and in the embryo, through the placenta—than that the environment damages the genome of subsequent generations. This seems to be one of the lessons from experimental toxicology. Then, for delayed conception, as with smokers, we should first suspect irregular ovulation; if there is very early loss (of presumably chromosomally normal conceptuses), we might suspect hormonal inadequacy. However, hormonal inadequacy will be difficult to demonstrate without large numbers if, as we predict, most very early losses are chromosomally abnormal.

We know that observable chromosomal anomalies (at miscarriage and at term) seem related to maternal age (*30*) in a seemingly constant fashion and not, insofar as they have been studied, related to any of the usual environmental influences. These observations fit with laboratory findings: oocytes can be induced to atrese, anomalies of development can be induced during pregnancy, but prepregnancy exposure of the female cannot easily be demonstrated to result in miscarriage or genetic birth defects. In the female (mouse as well as human), the stored eggs seem to be exceptionally well insulated, even against the myriads of new products being synthesized daily and released into our environment.

In summary, we know through epidemiology that the following reproductive functions are responsive to environmental factors: the onset of the menarche; ovulation, for those with established cycles; the menopause; and the development of the chromosomally normal embryo and fetus. In terms of uncovering new environ-

mental hazards to female reproduction, the epidemiologist would be well served by the development of biological markers for these events, especially if markers can be designed to incorporate the desirable attributes enumerated earlier.

