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# **Malnutrition and Infection During Pregnancy**

**Determinants of Growth and Development of the Child**

**Agency for International Development  
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MALNUTRITION AND INFECTION DURING PREGNANCY

Determinants of Growth and  
Development of the Child

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## PREFACE

The Subcommittee on the Interactions of Nutrition and Infection of the Food and Nutrition Board, National Academy of Sciences/National Research Council held a symposium on Malnutrition and Infection During Pregnancy, at INCAP in Guatemala during January 1974. Scientists at the symposium reviewed existing knowledge and called attention to subjects which deserve further research.

In an attempt to share the results of the workshop with other scientists engaged in research on maternal factors which influence human growth and development, the Agency for International Development, Office of Health, Technical Assistance Bureau; and the National Academy of Sciences, Food and Nutrition Board, are pleased to make these papers available.



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## Malnutrition and Infection During Pregnancy: Determinants of Growth and Development of the Child

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### Editors' Comments

Organization of this workshop was suggested by the Subcommittee on the Interactions of Nutrition and Infection of the Food and Nutrition Board, National Academy of Sciences/National Research Council (NAS/NRC). The meeting was convened in Guatemala City at the Institute of Nutrition of Central America and Panama (INCAP) in January 1974 as the first of many events marking the 25th anniversary of the founding of the institute. We were very pleased to participate in this workshop, because each of us has had a long and close relationship with INCAP. The success of the meeting, documented in these pages, is a tribute to this great institution of nutritional research where many of the ideas to which the workshop addressed itself originated.

The following persons participated in the workshop: Juan Rodolfo Aguilar, MD, División de Nutrición Aplicada, INCAP; Javier Aguja, MD, Departamento de Pediatría, Hospital General del IGSS, Guatemala City; Charles A. Alford, Jr., MD, Department of Pediatrics, University of Alabama in Birmingham; Victor Argueta von Kaenel, MD, Hospital General San Juan de Dios, Guatemala City; Guillermo Arroyave, PhD, División de

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Presented before the symposium held at the Institute of Nutrition of Central America and Panama, Guatemala City, Jan 10-12, 1974.

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## Introduction

Moisés Béhar, MD

The Institute of Nutrition of Central America and Panama (INCAP) was created 25 years ago as a research and advisory organization in the field of human nutrition to serve the countries of the Central American Isthmus.

Our first efforts in defining the nutritional problems of the population were directed primarily at school-age children. It took us a few years to realize that at that age most of the effects of malnutrition have already taken place and were irreversible. Hence, our studies were reoriented to preschool children from 1 to 4 years

of age. Working primarily with overt clinical cases of severe malnutrition, we learned that the critical age was the first two years of life; that inadequate diet interacting with frequent infections was the important mechanism responsible for malnutrition, and that overt clinical cases of severe malnutrition were only the readily visible part of a much greater problem. Although death and diseases were extreme manifestations of the problem, functional and behavioral alterations were perhaps no less important.

Current research indicates that we have not given enough attention to the important earlier formative periods, intrauterine life, and the period of lactation. We have, therefore, moved from the study of specific age groups to an interest in the total span

of man's life, from conception to adulthood.

It is most opportune to initiate the year of our 25th anniversary with a workshop devoted to possible adverse effects of problems occurring during intrauterine life. The main purpose of this workshop will be an analysis of information on the role of two important environmental factors, *nutrition* and *infection*, on the development of the human organism during intrauterine and extrauterine life.

The growth curve for weight of babies beginning 12 weeks after conception and continuing for the first year and a half of life is sigmoidal, as is true for most biological growth. The period of greatest acceleration of weight gain is from about three months before birth to about three months after birth. Consequently,

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Reprints not available.

birth is an incident that occurs at the middle of this important period.

Genetic factors have been considered the most important determinants of fetal growth and development because the uterus, the placenta, and the fetal membranes offer excellent protection against malnutrition and infection. To some extent this is true, but nevertheless many infections do occur in the fetus.

There is also experimental evidence in animals that severe nutritional deficiencies, particularly of vitamins, may induce serious teratogenic effects. Calorie and protein deficiencies in the pregnant mother also have a detrimental effect on growth, and possibly on development of the offspring.

Epidemiological observations in man in times of disaster suggest that severe calorie deprivation during pregnancy impairs fetal growth. However, controlled studies in industrialized countries fail to show significant correlation between nutritional status of the mother and birth weight of her baby. Such studies have reinforced the theory of "parasitism," that is, that the fetus takes from the mother whatever it needs for adequate growth and development—even at the expense of the mother's tissues and reserves.

Thus, low birth weight in developing nations has been attributed to genetic factors; nutritionists have not paid much attention to the problem. With the growing concern in chronic,

subclinical malnutrition, so prevalent in these areas, the effect of mild to moderate maternal malnutrition on intrauterine and subsequent growth and development remains largely unknown. It is this effect that will be analyzed in this workshop.

In a similar manner, there is evidence for the teratogenic effects of certain infectious agents on the embryo and fetus. However, studies of the possible effects of common infections of the pregnant woman in terms of fetal growth and development are less numerous and have received less attention. Nevertheless, this is particularly important in underdeveloped countries where the rate of infection in the general population, including pregnant women, is very high. The possible interaction of malnutrition and infection must be considered. Maternal and intrauterine infections may not only reduce fetal growth, but could also affect future growth and even more importantly interfere with adequate functional development; this damage may be subtle and therefore difficult to detect and to relate to causal phenomena, but not necessarily less important. We must ask ourselves how important it is, in terms of future performance, to weigh a few hundred grams more or less at birth. There is enough evidence to demonstrate that within the normal range of birth weight (that is, when the variation is primarily genetic and not influenced by environmental constraints) *there*

is *no* correlation between birth weight and future performance. Is the situation the same for babies whose low birth weight is due to the combined effects of malnutrition and infection? We do not know the answer, although there is clear indication that very low weight affects at least one gross measurement of future performance, the ability to survive.

We should examine subtle functional alterations in low-birth-weight babies and their importance in terms of future performance. Birth weight may be very important as an indicator of inadequate development, but we must remember that growth is only a gross indicator. Development is a complicated process with a definite chronology of *differentiation* and *maturation*, while important *structural* and *functional* alterations can take place without substantial changes in growth. We must define the possible effects of unfavorable environmental circumstances in which the majority of babies in underprivileged population groups are born, and determine whether they contribute unfavorably to the future health and well-being of the children. Finally, if there is a problem, we must decide what can be done to correct it.

Indeed, INCAP is very pleased to open its 25th anniversary year with this meeting. I hope that it turns out to be as useful to you as I know it will be for us at INCAP.

# Nutritional Individuality

John E. Gordon, MD, PhD

By way of introduction to this workshop, I choose to speak on the nutritional individuality of affected population groups. In so doing, I view some results that are not so latent, and by placing these causes among other agents instrumental in deficits of growth and development of young children, I reflect on the manner and the extent to which they interact.

In developing countries where childhood malnutrition is common, most children are breast-fed. They do well for the first four months or so, with gains in height and weight comparable to children whose nutritional situation is more favorable. The difficulty comes with the beginning of weaning and continues until transition to an adult diet is completed, which in general is long postponed. The result is that the first two or three years of life are the critical period.

Older preschool children have usually proved easier to enlist in nutritional intervention programs than the littler ones. As the principal source of nutrients for the younger preschool population, nursing mothers logically were added to the target group, although rarely rating equal attention. The current outgrowth of nutritional policy to include the pregnant mother and fetus is a logical evolution of a combined attention to mother and child.<sup>1</sup>

As valuable as cumulating evidence suggests that move to be, attention to fetal nutrition in no sense substitutes for postnatal action, and for good reasons.<sup>2</sup> Nutritionally, the first year or two of life mark in major degree a continuum of fetal conditions, a transitional stage to be sure, but with maintained dependence on a largely

maternal environment for food and protection, and a social adaptation in food habits and otherwise that permits successful coexistence with fellow humans.

During gestation the mother of course provides the whole of the physical and biological environment for the fetus. Maternal anthropometric, psychological, pathologic, and obstetric characteristics have long served to express the nature of that environment. Recognition grows, however, that the third classical component of environment, the sociocultural, also provided by the mother, has equal significance if not an overriding importance in development of the fetus. Poverty commonly governs food supply, education determines the direction of health maintenance and the management of illnesses, and the extent of child training and the experience derived from preceding pregnancies affect the care afforded the as yet unborn. Social and economic influences acting on the mother are thus reflected directly or indirectly on the fetus. The resulting biological and social complex has been termed the "matroenvironment."

In a search for contemporary influences, for fresh incitants of malnutrition or other human difficulty, the social environment thereby gains in priority. These circumstances extend the activity of the maternal environment past fetal life in a process of postnatal adaptation in which the mother is the dominant influence.

No assumption is offered that the process ends there. It lessens as the individual gains in his own experience and, in combination with a continuing maternal influence, progressively attains an ability for formal logical operation by about age 12 years. Beyond that, the astute individual continues to profit from parental counsel.

In considerations of malnutrition, growth, and development, the addition of the prenatal period to the crit-

ical first three years of life marks progress. All too commonly deficiencies originate during gestational life, which makes child health more than a concern of those already born. In most instances the underlying causes are much the same: too little or inadequate food, acute infectious diseases, and low birth weight.<sup>3</sup> With onset early in that period, evidence exists that retarded growth and development are past full relief, that the damage done is likely irreversible.<sup>4</sup> In the best of circumstances, medical care takes the place of the more decisive preventive approach.

The Chinese acknowledge the reality of this initial stage, that life begins in utero; the child is recognized as 1 year old at birth.

## Maternal Environment

With the whole of external influences concentrated in the mother—physical, biological, and social—the maternal environment attains in gestation an importance unmatched at any other time in human development. Parturition may impose a heavier strain on that environment; the child also encounters a set of external risks from which it was previously isolated; and yet critical as it is, the period is relatively brief. Following birth, the physical and biological environments impinge directly on the child instead of being mediated through the mother. The responsibility for protection and guidance in the socialization by which the infant acquires his own ability and experience continues to rest, however, in the maternal environment—and for a longer time than at any other stage.

**Gestation.**—During gestation of the fetus, the environment provided by the mother was long regarded as a shelter against potential risks; the nutritional reserves of the mother might suffer, but not those of the child. The exaggerated frequency of low birth weights in regions with a prevailing malnutrition establishes

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the fallacy of that view. Mild infections of the prototype of rubella, themselves essentially innocuous to the maternal host, may elicit a devastating fetal effect. In general, maternal infection induces a nutritional deficit in both organisms, and all too often specific infectious diseases extend the harm to both.

If nothing else, early fetal losses through spontaneous abortion, crudely estimated at half of all pregnancies, suffice to establish a high degree of risk. As a later manifestation, the stillbirth rate is itself high. How much of fetal wastage is attributable to faulty genetic inheritance and how much to the impact of malnutrition, infectious disease, and other biological influence within the maternal environment is not known.

The infectious diseases, the other illnesses, and the malnutrition so largely responsible in technically underdeveloped regions for a poor preparation for pregnancy continue as active determinants of an insecure result in that event, with the added consideration that they react on the developing fetus.

An assessment of maternal qualities requires a backward look at the childhood upbringing of the mother herself. Origins are both remote and immediate. Faults in early growth and development, and continuing deficits thereafter, are reflected among other things in short stature and an excess frequency of small-for-date babies; thus, there is a continuing cycle, transmissible in series, to be remedied only when women enter pregnancy in an adequate nutritional state. Age at pregnancy, short intervals between births, and racial differentials are basically biological, yet so strongly admixed with social elements as to rival the second major feature of the maternal environment, the social setting to which the child is introduced.

Accruing knowledge has enlarged the concept of social environment in disease causality past conventional social, cultural, and economic influences to include a fourth behavioral component. Conviction has grown that the biological and reproductive causes so largely resident in malnu-

trition and infectious disease have a coordinate effect in early childhood on mental as well as physical growth and development.<sup>6</sup> Psychological measurements designed to establish the added effect supported earlier observations that the behavioral environment itself possessed definitive capacities in origin of growth disturbances, particularly the so-called failure-to-thrive and the battered-child syndromes. The result was recognition of adequate caretaking in early childhood as a reasonable and necessary complement to measures directed toward control of biological influences. How much of a deleterious effect on the child in utero arises from family crises and their inherent stresses, from disturbed parental relations, or maternal addiction to alcohol, drugs, and tobacco remains problematical. They do act in postnatal life, and likewise so do such limitations as young parental age and inexperience, weaning practices, and the lack of mutual contribution by parents in socialization. Food habits established in childhood commonly set the pattern for adult life.

The expression "socioeconomic" is evidence in itself of the weight accorded the economic component of the social environment. Few established causes of failure in growth and development, including malnutrition and infectious disease, escape a strong financial association. Special economic challenges exist in the working mother, in welfare systems and welfare recipients, in food prices, and in the policies and practices that guide food purchases. Basic to all are the lessons of history, that so many health difficulties (to name only two, diphtheria and tuberculosis) have yielded to economic advances and an improved way of life long before specific preventive measures became available.

**Perinatal Period.**—From the abrupt transition at parturition, mother and child emerge as independent organisms, now sharing exposure to common external surroundings but with highly different responses, because stimuli act selectively on the two hosts and some more forcefully than others. The child now encounters a

whole set of influences from which it was previously isolated. Others are a carry-over from gestation, notably a small birth weight. Developmental anomalies may prove incompatible with new calls on physiological function. Birth injuries during delivery may interfere with nutrition.

The mother encounters her own particular difficulties in an abnormal pregnancy, or in multiple births, each with its attendant complications. In puerperal sepsis, she too finds a problem of infection. Her indispositions furthermore react on the infant.

Most of the risks during the perinatal period are reproductive and biological, which is not to discount such social influences as the quality of available obstetric care, the prevailing poverty, large families, birth order of the child, and stresses arising from social casualties.

Judged by deaths, the first birthday of a child is the most hazardous he will ever face. Granted recovery, the quality of survival in developing regions of the world is sufficiently impaired that it contributes heavily to later malnutrition.

**Postnatal Period.**—Child development has its own objectives, variously physical, mental, and behavioral, but all stand in relation to food supply. For infant and toddler, food supply draws heavily on the maternal environment. During many months and in many areas, breast feeding makes that relationship exceedingly close; and the dependency on maternal care in food preparation continues throughout early childhood. A further connection is that food habits and customs instilled in childhood, whether good or bad, tend to persist into adult life. The responsibility lies mainly with the mother. Mimicry of older siblings contributes, yet all too often this represents no more than what they too have learned through maternal influence. Perhaps nothing is more inhibitory than a sick, a disinterested, or an ignorant mother. A fair index of maternal competence is to be had from the number of deaths among siblings, and also their food behavior.

Errors in behavioral development relating to food commonly trace to

three sources. Some are inadvertent, a product of the mores of the community. Others are due to deficient knowledge of weaning practices and the principles of nutrition. Some failures refer directly to behavioral faults: family crises, entanglement with the law, transversion of accepted societal rules, or addiction to drugs or alcohol. Overwhelmingly, they all relate to poverty, poor education, and provincialism. The more serious are those that end in separation, desertion, or divorce, and most of all, the death of a parent.

**Mortality.**—Progress in lowering death rates has been from older ages toward younger. Second-year death rates in Britain began to decline 40 years before infant mortality. In developing countries the proportion of postneonatal to neonatal deaths of the first year is about 3:2; in industrial regions the ratio is 1:2. Perinatal deaths in the United States are four times those at any other age.

**Morbidity.**—Such information as exists relates mainly to more serious disease, with little known of effects from the milder and more common conditions and from latent infections without discernible signs or symptoms.

**Pregnancy Wastage.**—The extent of losses from spontaneous abortion is not accurately known. Those from induced abortion are of course increasing. Causes and consequences need further study. In developing regions, stillbirths are still confused with live births.

**Low Birth Weight.**—Conditions are well defined for hospital populations. For open populations and especially in developing countries where incidence is excessive, data on frequency and cause are sparse.<sup>7</sup>

**Congenital Anomalies.**—In developing regions, even deaths from this cause are poorly recorded, and developmental consequences still less so.

**Postpartum Amenorrhea.**—The relative contributions of biological and social determinants are poorly understood; duration of amenorrhea and contraceptive effectiveness are practical questions for planned parenthood. Closely spaced pregnancies bring high infant mortality.

**Lactation.**—How much the vicissitudes of gestation affect subsequent performance in lactation is largely uninvestigated in general populations, despite its evident importance in growth and development.

#### Applied Prevention and Control

The outstanding objective is prevention to the extent feasible and practical, and reasonable control if that must suffice.<sup>8</sup> Because growth and development are progressively evolving characteristics, field investigative procedure profits most from long-term prospective observations. Field study is important because there is no substitute for contact with reality, which is to be found in the homes of people. Prevalence data yield facts about a population at a selected time that may or may not be representative. Retrospective study starts with the observed event as the independent variable, with cause the dependent item. That is reversed emphasis.

With such close interaction of biological and social determinants, there is need for multidisciplinary effort, not so much through concerted group activity as individual effort by authorities skilled in a particular field. The same principle holds in the organization for action. Planning for in-

tervention is a matter of priorities, of feasibility, practicality, potential accomplishment toward a defined goal, and the sympathy and cooperation of the population concerned. Therefore, it brings together the skills of physician, biologist, sociologist, anthropologist, political scientist, business manager, and economist. Implementation of the program is best delegated to a specified agency suited to the eventual schedule, but proper evaluation, so often omitted, demands an assessment of separate aspects: biological value, educational profit, cost-benefit analysis, and the ultimate contribution toward a basic goal of social and economic progress.<sup>9</sup> Again, expertise prevails.

And the individuality of nutrition? It is the extent to which it penetrates all facets of human life.

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# Placental Function and Malnutrition

Richard E. Behrman, MD

An appreciation of the physiologic mechanisms that play a major role in the transfer of substances across the placenta is basic to understanding the capacity of the placenta to respond to the stress of maternal malnutrition during pregnancy and what the nature of this response may mean for the developing fetus.

In general, the homeostatic responses of the mother are geared primarily to her survival. For example, many maternal physiologic adjustments are not only in a direction that will not prevent an untoward change for the fetus, but may even result in a change clearly unfavorable for the fetus. This is exemplified by vasoconstriction of the uterine circulation by catecholamines released into the maternal blood.

Alternatively, the biological capacity of the fetus to adjust to changes in the maternal environment and to maintain an optimal fetal environment is to a considerable degree dependent on and limited by placental mechanisms for maintaining relatively constant conditions for the transfer of nutrients and gases between the maternal and fetal blood flowing through the placenta. Thus, a key measure of placental function is the rate of placental transfer of a substance per unit of concentration difference between maternal and fetal plasma, known as the "diffusion capacity" of the placenta for the substance.<sup>1</sup> The diffusion capacity of the placenta for a number of substances increases proportionally with fetal weight during pregnancy. The trophoblastic surface area of the placenta grows during gestation proportional

to fetal weight. At term it is approximately 3 to 4 sq m/kg. In comparison to the human lung, the placental membrane has a larger surface-to-body-weight ratio and is much thicker and metabolically more active.

Uterine blood flow increases enormously during pregnancy, most of it going to the cotyledons. The critical physiologic factors producing and potentially controlling this increase in man are unknown. Similarly, there is a progressive increase in umbilical blood flow with fetal age, with most of this flow (94%) also perfusing the placental cotyledons. There is a parallel increase in fetal blood pressure and the growth of the vascular bed of the placenta. The critical factors controlling changes in normal umbilical blood flow during gestation are also unknown. Mild changes in the partial pressure of respiratory gases have little effect on the umbilical vascular bed.<sup>2,3</sup> However, moderate fetal hypoxia and acidosis result in decreased umbilical blood flow, decreased fetal cardiac output going to the placenta, and increased shunting of umbilical vein blood into the fetal inferior vena cava, bypassing the liver.<sup>4</sup> The vascular mechanism by which the flow of umbilical vein blood returning from the placenta is distributed between the fetal liver and vena cava may be particularly relevant for fetal nutrition. Although many potential nutrients are carried by the umbilical vein, metabolism of the brain and limbs of the fetus is predominantly dependent on glucose. Presumably, the nonglucose nutrients are stored and converted in the liver under normal circumstances. Chronic hypoxic stress that substantially alters the continuing distribution of the umbilical bloodstream to the liver vs the systemic circulation might substantially change the pattern of substrates presented to the hepatic tissues.

The critical mechanisms involved in transfer functions of the placenta are best illustrated in terms of the concept of placental clearance, which is a function of the specific permeability of the placental membrane to a substance and the magnitude of the fetal or umbilical and maternal or uterine placental blood flows.<sup>5</sup> If the placental permeability to the diffusion of a substance is very low, the clearance in this instance can be characterized primarily as *diffusion limited*. The clearance becomes increasingly higher for substances to which the placental membrane is progressively more permeable. At some maximal value at which the permeability no longer limits the rate of transfer of a particular substance, the transfer of this substance would become *flow limited*. Experimental evidence in sheep and monkeys indicates that tritiated water and antipyrine are substances whose clearance represents the maximum flow-limited clearance.<sup>6</sup> This transplacental flow-limited clearance is approximately 100 ml of blood per minute per kilogram of fetal body weight. Thus, changes in the composition of maternal plasma lead to a rapid change in fetal plasma. The half time of equilibrium is less than ten minutes. In contrast, the clearance of urea is to a large degree diffusion limited. In regard to substances that are diffusion limited because of relatively low placental permeability, it must be kept in mind that the composition and molecular structure of the placenta is not static through gestation.

In terms of these mechanisms it is possible to indulge in some speculations about placental function during maternal malnutrition. Transfer of glucose has been characterized as facilitated diffusion, whereas transfer of amino acids involves an active metabolic process. If these tend to act like

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substances whose transfer is also flow limited to a considerable extent, then nutritional and, perhaps, infectious factors that have an untoward effect either directly or indirectly on the development and function of umbilical or uterine vascular beds may be more

critical for normal fetal growth and development than actual deficiencies in the transfer of specific nutrients to the fetus, or the transfer of an abnormal pattern of nutrients or metabolites or both. This hypothesis is consistent with observations of the basic

similarities in the cellular, histologic, and chemical pattern of human intrauterine fetal growth retardation associated with a wide variety of clinical problems.<sup>6</sup>

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## Nutrition of Pregnant Women in a Developing Country—Thailand

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The commonly held belief that the birth weight of a human infant is determined solely by genetic and racial differences was challenged by a World Health Organization (WHO) technical report that showed that birth weight varied within a number of ethnic groups in relation to the socioeconomic status of the mother.<sup>1</sup> Later studies showed that the nutritional status of pregnant women as reflected by base line body weight and weight gain during pregnancy had a positive correlation with fetal growth.<sup>2-5</sup>

Our studies in Chiang Mai, Thailand, were designed to assess the nutritional pattern of pregnant women. In comparison with the diet of women in the industrialized societies, the Thai diet is generally low in total calories, protein, calcium, and iron (Table). These deficiencies are similar

to those reported from other developing countries, for example, India.

Examining specific nutrients, we noted that intake of vitamins A, B<sub>12</sub>, niacin, and ascorbic acid was also deficient in comparison with that in the industrialized societies and substantially below that recommended by WHO for pregnant women. Thiamine intake, on the other hand, appeared adequate. This may be illusory, however, because the primary source of calories among pregnant Thai women is carbohydrate, which leads to a higher demand for thiamine, and therefore may result in relative thiamine deficiency. Indeed, cases of infantile beriberi are still seen in our population.

Although clinical signs of thiamine deficiency were observed in only 7% of the pregnant women, 20% had 24-hour urinary thiamine excretion of less than 40 $\mu$ g. Moreover, 13% excreted less than 40 $\mu$ g of riboflavin per day. Twenty percent had serum folate levels below 4 mg/ml and 6% had serum vitamin B<sub>12</sub> levels below 150 pg/ml; 6% were deficient in vitamin E, with serum levels of 0.4 mg/100 ml.

In addition, vitamin A deficiency was noted both in pregnant women and newborn infants.<sup>6</sup> In Chiang Mai, this deficiency is especially common in cases of protein-calorie malnutrition, and almost certainly is a result of early deprivation of vitamin A in infants fed breast milk low in vitamin A. However, the original endowment of the fetus with vitamin A during pregnancy is probably also deficient. Venkatachalam et al<sup>7</sup> showed that serum vitamin A levels of pregnant women decreased with each consecutive trimester. The cord blood levels of vitamin A were quite low in all groups.

Hemoglobin values less than 11 gm/100 ml were found in 21% of pregnant women, and 3% had values of less than 9 gm/100 ml. Serum iron levels below 50 $\mu$ g/100 ml were noted in 63%, and in 40% the serum iron binding capacity was in excess of 45 $\mu$ g/100 ml.

Pregnant women in rural areas in Thailand consume inadequate nutrients and are by all reasonable criteria malnourished. The average birth weight of 2.9 kg (6.4 lb) is lower than

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Reprints not available.

Comparison of Daily Intake of Selected Nutrients by Pregnant Women in Several Countries				
	Calories	Protein, gm	% Calories From Protein	Calcium, gm
Scotland (Aberdeen)	2,354	72	12.2	0.9
United States (Tennessee)	2,200	75	13.5	1.1
Holland	2,770	81	12.0	0.9
India (Calcutta)	1,920	48	10.0	0.5
Thailand (Soong Nern)	1,980	40	8.0	0.5

that in the industrialized world, and the infant mortality remains quite high. Since maternal nutrition is an important factor influencing the well-being of the newborn infant, it is mandatory that special attention be paid to proper nutrition of pregnant women to supply them with nutrients that easily traverse the placenta, for example, iron, protein, and water-soluble vitamins. Substances such as vitamin A, which have a relatively poor placental transfer rate, should be administered directly to infants postnatally.