REFERENCES

1. Maclure, K. M., and MacMahon, B. A. An epidemiologic perspective on environmental carcinogenesis. Epidemiol. Rev. 2: 19–48 (1980).
2. Hatch, M. C., and Stein, Z. A. The role of epidemiology in assessing chemical-induced disease. In: Mechanisms of Cell Injury (B. Fowler, Ed.), John Wiley, Chichester, England, 1987, pp. 303–314.
3. Fein, G. G., Jacobson, J. L., Jacobson, S. W., Schwartz, P. M., and Dowler, J. K. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. J. Pediatr. 105: 315–320 (1984).
4. Canfield, R. E., O'Connor, J. F., Birken, S., Krichevsky, A., and Wilcox, A. J. Development of an assay for a biomarker of pregnancy and early fetal loss. Environ. Health Perspect. 74: 57–66 (1987).
5. Faulk, W. P., Coulam, C. B., and McIntyre, J. A. Reproductive immunology: biomarkers of compromised pregnancies. Environ. Health Perspect. 74: 119–127 (1987).
6. Hertig, A. T., and Rock, J. A. A series of potentially abortive ova recovered from fertile women prior to the first missed menstrual period. Am. J. Obstet. Gynecol. 58: 968–993 (1949).
7. Kline, J., Stein, Z., Strobino, B., Susser, M., and Warburton, D. Surveillance of spontaneous abortions: power in environmental monitoring. Am. J. Epidemiol. 106: 345–350 (1977).
8. Kline, J., and Stein, Z. Environmental causes of aneuploidy: why so elusive? In: Aneuploidy: Etiology and Mechanisms (V. L. Dellarco, P. E. Voytek, and A. Hollaender, Eds.), Plenum Press, New York, 1985, pp. 149–163.
9. Kline, J., Stein, Z., Susser, M., and Warburton, D. Environmental influences on early reproductive loss in a current New York City study. In: Human Embryonic and Fetal Death (I. H. Porter and E. B. Hook, Eds.), Academic Press, New York, 1980, pp. 212–240.
10. Kline, J., Stein, Z., Susser, M., and Warburton, D. Fever during pregnancy and spontaneous abortion. Am. J. Epidemiol. 121: 832–842 (1985).
11. Kline, J. Maternal occupation: effects on spontaneous abortions and malformations. Occup. Med. State Art Rev. 1: 381–403 (1986).
12. Warburton, D., Stein, Z., Kline, J., and Susser, M. Chromosome abnormalities in spontaneous abortion: data from the New York City study. In: Human Embryonic and Fetal Death (I. H. Porter and E. B. Hook, Eds.), Academic Press, New York, 1980, pp. 261–287.
13. Ford, C. E., and Evans, F. P. Nonexpression of genome unbalance in haplophase and early diplophase of the mouse and incidence of karyotype abnormality in postimplantation embryos. In: Les Accidents Chromosomiques de la Reproduction. (A. Boue and C. Thibault, Eds.), INSERM, Paris, 1973, pp. 271–285.
14. Stein, Z., Stein, W., and Susser, M. Attrition of trisomies as a maternal screening device: an explanation of the association of trisomy 21 with maternal age. Lancet i: 944–947 (1986).
15. Baird, D. D., and Wilcox, A. J. Effects of occupational exposures on the fertility of couples. Occup. Med. State Art Rev. 1: 361–374 (1986).
16. Frisch, R. E. Influences on age at menarche. Lancet i: 1007 (1973).
17. Jacobs, H. S. Amenorrhea in athletes. Br. J. Obstet. Gynecol. 89: 498–499 (1982).
18. Knuth, U. A., Hull, M. G. R., and Jacobs, H. S. Amenorrhea and loss of weight. Br. J. Obstet. Gynecol. 84: 801–807 (1977).
19. Swigar, M. E., Naftolin, F., and DeCherney, A. H. Psychogenic factors in amenorrhea. In: The Gonadotropins: Basic Science and Clinical Aspects in Females (C. Flamingi and J. R. Givens, Eds.), Academic Press, New York, 1982, pp. 259–283.
20. Cai, S. X., and Bao, Y. S. Placental transfer, secretion into mother's milk of carbon disulfide and the effects on maternal function of female viscose rayon workers. Ind. Health 19: 15–29 (1981).
21. De Rosis, F., Anastasio, S. P., Selvaggi, L., Beltrame, A., and Moriani, G. Female reproductive health in two lamp factories: effects of exposure to inorganic mercury vapour and stress factors. Br. J. Ind. Med. 42: 488–494 (1985).
22. Harrington, J. M., Stein, G. F., Rivera, R. V., and de Morales, A. V. The occupational hazards of formulating oral contraceptives: a survey of plant employees. Arch. Environ. Health 33: 12–14 (1978).
23. Warne, G. L., Fairley, K. F., Hobbs, J. B., and Martin, F. I. R. Cyclophosphamide-induced ovarian failure. N. Engl. J. Med. 289: 1159–1162 (1973).
24. McKinlay, S. M., Bifano, N. L. and McKinlay, J. B. Smoking and age at menopause in women. Ann. Intern. Med. 103: 350–356 (1985).
25. Michnovicz, J. J., Hershcopf, R. J., Naganuma, H., Bradlow, H. L., and Fishman, J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N. Engl. J. Med. 315: 1305–1309 (1986).
26. Anderson, K. E., Kappas, A., Conney, A. H., Bradlow, H. L., and Fishman, J. The influence of dietary protein and carbohydrate on the principal oxidative biotransformations of estradiol in normal subjects. J. Clin. Endocrinol. Metab. 59: 103–107 (1984).
27. Fishman, J., and Bradlow, H. L. Effects of malnutrition on the metabolism of sex hormones in man. Clin. Pharmacol. Ther. 22: 721–728 (1977).
28. Samarajecwa, P., Cooley, G., and Kellie, A. E. The radioimmunoassay of pregnanediol-3α-glucuronide. J. Steroid Biochem. 11: 1165–1171 (1979).
29. Zorn, J. R., McDonough, P. G., Nessman, C., Janssens, Y. and Cedard, L. Salivary progesterone as an index of the luteal function. Fertil. Steril. 41: 248–253 (1984).
30. Hook, E. B. Rates of chromosome abnormalities at different maternal ages. Obstet. Gynecol. 58: 282–285 (1981).