### Conclusion

Nutritional pattern of pregnant women in Thailand was evaluated and compared in selected respects with that in other countries. The Thai diet was found to be deficient in vitamin, protein, calcium, and iron and was similar to diets reported from other developing countries, but distinctly inadequate in comparison to those in the industrialized societies.

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# Nutrition in Pregnancy in Central America and Panama

Guillermo Arroyave, PhD

The objective of maternal diet is provision of sufficient nutrients to maintain mother and fetus in good health, to support an adequate flow of breast milk without detriment to maternal nutritional reserves, and to maintain maternal health between pregnancies.<sup>1</sup> Daily dietary recommendations for Central American women are presented in Table 1.<sup>2</sup> The recommended dietary pattern for pregnant women is different from that for nonpregnant women, and the values are set at the upper level of the distribution curve of requirements. Most individuals consuming them would, in theory, be amply nourished.

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Although the consumption of more energy than is actually spent is undesirable, the recommended caloric intake for pregnant women is set to provide for storage of about 36,000 kilocalories during pregnancy as adipose tissue reserves. This is because "a safe level of energy intake is a basic requirement to insure satisfactory nutrition for the fetus and breast-fed infant."<sup>3</sup>

A diet adequate for nonpregnant women must be increased by 17% during pregnancy to satisfy the increase in required calories and nutrients, especially calcium, proteins, ascorbic acid, folate, and vitamin B<sub>12</sub>. The gap between prepregnancy and pregnancy diets can often be filled by foods readily available. For example, in rural villages in Guatemala, four spoonfuls of cooked black beans, two

tortillas, one-half ounce of cheese, one-half tomato, and one leaf of cabbage would nearly accomplish the task, except for vitamin A, which would be inadequate, and niacin, which would be marginal. Under these ecological conditions, the gap in vitamin A can be filled only by supplementation.

### Dietary Patterns and Nutrient Intake in Pregnancy

In two rural ladino villages of Guatemala, an increase in food consumption (corn and vegetables) during the last two trimesters of pregnancy was determined by 24-hour recall dietary intake surveys (Table 2) (M. Flores et al, unpublished data). This represents an increment in the caloric intake of about 400 calories per day; however, the intake of calo-

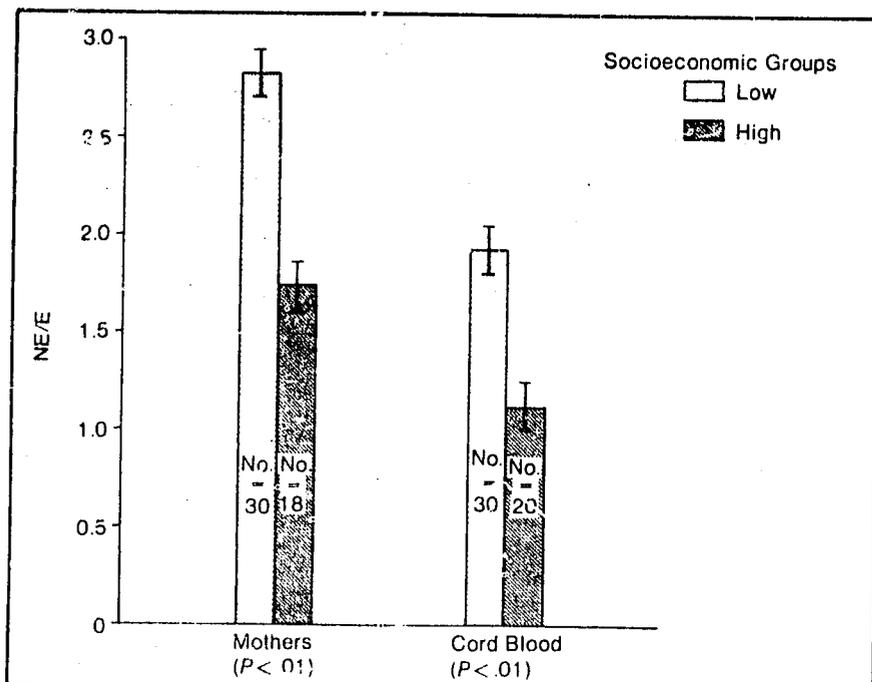


Fig 1.—Ratios of serum nonessential (NE) to essential (E) amino acids in Guatemalan mothers and newborns of two socioeconomic groups, measured at delivery.

Table 1.—Daily Dietary Recommendations\*

Nutrient	Pregnant* (2nd & 3rd Trimester)	Non-pregnant	Allowance for Pregnancy	% Increase
Energy, kilocalories	2,400	2,050	350	17
Protein, gm	60	45	15	33
Calcium, mg	1,100	450	650	144
Iron, mg	28	28	...	...
Vitamin A, $\mu$ g	900	750	150	20
Thiamine, mg	1.0	0.8	0.2	25
Riboflavin, mg	1.3	1.1	0.2	18
Niacin equivalent, mg	15.8	13.5	2.3	17
Ascorbic acid, mg	50	30	20	67
Folate (free), $\mu$ g	400	200	200	100
Vitamin B <sub>12</sub> , $\mu$ g	3.0	2.0	1.0	50

\* More than 18 years old.

Table 2.—Adequacy of Dietary Intake\*

Nutrient	Intake			
	P. egnant			Lactating (No. = 36)
	1st Trimester (No. = 20)	2nd Trimester (No. = 57)	3rd Trimester (No. = 57)	
Energy, kilocalories	1,418	1,723	1,819	1,599
Protein, gm	39	50	54	58
Animal protein, gm	8	7	9	10
Calcium, mg	768	967	1,012	897
Iron, mg	17	17	20	21
Riboflavin, mg	0.68	0.71	0.79	0.58
Thiamine, mg	0.81	0.99	1.07	1.03
Vitamin A, mg	0.47	0.53	0.75	0.34
Vitamin C, mg	36	29	39	13

\* For a low socioeconomic, rural population in Guatemala.

ries was insufficient for even a non-pregnant, nonlactating woman. There was consistent decrease in food intake in the lactating women in comparison to those in the third trimester of pregnancy. Calories, riboflavin, vitamin A, and vitamin C were all affected. The reasons for this are unknown, but the change in dietary pattern may have a cultural basis.

The question of whether dietary habits of women change during pregnancy has been investigated by Arroyave et al in Guatemala. They surveyed 14 pregnant women from high and low socioeconomic groups. The women in the high socioeconomic group change their dietary intake during pregnancy by introducing more milk, eggs, fruits, and vegetables and by reducing cereals and fats. In contrast, few women in the low-income group change their intake habits. Although an increase in mean intake was recorded with advancing pregnancy, this was accounted for by the improved food intake of only a few of the women. With respect to diet within households, the presence of pregnant or lactating women reduces the adequacy of the diet for the whole family.

#### Biochemical-Nutritional Characteristics

Studies on the nutrition of pregnant and lactating women carried out by the Institute of Nutrition of Central America and Panama (INCAP)<sup>14,15</sup> show that only 40% of women of low and medium socioeconomic levels have adequate intakes of riboflavin. Red blood cell levels of riboflavin, serum levels of vitamin A and carotene, and hemoglobin and hematocrit values are also substantially lower in these women. Evidence of inadequate protein intake as disclosed by a low ratio of serum valine to glycine (Table 3) has been verified in a group of pregnant women from a low socioeconomic rural ladino village.<sup>16</sup> Newborn infants of these mothers also showed this alteration, indicating a direct effect of maternal nutrition.

Two groups of urban Guatemalan mothers of low and high socioeconomic levels were matched for age, parity, interval since previous delivery, and absence of severe disease

during pregnancy; they all had uncomplicated, full-term pregnancies resulting in male newborns. Studies at INCAP<sup>7</sup> showed that urinary excretion of urea per gram of creatinine was adequate in 12 of 14 high-socioeconomic-group women, but in only 16 of 26 of those in the low socioeconomic group. Adequate urea excretion was set at 4 gm/gm of creatinine. Since the creatinine excretion per 24 hours is about 1.0 gm, excretion of less than 4 gm of urea nitrogen per 24 hours indicates inadequate protein intake. An immediate biochemical consequence is the elevated plasma ratio of nonessential to essential amino acids in women of the low socioeconomic group (Fig 1). The amino acid ratio of the newborns in this group is also severely affected.

Nutritional inadequacies of protein and other nutrients are reflected in physical measurements. Weight for height and tricipital skinfold thickness of women from low socioeconomic groups are substantially lower than those in women from high socioeconomic groups.<sup>7</sup> This indicates that a deficit in calories is present. Creatinine excretion per 24 hours is a reflection of total muscle mass. For comparative purposes we have chosen an arbitrary reference point, the median value for the high socioeconomic group. Twenty-four-hour creatinine excretion in 22 out of 29 low-socioeconomic-group women fell below the reference value. This could be due to differences in height, since averages were 150 cm (59 in) for the low and 162 cm (64 in) for the high socioeconomic groups. Expressing creatinine excretion per centimeter of body height to correct for this variable does not alter results, thus indicating a relative protein depletion in the low socioeconomic group.

If the caloric reserves (adipose tissue) were the same in both groups, one would expect the creatinine coefficient (milligrams of creatinine per kilogram of body weight) to be lower among women of low socioeconomic class. However, 74% were above the set reference point. This indicates that the lower weight/height ratio of such women is due partly to decreased protein mass, but even more

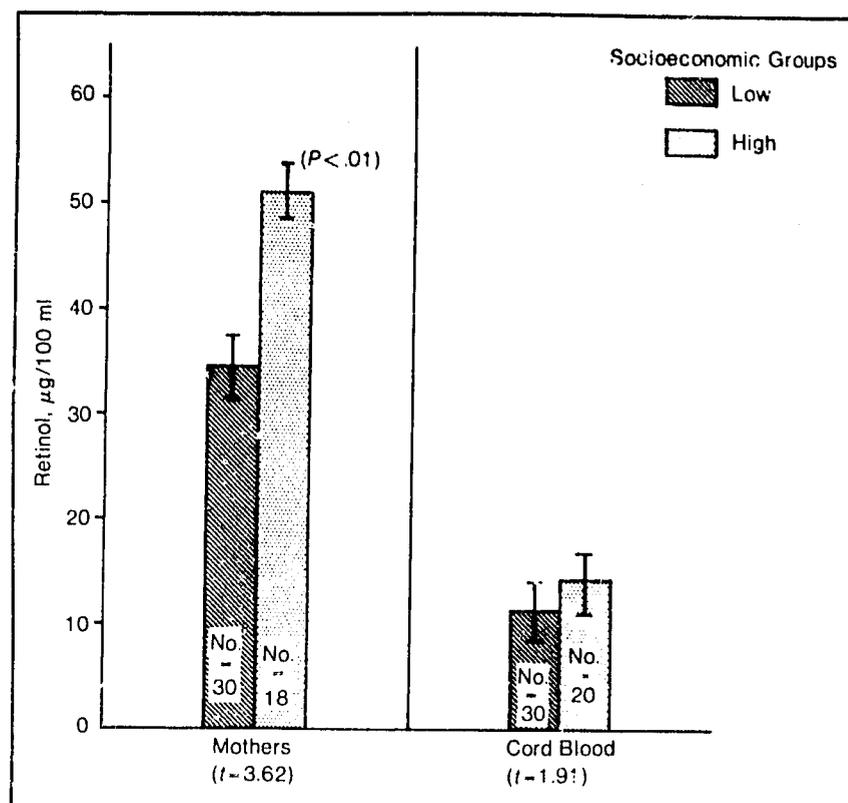


Fig 2.—Average serum retinol levels ( $\mu\text{g}/100\text{ ml}$ ) in maternal and cord sera in two socioeconomic groups, measured at delivery.

Table 3.—Plasma Valine and Glycine Levels in Population Groups Having Different Nutritional Characteristics

Group	No.	Valine, mg/100 ml	Glycine, mg/100 ml	Ratio
1. Pregnant women, Guatemala City, UIU*	5	1.491	1.210	1.298
2. Pregnant women, San Antonio la Paz, LIR*	6	0.998	1.599	0.636
3. Nonpregnant women, San Antonio la Paz, LIR	7	1.447	2.643	0.587
4. Newborn children, Guatemala City, UIU†	5	2.392	2.531	0.947
5. Newborn children, San Antonio la Paz, LIR‡	6	2.002	2.970	0.710
6. Well-nourished children, 3-6 yr old	5	1.679	1.606	.093
7. Children with kwashiorkor, 2-6 yr old	6	0.275	1.577	0.184
8. Children with marasmus, 1 yr old	1	0.456	1.266	0.360
	1	0.584	1.596	0.366

\* UIU signifies an upper income, urban group and LIR signifies as low income, rural population. Data taken from women in their ninth month of pregnancy.

† Data taken from cord blood of group 1.

‡ Data taken from cord blood of group 2.

to a decrease in caloric reserve. The protein-calorie deficit noted does not, however, result in abnormal plasma protein values. Normal or increased levels of plasma proteins in chronically undernourished pregnant women have been described before and are attributed to a suboptimal increase in blood volume during the final weeks of pregnancy. A relative predominance of the caloric deficit

over that of protein may be responsible for the maintenance of plasma protein levels as is the case in marasmic children.<sup>8</sup>

Serum retinol levels of the mothers during delivery and in the corresponding cord blood are presented in Fig 2. The average values differ significantly in the mothers, but not in the newborns. For general populations, values below  $10\mu\text{g}/100\text{ ml}$  are

considered "deficient."<sup>10</sup> On this basis, 11 out of 30 individuals in the low socioeconomic group, and three out of 20 in the high socioeconomic group are in the "deficient" category.

Urinary excretion of riboflavin, which is related to intake,<sup>11</sup> is abnormally low in more than half of the women in the low socioeconomic group,<sup>12</sup> whereas 90% of values in the high socioeconomic group are either "acceptable" or "high."

Pregnant women in all Central American countries exhibited a higher prevalence of iron deficiency than nonpregnant women as indi-

cated by the degree of transferrin saturation. Although serum folate levels were less than 3 ng/100 ml in 17% of pregnant and 33% of nonpregnant women in the low socioeconomic group, this is not the limiting hematopoietic factor. When iron is administered, however, the already low folate level falls even further and folate does become the limiting hematopoietic nutrient.<sup>11</sup>

#### Conclusion

Nutritional needs during pregnancy are increased and vary for each nutrient. Therefore, recommended di-

etary intake for pregnant women must be different from that for nonpregnant women.

In countries where malnutrition and infection are prevalent, such as those of Central America, pregnant women and the general population exhibit nutritional deficits of calories, protein, vitamin A, riboflavin, iron, and folates. However, deficits of iron and calories are greater in pregnant women than in the general population. Newborn infants of malnourished mothers reflect in some respects the biochemical abnormalities of their mothers.

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## Maternal Nutrition During Pregnancy in Industrialized Societies

David Rush, MD

Low social status is associated with depressed fetal growth, high perinatal mortality, and disadvantageous mental development. Suboptimal calorie and protein nutrition during pregnancy is a likely causal link in this chain: it is coherent

with the relationship of low birth weight with famine conditions, with low maternal weight and weight gain, with the economic constraints of poverty, and with reported gradients of dietary intake of protein by social status during pregnancy.<sup>1-3</sup> It is possible that intervention to improve nutrition during the last few months of pregnancy when most women receive prenatal care and are thus accessible to treatment, may be of considerable benefit.<sup>4</sup>

We are testing this hypothesis in a

randomized, double-blind, controlled clinical trial of protein and calorie supplementation during pregnancy in a poor, urban, black, North American population.<sup>5,6</sup> The randomized, controlled study design was necessary for a number of reasons, such as covariation of poor nutrition with other aspects of poverty and the urgency of testing the question in a short time.

#### Evaluation

**Standards of Adequacy: The Recommended Daily Allowances.—Protein.—A**

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United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO) committee has recently recommended revised protein (and energy) intakes: 0.52 gm of egg or milk protein or its equivalent per kilogram per day, plus 1 gm/day in the first quarter of pregnancy, rising to an additional 9 gm/day in the last quarter.<sup>7</sup>

The Protein Advisory Group has taken issue with the above recommendation.<sup>8</sup> This is not surprising, for even in populations in which kwashiorkor is endemic, there are no accepted recommendations for the infant who has been weaned, even though there is a well-defined clinical deficiency syndrome against which adequacy might be judged.<sup>9-12</sup>

**Energy.**—The energy needs of the individual pregnant woman can be defined only in relation to her level of activity: she must receive enough for her own metabolic needs and those of the fetus. In contrast to the nonpregnant woman whose adequate nutrition is reflected in maintenance of body weight, the needs of the pregnant woman who is supporting growth of the fetus are not so easily assessed. There is wide variation in food intake within geographically defined groups and preferential allocation of food within the family. Goldberger observed that young adult men had a lower incidence of pellagra than young women.<sup>13</sup> The FAO/WHO committee has recommended 2,200 calories per day for the moderately active 55-kg (122-lb) woman, with an additional total of 80,000 kilocalories during the entire pregnancy.

**Clinical Evaluation.**—Classical clinical signs of malnutrition in the industrial societies are infrequently found, tend to be nonspecific, and vary widely with the observer.

**Biochemical Evaluation.**—Biochemical assessment of protein-calorie malnutrition has been applied extensively in infancy and childhood.<sup>14</sup> There are fewer data for pregnant women and other adults on diets containing suboptimal levels of protein that are not sufficiently low to cause clinical illness. Beaton et al<sup>15</sup> in Guatemala and Beydoun et al<sup>16</sup> in Syracuse, NY, reported that ratios of urinary urea to creatinine, or urea to total nitrogen, are reasonably good

indices of dietary protein intake during pregnancy. However, these values can become normal within 24 hours of the institution of adequate protein intake and can fluctuate irrespective of protein intake because of variation in creatinine<sup>17,18</sup> and urea<sup>19</sup> excretion. (For instance, in pregnancy less urea is excreted by a supine woman than one lying on her side; creatinine excretion is unaffected by position.<sup>19</sup>) Lindblad et al<sup>20</sup> found that both maternal and umbilical cord plasma glycine/valine ratios were abnormal in a poor Pakistani population with chronic low protein intake, whereas in an affluent Swedish group with hypertensive disorder of pregnancy only the cord plasma values were abnormal. Thus, fetal "malnutrition," secondary to compromised placental blood supply, can be contrasted with inadequate maternal protein intake. Churchill et al reported a strong relationship of serum  $\alpha$ -amino acid concentration to birth weight and cranial volume.<sup>21</sup> They ascertained by a single 24-hour quantitative dietary recall that  $\alpha$ -amino acid level was associated with low protein ingestion. It is unlikely, however, that the method of recall has sufficient reliability (see below).

**Dietary History.**—Assessment of diet intake, by history, is recommended by manuals on nutritional evaluation.<sup>22,23</sup> However, a critical assessment of this method suggests that it is unreliable. For instance, according to Young et al, "The hypothesis stating '24 hour recalls are an unbiased estimate of seven day record' was rejected without exceptions for all [ten] nutrients,"<sup>24</sup> and "[the dietary] history did not give the same estimate of intake for an individual as the seven day record."<sup>25</sup>

The seven-day food diary or extended self-weighing of food are not dependable methods either. Recording and weighing almost certainly modify the diet. Moreover, only some individuals are willing to cooperate in these tedious procedures, and fewer still are able to record the necessary data accurately. Thus, clerks and civil servants are among those with whom these methods are usually employed.

The correlation between a lengthy interview and brief recall decreases as the number of food items in-

creases. It is also decreased with lower educational attainment of the respondent,<sup>26</sup> and therefore becomes less useful in the groups at highest risk of disadvantageous fetal growth in industrial societies; urban dwellers of low educational level.

For repeated 24-hour dietary recalls, nine recalls would be needed for total calories and 27 for animal protein<sup>27</sup> in order to achieve a 95% probability that 90% of individuals have calculated mean values within 20% of their true means. Not only are such large numbers impractical, but the method is predicated on stable intake, and diet during pregnancy is known to change.

Reshef and Epstein reported quantitative similarity between lengthy diet histories taken six months apart.<sup>28</sup> Their finding does not validate the accuracy of such procedures, but rather confirms that, in their hands, it had good reliability (ie, repeatability). It is quite likely that systematic misreporting can occur. In studies of obesity, reported caloric intake, measured by various techniques, has been correlated inversely with body weight. This was observed by Maresh and Beal for American adolescents,<sup>29</sup> by Marr et al for British male civil servants,<sup>30</sup> and by Lincoln for middle-aged, middle-income, North American men.<sup>31</sup> In the initial analysis of our own 24-hour diet recall data from a poor black New York City population, we also observed lower reported caloric intake with increased body weight. The finding is not limited to the obese; we interviewed no one with a prepregnant weight over 63 kg (140 lb). Findings by A. J. Stunkard, MD (unpublished data), show that obese patients lose weight when given only as much to eat as they report having eaten in the past. However, the inverse relationship of caloric intake with body weight may be in part real, for decreased activity is consistently observed with increased body weights.<sup>32,33</sup> Quantitating how much the lower reported energy intake of those with higher body weight is due to conscious or unconscious misreporting of food intake and how much is a function of lower activity is not only technically difficult, but tangential to our needs. Whatever the

explanation, it is relevant that reported energy intakes are very poorly related to energy balance.

Assessment of nutrient intake must be based on a translation of foods eaten into their constituent nutrients. Calculated nutrient content of foods, using current tables, may bear little relationship to a direct chemical analysis.<sup>14</sup>

**Balance Studies.**—It is not known whether increases in the level of maternal protein intake beyond that needed to avoid signs of protein malnutrition are advantageous, given an adequate energy supply. Macy and Hunschner found an average of 2.83 gm of nitrogen per day retained in pregnancy.<sup>15</sup> More recently, Johnstone et al calculated a daily retention of about 0.8 gm of nitrogen per day.<sup>16</sup> This is in general agreement with Hytten and Leitch's estimate derived from studies of the composition of weight gain and the products of conception,<sup>7</sup> even though the potential errors in balance studies of nitrogen tend to overestimate retention.<sup>17</sup> Hytten and Leitch concluded,

It is generally believed that storage of protein, presumably in excess of measured increases in maternal structures and fetus, occurs in experimental pregnant animals. In default of any convincing quantitative evidence of similar storage for man, we propose to assume that no more protein is laid down than [can be] accounted for in the fetus and maternal tissues and fluids.<sup>7</sup>

Whether the consistently observed gradient of increased protein intake with improved social and economic conditions<sup>2</sup> is one of the mechanisms by which the affluent achieve improved fetal growth remains an open question.

**Activity and Energy Expenditure.**—Activity requires greater energy expenditure in pregnancy and efficiency decreases as pregnancy proceeds. The pregnant woman concomitantly limits her activity, balancing, in part, her increased obligatory energy expenditure.<sup>18</sup>

If variation in activity affects only energy balance and not other physiologic functions important for fetal growth, we could avoid the methodological problems of quantitating energy intake and activity, and could

limit scrutiny to their summation, energy balance. This may be the case, since, for example, there is little change in uterine blood flow with activity or exercise in the pregnant ewe.<sup>19</sup>

#### Energy Balance: Weight, Weight Gain, and Anthropometry in Pregnancy

Energy intake, metabolism, and expenditure are difficult to measure and thus difficult to relate to outcome. Changes in maternal weight and fat pad thickness are easily measured, and the components of these changes (excluding the products of conception) are, for practical purposes, limited to water and fat. It may be possible therefore to calculate changes in water and fat if changes in body weight and fat pad thickness are known.

We do not yet know how constant is the proportion of fat in gains of maternal weight in pregnancy. The proportion of water does vary: with clinical edema, the proportion is obviously higher.

The relationship of maternal fat deposition to birth weight has not been studied, even though an excellent study of fat pad changes in pregnancy has supplied us with much of the requisite technology.<sup>20</sup>

The correlation of weight gain with increased energy storage (as fat) must be high, and it seems worthwhile to explore some of the known relationships of fetal growth to changes in maternal weight.

In the poor, urban, American community in which our group works, there was a consistent strong additive association with birth weight of both prepregnancy weight and weight gain in pregnancy,<sup>11</sup> with negligible relationships of maternal age, parity, or height, after controlling for prepregnant weight. This is similar to the findings in the study of Weiss and Jackson.<sup>22</sup> The collaborative perinatal study disclosed that among the 2,500 women who delivered two singleton infants, changes between pregnancies in either prepregnancy weight or weight gain were associated with remarkable differences in birth weight.<sup>43</sup>

For affluent populations, maternal weight gain continues only slightly diminished to term,<sup>2</sup> with parallel regular fetal growth. This may not be true in developing countries. Jurado-Garcia et al in Mexico City have shown that for the infants of impoverished mothers delivered at a university hospital, there was no increase in birth weight for those beyond 35 weeks' gestation.<sup>11</sup> The most plausible explanation for this striking finding is deprivation of energy, and data on maternal weight change would be most helpful.

Fat pad thickness increases to the end of the second trimester, then stays stable until delivery, and returns to normal very shortly after delivery.<sup>20</sup> This sequence fits well with the observation that group differentiation of fetal growth begins only at the start of the third trimester. Thus, adequate fat deposited up to 30 weeks may be protective of continued optimum fetal growth, as is third-trimester refeeding following starvation.<sup>4</sup>

In the collaborative study population, there was a strong gradient of increased birth weight with increased prepregnant weight and pregnancy weight gain among both races.<sup>42</sup> However, for any given maternal weight and weight gain, birth weight for whites was consistently higher. Thus, there are other determinants of the differences between blacks and whites. Whether the invariant increase in mean birth weight with rising social status (in geographically and racially homogeneous populations) can be accounted for by differential weight and weight gain remains to be explored, but it is a compelling hypothesis.

At least half of the variance of birth weight associated with maternal cigarette smoking is shared jointly with depressed maternal weight gain.<sup>46</sup> Thus, the predominant mechanism by which smoking affects fetal growth may be by depressed energy intake.

#### Goals for Further Study

Future standards for energy intake in pregnancy would be more precisely defined by energy balance, measured

by weight gain and fat pad thickness, than by dietary intake. The question whether induced or volitional increases in energy balance would generate higher birth weight, and subsequent lowered perinatal mortality, is now under study.<sup>5,6</sup>

The exploration of the interaction of nutrition and smoking requires urgent attention, for smokers have infants whose perinatal mortality is about a third higher than non-smokers.<sup>47</sup>

The most efficient approach to the definition of optimal protein intake may be to study energy as a covariate. The attention to weight gain and the wider use of skinfold calipers could be helpful clinically. It is easier to counsel proper weight gain than precise calorie intake. A recent interpretation of the US Ten-State Nutrition Survey is germane:

Although the diets of low income families did not differ in the concentration of essen-

tial nutrients from those of middle income groups, the availability of calories, ie, the amount of food available, was directly related to the family income. The total food intake of children in low income families was limited, and this was reflected in growth performance.<sup>48</sup>

The Ten-State Survey included too few pregnant women to test whether these conclusions hold specifically for the pregnant woman.

#### Conclusion

The most fruitful path to the understanding of the consequences of maternal nutrition for the offspring, at least for the immediate future, would seem to be concentrated on effects on fetal growth.

Techniques for assessment of maternal nutritional status are highly unsatisfactory: the lack of universally acceptable standards for nutrient intake and balance follows from these technical inadequacies.

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# Maternal Nutrition and Fetal Growth in Developing Societies

## Socioeconomic Factors

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Developing societies share several common characteristics, including low gross national product per capita, heavy dependency on the export of raw materials, inefficient systems of land tenure, and deficient technology. Malnutrition and infectious disease are highly prevalent, especially during the first five to seven years of life. Such societies moreover are characterized by sharp differences between upper and lower socioeconomic status groups in terms of income and living conditions.

Figure 1 summarizes results of several studies comparing height of adult women from high and low socioeconomic groups. Women from low socioeconomic groups in rural and urban populations are shorter; those from the high socioeconomic group resemble the white urban population in the United States. With regard to height or prepregnancy weight, similar facts obtain.

In developing societies, socioeconomic status is also associated with other maternal characteristics. Dietary intake of proteins and calories<sup>1,2</sup> and weight gain during pregnancy<sup>4,11,12</sup> (L. J. Mata, ScD, unpublished data) are low in women from rural populations. Comparisons in terms of birth weight show a similar pattern. In developing countries, the proportion of infants with low birth

weight is greater in rural and urban groups from low socioeconomic strata than in high socioeconomic groups from the same countries.<sup>1,11,12,24</sup>

In most studies, socioeconomic status has been defined exclusively by family income. There are very few reports concerning other sociocultural factors that could explain the observed differences in maternal nutrition and birth weight. Maternal malnutrition, whether secondary to dietary deficiency, increased losses, or demands due to infectious disease, is an important cause of fetal growth retardation, which is perpetuated through generations.

### Study of Nutrition and Mental Development

We are exploring these interrelations in an INCAP study on nu-

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Reprints not available.

trition and mental development<sup>4</sup> in four rural ladino villages in Guatemala. The total population of the four villages is around 3,000, half of whom are below 15 years of age. The villages have a subsistence agriculture economy, producing corn, beans, and mango. The median annual income is \$200 per family, most of which is allocated to food and clothing. Sanitation is extremely poor; drinking water derives from public wells or creeks and only 6% of the houses have latrines.

We have devised a socioeconomic scale based on characteristics of the house, clothing, and education of children. As the score increases, the percentage of low-birth-weight infants decreases (Fig 2). Even in small rural villages in which almost all inhabitants are poor and illiterate, very simple socio-cultural scales can identify groups of mothers with sharp differences in incidence of low-birth-weight infants.

Socioeconomic score also shows a significant association with maternal height and head circumference, third-trimester maternal weight, and indicators of maternal morbidity during pregnancy. Figure 3 shows the relationship between socioeconomic score and low birth weight of infants according to maternal height. The magnitude of this association is greater in mothers with low stature than in those with high stature. A similar pattern appears when maternal weight, head circumference, or

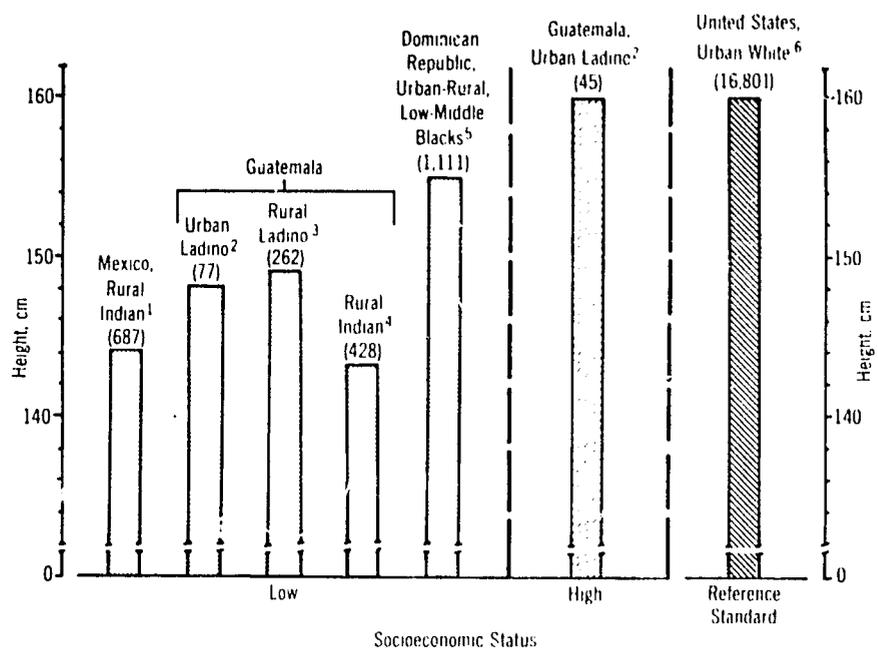


Fig 1.—Relationship between socioeconomic status and height of adult women in pre-industrialized societies. Number of cases shown in parentheses. Data obtained from following sources: (1) Faulhaber<sup>1</sup>; (2) Arroyave et al<sup>2</sup> and Lechtig et al<sup>3</sup>; (3) Lechtig et al<sup>3</sup>; (4) L. J. Mata, ScD, unpublished data; (5) Sebrell et al<sup>5</sup>; (6) Niswander et al.<sup>6</sup>

morbidity is studied. Consequently, these maternal characteristics may explain an important part of the relationship between socioeconomic score and low birth weight.

A reasonable interpretation of this finding is that the socioeconomic score reflects economic and cultural conditions resulting in maternal malnutrition and disease, which in turn produce fetal growth retardation. There are, of course, alternative ex-

planations for these findings. It is possible, for example, that the socioeconomic score and maternal characteristics are risk indicators not causally related to the mechanisms responsible for fetal growth retardation. This, however, is an unlikely possibility since there is evidence that improvement in maternal nutrition is associated with higher birth weight.<sup>23</sup> Whatever the causal relations among these variables, it is clear that the so-

Fig 2.—Relationship between socioeconomic score and proportion of children with low and high birth weights in four rural Guatemalan villages. Number of cases given in parentheses; total number is 364.

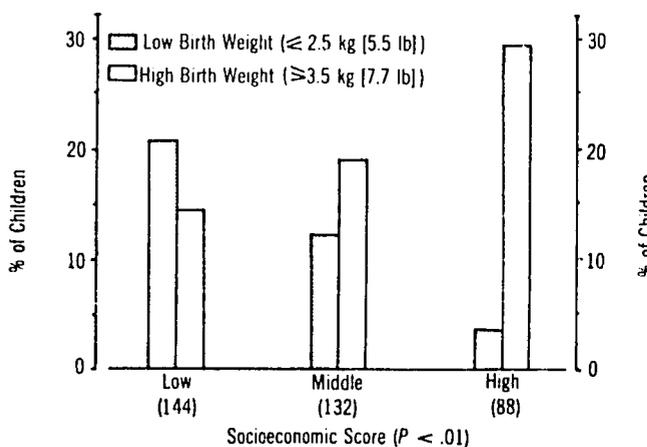
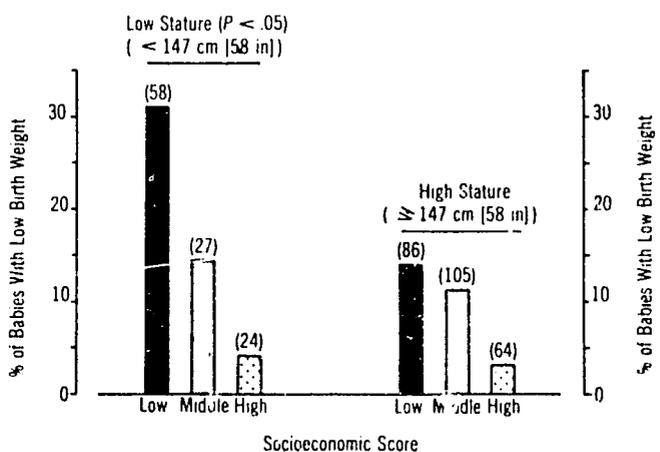


Fig 3.—Influence of maternal height on relationship between socioeconomic score and proportion of babies with low birth weight ( $\leq 2.5$  kg [5.5 lb]). Number of cases given in parentheses.



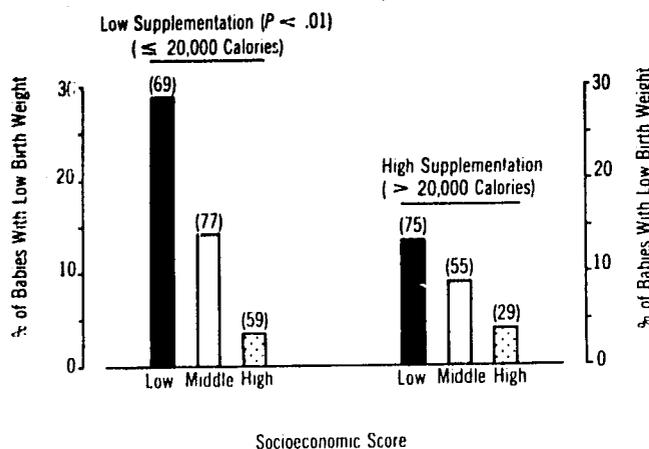


Fig 4.—Influence of caloric supplementation on relationship between socioeconomic score and proportion of babies with low birth weight. Number of cases given in parentheses.

socioeconomic score has biological importance in these villages, since the risk of low birth weight is seven times greater in mothers with a low socioeconomic score than in those with a high score.

Figure 4 shows the relationship between the socioeconomic score and low-birth-weight infants according to maternal caloric supplementation during pregnancy. The magnitude of the association is greater in mothers with low supplementation than in those with high supplementation. The group with a high socioeconomic score showed no difference in proportion of low-birth-weight infants whether supplementation was high or low. These results indicate that differences in the incidence of low birth

weight according to the socioeconomic score can be reduced if mothers are well supplemented during pregnancy, and that the effects of caloric supplementation are strongest in the group with a low score.

Figure 5 presents the relationship between the socioeconomic score and proportion of low-birth-weight infants according to stature of the mother and caloric supplementation during pregnancy. The association of socioeconomic score and low birth weight is significant for short mothers with low supplementation. No association between the socioeconomic score and low birth weight is evident in tall, well-supplemented mothers. The two intermediate groups show an intermediate association.

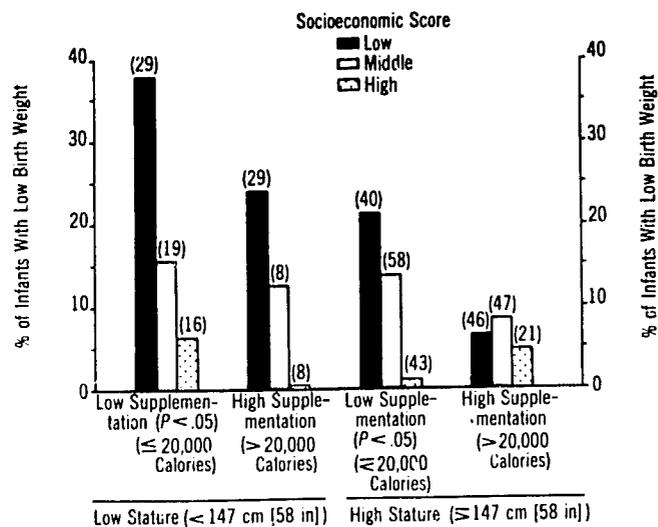


Fig 5.—Influence of maternal height and caloric supplementation during pregnancy on relationship between socioeconomic score and proportion of infants with low birth weight. Number of cases given in parentheses.

### Conclusion

Maternal height and caloric supplementation seem to have little effect on the incidence of low-birth-weight infants in mothers from high socioeconomic groups within rural ladino villages in Guatemala. On the other hand, in mothers from low socioeconomic groups, maternal height and food supplementation have a strong effect on the frequency of low-birth-weight infants. Maternal nutrition appears to be one of the intermediate steps in the causal chain between socioeconomic factors and fetal growth.

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### Summarized Discussion of Session I

Dr. Behrman inquired whether there was an increase in mortality of infants who weigh more than 4,400 gm (10 lb) at birth, commenting that in an earlier study in the higher socioeconomic classes, heavy infants had lower intelligence quotients when tested at school age. He also wanted to know whether one could distinguish in the upper socioeconomic classes infants who weigh more than 4,400 gm at birth. Dr. Rush responded that there was indeed some increased risk at the higher birth weight. He pointed out that in infants with weights up to 3,500 gm (8 lb), there was better survival with the higher weight, followed by a plateau for about 500 gm (1 lb) and then a rise in mortality. He pointed out further than mean birth weight for Western population varied between 3,200 and 3,500 gm (7 and 8 lb), and that 4,400 gm was more than 2 standard deviations from the mean and therefore might represent, in fact, pathologic states of pregnancy. He continued that it is possible to distinguish between types of fetal gestation in upper social status, but that there was differentiation of nutrition within the upper-class mothers as well, because ability to purchase food does not necessarily assure purchase of proper nutrients. The important point is that there is a variation in nutritional status, independent of social status. However, there is an interaction between social status and effects on fetal growth. For instance, in some studies, low-birth-weight infants in an upper class incurred little or no disadvantage in eventual mental performance, whereas there was such a disadvantage among the poor. This also holds for the increase in perinatal mortality due to maternal smoking, which is greater among the poor than among the well-to-do.

Dr. Sinclair then continued the discussion, raising the issue that the correlation between pregnancy weight gain and birth weight may be somewhat spurious because

one weighs the pregnant mother together with the fetus and one therefore may be measuring, at least in part, the same thing twice. Would it not be more accurate, he wondered, to subtract fetus weight from pregnancy weight gain itself. Dr. Rush responded that the proportionate component of fetal weight gain is so small as to be negligible. He continued that fetal weight of a few hundred grams is contrasted with the pregnancy weight gain of approximately 25 kg (55 lb) or so. Dr. Sinclair disagreed and stated that if one took weight gain of some 12 kg (26 lb), the fetal weight and that of the products of conception would constitute approximately one third of the total weight. Dr. Rush, on reflection, agreed and suggested that perhaps such calculations would be valid in analyzing data of pregnancy weight gain and birth weight. Dr. Ousa added that in Thailand, at least, one must account for the influence of toxemia of pregnancy on the data of weight gain during pregnancy, because in that country there are many toxemic women.

Regarding Dr. Lechtig's data, Dr. Plotkin wondered about the infection rate in mothers, for example, urinary tract infections. Dr. Lechtig responded that in their studies, infection correlated negatively with birth weight. In view of the statement by Dr. Rush that maternal smoking correlated with lower birth weight, Dr. Plotkin wondered whether there was an animal model in which this influence could be tested. Dr. Sinclair recalled that there was and referred to a Winnipeg, Canada, study by Dr. Howarth in which rats were placed in a smoke-filled box for many hours each day. The effect was a decrease of mean fetal weight, but no reduction in either length of gestation or in litter size. However, the exposed rats tended to eat less. Dr. Beisel wondered whether transplacentally transmitted glucose and other nutrients may be reduced or otherwise af-

ected in states of infection. Dr. Behrman reported that he knew of no studies that tested this question. There then followed a discussion about the importance of placental size in relation to birth weight, and Dr. Lechtig pointed out that it has some influence on the birth weight, but that there is a large margin of safety. Dr. Rush disagreed somewhat and recalled that in the Dutch famine studies, it had been determined that the placental size depended on the size of the fetus and not the other way around.

Next, the discussion turned to maternal height as a possible determinant of birth weight of the infant, prompted by a question from Dr. Keusch. Dr. Behrman responded that in the Leningrad famine, no such correlation between height and birth weight could be made. However, Dr. Rush commented that with the available data it is not yet possible to isolate the question of height as an influence and thought that height was associated with the social status as well. Dr. Lechtig disagreed and stated his belief that there was a relationship between maternal height and birth weight. Dr. Keusch continued wondering, if height had an influence, why it was that in Dr. Lechtig's studies of caloric supplementation, the high caloric intake did eliminate the difference of birth weights between short mothers and the taller ones. Dr. Béhar then pointed out that caloric supplementation in that study affected only the pregnancy period, whereas height of the mother was really a reflection of the nutritional influences of her entire earlier life. Dr. Mata added that taller women in every society produce larger babies. Finally, Dr. Beisel added that perhaps height would be more properly defined not in some absolute, or even relative terms, but rather as achievement of optimal height level.

# Fetal Defense Mechanisms

E. Richard Stiehm, MD

The function of the immune system of the fetus and the newborn is suspect because of an increased incidence of infection, poor clinical response to infection, diminished lymphoid tissue, and sluggish immunologic responses to a variety of natural or test antigens.

Fetal immune mechanisms can be classified in a similar fashion as the human primary immunodeficiencies are classified that is, into antibody deficiencies, cellular immune deficiencies, phagocytic and macrophage deficiencies, and opsonin and complement deficiencies. Since most primary immunodeficiencies result from developmental arrest, the immunologic abnormalities in primary immunodeficiencies will occur at some stage of normal ontogenesis, and the methods used in the diagnosis of primary immunodeficiencies are valid in delineating defects or immaturity in fetal immune development.

This discussion will consider the following: (1) normal development of each of the major components of the human immune system, (2) factors that may activate the fetal immune system and the consequences of such activation, and (3) factors that may impair the development of the immune system, and the consequences of such impairment.

## Development of the Human Immune System

### Development of Cellular Immunity.—

The cellular immune system plays a major role in the defense against vi-

ral infection, certain intracellular bacterial infections, fungal and protozoal infections, mutant or malignant cells, and histoincompatible human cells. The cellular immune system originates from stem cells within the liver or the spleen; these enter the thymus about the eighth week of gestation.<sup>1</sup> In the thymic cortical area, an active site of lymphoid proliferation, distinctive surface antigens (such as  $\theta$  antigen in mice and possibly analogous antigens in humans) are acquired and acquisition of immunocompetence is achieved. During cellular maturation, many of the lymphocytes die in situ in the cortical areas; others migrate to the medulla of the thymic lobule. Mature T cells (thymus-derived cells, the functional cells of the cellular immune system) leave the thymus via the bloodstream and are distributed throughout the body but with particularly large concentrations in the paracortical areas of lymph nodes and the periarteriolar areas of the spleen. Some T cells enter the lymphatics and return to the circulation via the thoracic duct.

Functional assessment of the cellular immune system can be accomplished by assessing the number of T lymphocytes on the basis of their ability to form rosettes with sheep erythrocytes by *in vitro* tests of lymphocyte proliferation, by mediator formation and direct cell-to-cell killing capabilities, and by delayed hypersensitivity responses to certain skin test antigens.

**Development of the Antibody Immune System.**—Immunoglobulin synthesis begins in human fetuses at about 10½ weeks, with the synthesis of IgM in lymphoid tissues, as shown by tissue culture studies of incorporation of amino acids labeled with radioactive

isotopes into immunoglobulins, identified by radioimmunoelectrophoresis.<sup>2</sup> Initially, the spleen is the site of most active synthesis. By the same technique, IgG synthesis begins at 12 weeks and IgA at 30 weeks of gestation. Van Furth et al<sup>3</sup> detected IgG- and IgM-fluorescing cells in lymphoid tissue in human fetuses by 21 weeks. Lawton and Cooper<sup>4</sup> found IgM, IgG, and IgA surface determinants on lymphoid cells (B cells) in peripheral blood, bone marrow, liver, and spleen by 11½ weeks. By 14 weeks the distribution of immunoglobulin-staining B cells was similar to that in adult tissues. Cells with cytoplasmic immunoglobulin (those synthesizing immunoglobulins) were not found in early fetuses and did not appear until the time when endogenous immunoglobulin synthesis commences.

Lawton and Cooper<sup>4</sup> have proposed a two-stage model for differentiation of plasma cells. The first stage (clonal development) involves conversion of stem cells into antigen-reactive B cells with surface immunoglobulins. These B cells initially synthesize IgM, but some then switch to IgG and IgA production, maintaining the same antibody specificity. This stage is independent of exogenous antigen stimulation and fits the clonal selection theory. The second stage (clonal proliferation) commences when the B cells enter circulation; on exogenous antigenic stimulation they will proliferate to form antibody-producing cells with cytoplasmic immunoglobulins. Developmental arrests resulting in congenital antibody immunodeficiencies can occur at either stage; in stage-1 defects, peripheral B cells are lacking, but in stage-2 defects, B cells are present without immunoglobulin synthesis.

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**IgG.**—Although IgG synthesis does not commence until the 12th week of gestation, IgG may be present in the fetal circulation as early as the 38th day of gestation as a result of passive transport of maternal IgG across the placenta.<sup>1</sup> The IgG levels remain low (<100 mg/100 ml) until about 17 weeks, at which time they increase gradually in proportion to the gestational age (Figure). At term (40 weeks), the newborn IgG level usually exceeds the maternal IgG level by 5% or 10%.<sup>2</sup> Although Gitlin described an abrupt onset of maternal-fetal transfer at about 22 weeks,<sup>1</sup> with the result that most fetuses between 25 and 40 weeks had IgG levels equivalent to the maternal levels, most other investigators have noted a gradual increase of fetal IgG levels throughout late gestation.<sup>3-10</sup> For example, we recorded IgG levels of  $330 \pm 61$  mg/100 ml (mean  $\pm$  1 SD) at 26 to 27 weeks,  $556 \pm 107$  mg/100 ml at 32 to 33 weeks,  $823 \pm 135$  mg/100 ml at 36 to 37 weeks, and  $937 \pm 175$  mg/100 ml at 40 to 41 weeks.<sup>10</sup> This increase allows an estimation of gestational age by the cord blood IgG level. Nearly all the cord IgG is of maternal origin. Mårtensson and Fudenberg<sup>11</sup> detected trace quantities of endogenously synthesized IgG by assaying genetic IgG globulin (Gm) types, but this contributes negligibly to the IgG serum level.

There are two principal mechanisms by which the maternal-fetal transfer of IgG is accomplished.<sup>1,12</sup> The first is a passive transfer, in which the fetal IgG is proportional to the maternal IgG level; this mechanism undergoes maturation with increasing gestational age to permit increased amounts of IgG to cross the placenta during gestation. The second is an active enzymatic mechanism that actively transfers IgG from mother to fetus. This enzymatic mechanism is inhibited at high maternal IgG levels and is increasingly activated at low maternal IgG levels, and thus it serves to bring to normal fetal IgG levels; infants born to mothers with high or low IgG levels tend to have near normal IgG levels.

**IgM.**—The synthesis of IgM commences at 11 weeks of gestational

age and continues at low levels throughout gestation. The normal fetus has sufficiently well established IgM synthesis so that nearly all fetuses have detectable levels of IgM in their serum by 30 weeks. Maternal IgM normally does not cross into the fetal circulation. Levels of IgM increase gradually during late gestation; at term birth, the mean level of IgM is  $10 \pm 5$  mg/100 ml.<sup>1</sup> Buckley et al<sup>13</sup> reviewed the results of seven studies of cord blood IgM levels and found that mean levels varied from 5.8 to 15.3 mg/100 ml and that the limits of two standard deviations from the mean varied from 1 to 10 mg/100 ml (lower limit) to 12.9 to 27.4 mg/100 ml (upper limit). Most authorities consider the normal mean level to be 10 mg/100 ml, with an upper limit of normal ( $+2$  SD) of 20 mg/100 ml.

**IgA, IgD, and IgE.**—Synthesis of IgA is delayed until 30 weeks and then proceeds to such a limited degree that serum IgA is usually not detected until several days after birth, when levels are usually 1 to 5 mg/100 ml. Higher levels of IgA in cord blood are often indicative of a maternal-fetal transfusion. Secretory IgA, composed of two serum IgA molecules and secretory component, synthesized by glandular epithelial cells, is not detected in the fetus. However, free secretory component can be synthesized by the fetus as evidenced by its presence in the urine of premature infants.<sup>14</sup>

The immunoglobulins IgD and IgE do not cross the placenta and are found only in trace quantities in the fetus and in cord blood.

**Development of Phagocyte and Macrophage Immunity.**—The importance of the granulocyte in defense mechanisms is amply illustrated by the severe illness, chronic granulomatous disease, that results from a defect in the intracellular killing of certain bacteria.

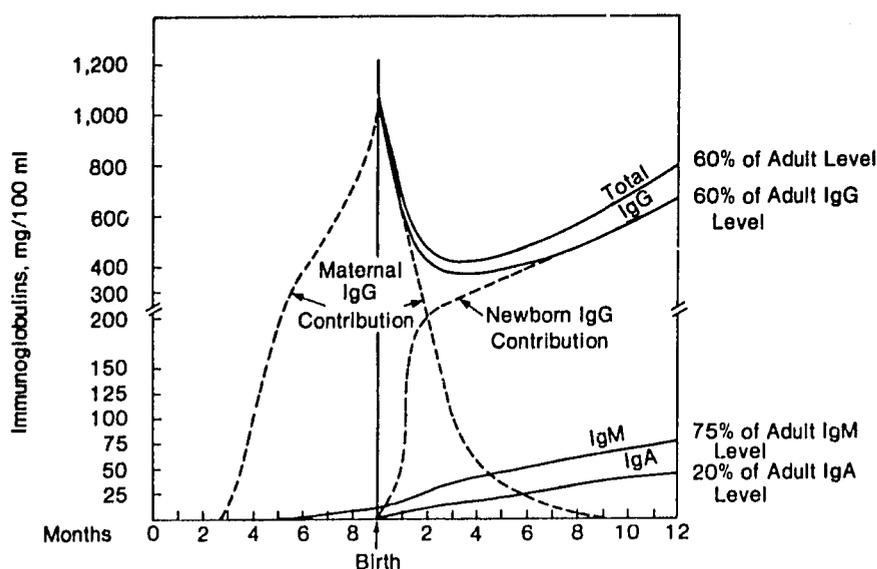
Granulocytic cells are first noted in the liver at about two months of gestation. By five months, the bone marrow becomes the chief synthetic organ. However, the functional capabilities of fetal granulocytes to phagocytize and kill microorganisms

are not well studied.

Several studies of neonatal leukocytes are available. Tunnicliff in 1910, using various bacteria,<sup>15</sup> Matoth in 1952, using starch particles,<sup>17</sup> and Miyamoto in 1965, using killed streptococci,<sup>16</sup> noted diminished phagocytosis of cord blood leukocytes in the presence of their own serum. However, these studies did not define with clarity the contribution of the newborn serum to the phagocytic defect. Gluck and Silverman in 1957, using India ink particles, first suggested that neonatal phagocytic deficiency might be due to a lack of serum factor, inasmuch as diminished phagocytosis of premature leukocytes could be almost completely corrected in the presence of adult serum.<sup>18</sup> Recent studies have confirmed these observations.<sup>19,21</sup>

In contrast to these studies, Coen et al<sup>22</sup> found diminished bactericidal capacity in the leukocytes of nine of 25 infants in the first 12 hours of life, utilizing *Staphylococcus aureus* as a test particle. They attributed this to a defect in glucose metabolism through the hexose monophosphate shunt (HMP), since carbon dioxide production from glucose-1-<sup>14</sup>C was diminished, confirming similar observations by Donnell et al.<sup>23</sup> However, Park et al<sup>24</sup> and McCracken and Eichenwald<sup>25</sup> found normal or increased nitroblue tetrazolium (NBT) dye reduction in the leukocytes of newborns, a procedure used to measure the functional capacity of the HMP shunt of leukocytes, and as a screening test for chronic granulomatous disease of childhood. Further, NBT dye reduction was only slightly reduced in the leukocytes of premature infants.<sup>26</sup>

Macrophage function has not been studied in the human fetus or newborn, but elegant studies in neonatal rats suggest an immaturity of neonatal macrophage function, as evidenced by increased susceptibility to *Listeria monocytogenes* infections, ready establishment of immunologic tolerance, and diminished antibody production.<sup>27</sup> These defects can be reversed by infusion of adult rat macrophages or activation of the neonatal animals' own macrophages.



Immunoglobulin (IgG, IgM, and IgA) levels in fetus and infant in first year of life. The IgG of fetus and newborn infant is solely of maternal origin. Maternal IgG disappears by age of 9 months, by which time endogenous IgG synthesis by infant is well established. The IgM and IgA of neonate are entirely endogenously synthesized, since maternal IgM and IgA do not cross placenta.

Author & Year	Population Studied	IgM Levels*	
		No. Elevated/ No. Studied	% With Elevation
Stiehm et al (1966) <sup>43</sup>	Random newborns (mixed income)	7/129	5.4
Stiehm & Nichol (1968), unpublished data	Consecutive newborns (middle income)	2/1,100	0.2
Alford et al (1967, 1969, 1971) <sup>44,47</sup>	Consecutive newborns (mixed income) (1967-1968)	123/2,916	4.2
Alford et al (1967, 1969, 1971) <sup>44,47</sup>	Consecutive newborns (mixed income) (1968-1969)	69/3,035	2.3
Hardy et al (1969) <sup>52</sup>	Consecutive newborns (mixed income)	132/2,623	5.0
Sever et al (1969) <sup>50</sup>	Consecutive newborns (mixed income)	14/1,768	0.8
Miller et al (1969) <sup>46</sup>	Consecutive newborns (mixed income)	135/5,006	2.7
Lechtig & Mata (1970, 1971) <sup>53,54</sup>	Random newborns (mixed income, urban, developing country)	1/16	6.3
Lechtig & Mata (1970, 1971) <sup>53,54</sup>	Random newborns (poor income, rural, developing country)	55/115	48.7
Dent et al (1971) <sup>51</sup>	Consecutive newborns (mixed income)	4/100	4.0
Gotoff et al (1971) <sup>46</sup>	Random newborns (low income)	41/1,659	2.5
Gotoff et al (1971) <sup>46</sup>	Random newborns (middle income)	27/2,626	1.0

\* All the studies have used an IgM level of 20 mg/100 ml or above to be abnormally elevated except for Gotoff et al<sup>46</sup> who used 17 mg/100 ml, and Hardy et al<sup>52</sup> who used 30 mg/100 ml.

**Development of the Complement and Opsonic Systems.**—Complement (C) synthesis begins early in ontogeny and precedes immunoglobulin synthesis. The synthesis of C1-esterase inhibitor, C2, C4, and C5 begins as early as eight weeks of fetal life, considerably earlier than the onset of IgM or IgG synthesis.<sup>28,29</sup> Synthesis of

C3 and C4 is well established by 11 to 14 weeks and to a degree greater than that of immunoglobulins at the same time. Synthesis of C1q occurs later, principally in the spleen, or possibly in the colon and ileum. This early synthesis of C is evidently not related to the presence of antigen.

Despite the early onset of comple-

ment synthesis, substantial quantities of complement do not appear in the serum until 12 to 14 weeks and then gradually increase; at term, total hemolytic complement titers and levels of individual components are 50% to 65% of the corresponding levels in normal adult serum or paired maternal serum. Complement components do not cross the placenta. Low-birth-weight infants have much lower levels of hemolytic complement and individual complement components and these are reduced in proportion of the degree of immaturity.<sup>30-32</sup>

Complement plays an important role in the heat-labile opsonic system, enhancing phagocytosis of organisms to which the body has never been exposed.

Several studies suggest deficient opsonic activity of low-birth-weight and term infant sera and this may contribute in large measure to the abnormal phagocytic function of their whole blood or unwashed leukocytes. Gluck and Silverman were able to correct the opsonic defect of premature leukocytes by the addition of fresh adult serum.<sup>19</sup> Much of this phagocytic-restoring activity resided in  $\alpha$  and  $\beta$  globulins. Miller noted a diminished opsonic activity of newborn serum, utilizing adult leukocytes and yeast particles.<sup>31</sup> This abnormality was most severe at 2.5% plasma concentration but minimal at 10% plasma concentration. Premature serum was not assessed.

In contrast to these studies, Forman and Stiehm,<sup>30</sup> McCracken and Eichenwald,<sup>30</sup> and Dossett et al,<sup>31</sup> using live *S aureus* and *Serratia marcescens* as the test particles, in the quantitative phagocytosis assay of Quie et al,<sup>34</sup> noted no abnormality of the opsonic activity of the sera of term infants. Term newborn serum apparently has diminished opsonic activity only to certain organisms, primarily Gram-negative ones, whereas serum from low-birth-weight infants has defective opsonic activity when any organism or particle is used.

#### Activation of the Fetal Immune System

The components of the immune system are established by the 12th week of gestation, and for selected

functions, considerably earlier. Under special circumstances, the fetal immune system may be activated prior to birth.

**Cellular Immunity.**—The cellular immune system may be functioning in every normal fetus to eliminate maternal lymphocytes that leak across the placental barrier in every pregnancy and have the potential of causing a fatal graft-vs-host reaction. The very early capability of reacting to allogeneic cells as demonstrated by mixed leukocyte culture reactivity<sup>35</sup> may be an important fetal defense against this occurrence. A fatal graft-vs-host reaction may occur in infants with congenital cellular immunodeficiencies as a result of maternal lymphocyte engraftment and, if the infant is a boy, it may result in XX/XY chromosomal chimerism.<sup>36</sup>

Viral infections affecting the fetus during gestation may be limited by a functioning cellular immune system. This is exemplified by some infants with congenital infection (eg, rubella) who at birth no longer are shedding virus, presumably as a result of an intact and functioning cellular immune system.

The cellular immunocompetence of the fetus may also modulate the effect of intrauterine infections. According to Silverstein,<sup>37</sup> most of the manifestations of intrauterine syphilis are a result of an inflammatory and immune response to the *Treponema* organism, which in itself is not toxic to the tissues. In the absence of an immunologic response to the organism, typical congenital syphilis will not be present even though the organism is in the tissues.

**Antibody Immunity.**—The passive maternal antibody received throughout late gestation may help to prevent intrauterine infection to some degree; however, it seems unlikely that an infectious agent to which the mother has high-titered IgG antibodies could gain access to her fetus. Thus, the main role of passive antibody is prevention of infection after birth for the first months of life. Further, there is no evidence that either passive maternal antibody or endogenously synthesized fetal antibody (see below) has a role in combatin

established intrauterine infection.

Several animal studies attest to the ability of fetal experimental animals to synthesize antibodies following intrauterine antigenic challenge.<sup>38</sup> The normal human newborn makes small quantities of certain natural antibodies, presumably a result of intrauterine antigenic challenge.<sup>39-41</sup>

As noted, there is some immunoglobulin synthesis, particularly IgM synthesis, by every fetus in late gestation. On intrauterine antigenic challenges, IgM synthesis is accelerated and the result is elevated levels of cord blood IgM (exceeding 20 mg/100 ml).<sup>42-44</sup> Cord IgM is increased in congenital infection but not in all infected infants<sup>45-54</sup> (E. R. Stiehm, MD, and K. Nichol, MD, unpublished data).

The degree of elevation of IgM level depends on several factors. In general, the more affected the infant, the more likely he is to have an elevated IgM level. The type of congenital infection may be important; infants with rubella and syphilis have higher levels of IgM than do other infected infants. The maturity of the infant may be important; a very small premature may have a lessened IgM response than an older infant.

The Table lists the incidence of elevated IgM levels under various conditions. To a major extent it depends on the socioeconomic status of the newborn population. An obvious feature in these studies is the higher mean IgM level and the higher percentage of infants with IgM level elevations in low socioeconomic groups, particularly in the developing countries.<sup>54,55</sup>

**Inhibition of the Fetal Immune System.**—A variety of diseases are associated with depression of the immunologic system as indicated in the following list:

1. Primary immunodeficiencies
  - A. Antibody immunodeficiency
  - B. Combined immunodeficiency
  - C. Cellular immunodeficiency
  - D. Phagocytic immunodeficiency
  - E. Complement immunodeficiency
2. Secondary immunodeficiencies
  - A. Hematopoietic disorders (eg, leukemia, lymphoma, aplastic anemia, sickle cell anemia)
  - B. Burns
  - C. Exudative enteropathy
  - D. Nephrotic syndrome

E. Splenectomy

F. Uremia

G. Virus infection

H. Malnutrition

I. Prematurity and early infancy

Many of these factors—genetic, infectious, and nutritional disturbances—are operative during fetal life and may impair the fetal immune response.

Intrauterine viral infections, notably rubella, not only activate the fetal immune systems but may interfere with their normal development, with the result that a permanent immunodeficiency syndrome develops. The most common immunodeficiency associated with congenital rubella is immunodeficiency with excess of IgM (dysgammaglobulinemia: type I),<sup>56,57</sup> probably a result of interference by virus at the critical time when clonal differentiation from IgM B cells to IgG and IgA B cells is occurring. Cellular immunodeficiency syndromes,<sup>57</sup> and loss of rubella antibody with unreactivity to rubella vaccine<sup>58</sup> (suggestive of specific immunologic tolerance) may also result.

A variety of chromosomal and genetic disorders have associated immunologic defects, including well-defined, genetically transmitted, primary immunodeficiency syndromes (eg, X-linked infantile agammaglobulinemia, ataxia telangiectasia), chromosomal disorders with subtle immunologic abnormalities (eg, abnormal lymphocyte metabolism in Down syndrome, predisposition to autoimmunity in Turner syndrome), and other genetic diseases with increased susceptibility to infection (eg, galactosemia, Bloom syndrome). Only with profound cellular immunodeficiency will this be an important factor during intrauterine life; in this latter instance, engraftment of maternal lymphocytes<sup>56</sup> or exogenous lymphocytes from an intraperitoneal intrauterine blood transfusion<sup>59</sup> can result in a fatal graft-vs-host reaction.

Nutrition deprivation may impair the fetal defense system. Naeye et al<sup>60</sup> found on autopsy that low-birth-weight infants born to poor urban mothers and dying in infancy had disproportionately small thymus and spleen sizes when compared to dying

infants from nonpoverty families. In contrast, the brain, kidneys, heart, and skeletal bones did not differ in the two groups. He attributed this to maternal malnutrition.

In older infants and adults, malnutrition results in severe impairment of many of the body defenses, but particularly of the cellular immune system.<sup>21</sup> We have preliminary data<sup>22</sup> that suggest that small-for-gestational-age neonates have decreased T cells as estimated by a lower percentage of rosette-forming cells than premature infants who have appropriate weight for gestational age.

### Conclusion

The cellular immune system (T cell system) begins to develop in the 8th week of gestation in the thymus, and is complete by 15 to 16 weeks. Proliferative responses of fetal and neonatal lymphocytes to mitogens, antigens, and allogeneic lymphocytes are well developed, although direct cell-to-cell cytotoxicity and mediator production may be deficient (immature).

Rejection of maternal lymphocytes leaking into the fetal circulation may be an important physiologic role for this system. An augmented cellular immune response of the fetus to infectious agents may limit intrauterine infection and modulate the effect of the microorganism. The fetal cellular immune response may be impaired in certain genetic disorders, congenital infections, or maternal malnutrition.

The antibody immune system (B cell system) begins to develop in the 11th week of gestation but is completely established only after birth. A sequence of B cell development and immunoglobulin synthesis of IgM, IgG, and IgA is regularly observed. Although there is some IgM production during late gestation, the fetus and newborn rely on transplacental maternal IgG to prevent infection. Stimulation of IgM synthesis by the fetus occurs when there is intrauterine antigenic challenge such as may occur with congenital infection. This augmented IgM synthesis probably

does not help to limit established infection but is of some use in the diagnosis of congenital infection by analysis of cord IgM levels and antibody content.

The phagocytic and macrophage immune system precursors are present in the liver at eight weeks. No consistent developmental abnormalities of phagocytosis have been described, although several preliminary reports suggest some impairment of neonatal phagocytosis. Macrophage abnormalities are present in newborn experimental animals; human studies are not available.

The complement system begins to develop at eight weeks of gestation, before antibody synthesis. Complement activity and individual complement components increase slowly during gestation and reach only 50% to 75% of normal postnatal levels at the time of birth. This diminished complement activity may contribute to opsonic defects in the term neonates.

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# Routes of Fetal Infection and Mechanisms of Fetal Damage

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Severe infections of many kinds can lead to fetal death, abortion, or premature birth. Less severe infections lead to morphological malformations if they occur early enough in pregnancy or to functional disorders if they occur later; and mild infections lead to subtle disturbances or perhaps none at all. Hypothetically, there are at least five points at which the human fetus could be affected. These will now be considered.

## Unfertilized Egg or Germinal Epithelium

The possibility that viruses may infect unfertilized eggs is intriguing, raising the idea of vertical transmission from generation to generation. Mumps virus certainly infects the ovaries and coxsackievirus B and other agents are probably also capable of doing so. However, these agents are usually lytic and would be expected to destroy the eggs.

Lymphocytic choriomeningitis virus has been identified in the germinal epithelium of mouse ovaries, and this is presumably the means by which carrier mice pass on infection to their offspring.<sup>1</sup> Demonstration of a similar phenomenon in man has yet to be made.

## Fertilized Egg or Blastocyst

Fertilization and the cell divisions that follow make the egg more susceptible to virus infection. Type C particles associated with mouse leukemia have been demonstrated in cells of the blastocysts of mouse embryos<sup>2</sup> and in some normal human

placentas.<sup>3</sup> It has even been suggested that type C particles are present overtly or latently in all human cells.<sup>4</sup> Thus, congenital viral infection may be part of our heritage, and development of leukemia later in life or even autoimmune disease may be determined at the moment of conception.

Simian virus 40 (SV40) and polyoma virus are both DNA-bearing oncogenic viruses belonging to the papova group, native to the rhesus monkey and to mice, respectively. In their behavior in mouse embryos they differ in instructive ways. Although SV40 normally produces only abortive infection in mouse cell cultures, in early mouse embryos it produces a toxic effect without replication, while later stages of mouse embryo development are resistant.<sup>5</sup> In contrast, polyoma, which is a virus of mice, does not affect two-cell embryos, but does replicate in the endodermal cells of blastocysts. The blastocysts appear normal, however. Thus, we have here examples of an abortive heterospecific infection causing death, whereas a productive homospecific infection causes little or no effect on the embryo.

Infection during pregnancy with Sendai virus causes fetal wastage. Fluorescent antibody staining shows that both spontaneously infected and artificially infected fertilized eggs contain Sendai virus antigen. In both types of infection the infected eggs degenerate.<sup>6</sup>

It would be difficult to demonstrate lethal infection of the egg in women. If death of the ovum occurred after fertilization but before implantation, a pregnancy would not be known to have occurred, and no epidemiological connection between infection with a

particular agent and fetal wastage would be obvious. It would also be difficult to detect the results of abortive infection of embryos, since the usual traces of intrauterine infection in the form of virus antigens or antibody to those antigens might not be present.

## Uterine Endometrium

Examples of fetal infection by pre-existing endometrial disease have not been described, but it is possible that a bacterial abscess or a toxoplasmic cyst could rupture into the placenta.

## Infection Across or Through the Placenta

These will be considered together, since the distinction between the two routes is open to argument. The Table lists most of the agents that can traverse the placenta of humans.

**Bacteria.**—There are a number of routes by which bacteria might reach the placenta, aside from ascending through the cervix. Organisms might traverse the fallopian tubes in the course of peritoneal or tubal disease; or organisms in the maternal blood might cause abscesses in the uterine decidua, with subsequent direct invasion of the placenta; or finally, organisms carried in the blood might be deposited around the villi, there to begin invasion of the fetal side of the placenta. Passage of bacteria from maternal blood to placental villi seems the most likely route, a conclusion supported by histologic observations.<sup>7</sup>

A great many bacteria can infect the placenta<sup>8</sup> but the organisms that cause pyelonephritis, in particular *Escherichia coli*, are probably the most common. Clinically evident maternal pyelonephritis causes premature birth from 20% to 50% of

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the time, whereas subclinical pyelonephritis may also put the fetus at increased risk.<sup>9</sup> The mechanism by which bacteremia produces prematurity is not known. Endotoxin may play a role; at least in rabbits, endotoxin alters placental permeability.<sup>10</sup> Lymphocytes of infants born to bacteriuric mothers were found to be sensitized to *E coli* antigen.<sup>11</sup>

*Listeria monocytogenes* may be transmitted to the fetus by direct contact with the vaginal flora during parturition. Transplacental infection in association with placentitis also occurs. The mother may have an influenza-like illness and presumably has bacteremia at this stage. Infection during the first half of pregnancy may cause abortion during the second half, prematurity, and neonatal disease. The infant is either born ill or becomes so shortly after birth. The amniotic fluid is often frankly abnormal in appearance. The aborted fetus or dead infant often shows multiple granulomata.

Infection of the placenta with *Treponema pallidum* results from spirochetemia, which is an integral part of secondary syphilis. In severe cases, widespread placentitis with proliferative vasculitis, villous inflammation, and tremendous edema is found preceding fetal infection.<sup>7</sup> Syphilis acquired during the first 16 to 20 weeks of gestation does not affect the fetus, or even cause abortion.<sup>12</sup> Nevertheless, treponemes are found in fetuses infected early in pregnancy. Silverstein suggests that no disease occurs at this stage because the fetus lacks an immune response.<sup>13</sup> *Treponema pallidum* does not of itself damage human cells. The pathologic change of congenital syphilis is primarily that of an inflammatory response<sup>14</sup> related to the development of fetal antibody-producing cells after the fifth month of gestation.

**Fungi.**—Both *Candida albicans* and *Coccidioides immitis* have rarely been transmitted to the fetus. Either infection causes severe placental disease.<sup>7</sup>

**Protozoa.**—Although toxoplasmosis in the pregnant woman may cause fever with lymphadenopathy and rash

or other nonspecific symptoms, most acquired cases are asymptomatic. Congenital cases do not occur in infants born of mothers seropositive before pregnancy.<sup>15</sup> Thus, it appears that primary infection with *Toxoplasma* is necessary for congenital infection.<sup>16</sup> Some 75% of infections during the first and second trimester spare the fetus; in the third trimester infection, only 40% of fetuses are spared. On the other hand, fetal damage occurs most frequently in the first and second trimesters.

The simplest explanation for these findings is that *Toxoplasma* must penetrate the placenta first and the chances of an organism doing so increase as pregnancy progresses. As the size of the placental surface increases considerably during the latter part of pregnancy when the chorionic membrane becomes thinner, the chances of a *Toxoplasma* invading the placenta may become greater. However, infection of the conceptus early in pregnancy, when the central nervous system (CNS) is developing, has greater potential for producing damage.

An alternative explanation is that later in pregnancy, maternal antibody passing to the fetus results in encystment of the organisms before extensive damage can occur. The presence of unencysted organisms on microscopic examination of the brain of congenital cases makes this possibility less likely.<sup>17</sup> Furthermore, children born with subclinical congenital toxoplasmosis who were apparently normal at birth and who were untreated had an intelligence quotient 17 points lower than matched controls after two to four years.<sup>18</sup> Three treated infants were on par with their matched controls.

With regard to the mechanism of fetal injury in toxoplasmosis, the most obvious hypothesis is that the density of cysts occurring in the brain and the host's inflammatory response to the presence of this foreign material are directly related to the extent of disease. This process goes on after birth and the salutary results of treatment in the newborn period suggest that some of the CNS damage takes place postnatally.<sup>18</sup>

Transmission of malarial parasites to the human fetus has been proved, which implies infection by passage of maternal red blood cells across the placenta.<sup>19</sup>

**Mycoplasma.**—Several *Mycoplasma* strains have been isolated from the genital tract of man. Infection occurs at birth in about 8% of full-term infants.<sup>20</sup> Subsequently, there is little possibility for infection until the age of sexual exposure.

The importance of mycoplasmas is their possible causation of spontaneous abortion and prematurity. The T strains of *Mycoplasma* and *M hominis* have been isolated from several spontaneous abortions, and the presumption is that the infection occurred via the maternal bloodstream.<sup>20</sup>

The argument for causation of prematurity rests on the observation that neonatal infection rates are highest in prematurely born infants and that tetracycline administered to pregnant women seemed to have a salutary effect on the birth weight of their infants.<sup>21</sup> Moreover, birth weight was inversely related to the rate of isolation of mycoplasmas from the mothers and from the infants at birth.<sup>20,21</sup> One can speculate that mycoplasmal colonization of the mother has a deleterious effect on the fetus, but just how this effect is accomplished is unknown.

**Viruses.**—The best-described example of transplacental infection is congenital rubella. In this disease there is a prolonged viremia during the week before the development of the rash. When this happens in a pregnant woman, the fetus becomes infected. Many epidemiological observations testify to the increased spontaneous abortion rate that occurs after maternal rubella. Whereas the ten-year British study reported a threefold increase compared to controls,<sup>22</sup> no estimate can include abortions occurring in the several weeks after fertilization, before a woman knows herself to be pregnant, as may be true for any viral infection.

Whether viruses simply pass through the placenta into the bloodstream of the fetus, or whether placental infection is always the prelude

Infections Transmitted by the Transplacental Route		
Agents	Proved	Probable
Bacteria	Most species, particularly <i>Enterobacteriaceae</i> ,* <i>Listeria</i> ,* <i>Treponema</i> and other spirochetes, <i>Vibrio fetus</i> , <i>Mycobacterium tuberculosis</i>	
Fungi	<i>Candida</i> *	
Protozoa	<i>Toxoplasma</i> , malaria, trypanosomes	
Mycoplasmas		T strains*
Viruses	Cytomegalovirus, rubella, herpes simplex,* varicella, vaccinia, polio, western equine encephalomyelitis	Coxsackievirus B,* hepatitis B, mumps, influenza, measles

\* More commonly causes ascending infection from vagina.

to fetal infections is conjectural. The latter hypothesis seems more reasonable. Although leaks in the placenta probably occur, it is doubtful whether sufficient numbers of infecting organisms get across in this manner to explain the frequency of congenital infection. The direct route does not explain how there can be congenital rubella in only one of a set of twins.

Virus can be recovered more frequently from the placentas of rubella-exposed fetuses that were surgically aborted than from the fetuses themselves. These data counter an alternative explanation that placental infection follows multiplication in the fetus and a secondary fetal viremia.

A 90% incidence of fetal infection was noted when fetuses that were aborted after serologically proved maternal rubella in the first trimester were examined.<sup>21</sup> Virus recovery was reported in 90% of products of conceptions aborted because of proved maternal rubella<sup>22</sup>; the authors concluded that "there was no evidence that the placenta was any real barrier to fetal infection." On the other hand, it has been proved that there are infants congenitally infected with rubella virus who survive without demonstrable defects.<sup>23</sup> Fetuses exposed to maternal viremia nearly always become infected with rubella virus. However, the age of the fetus, the level of viremia, and the cellular response to infection all determine whether the rubella syndrome will occur.

Implicit in the above is the idea that congenital rubella is simply the result of maternal viremia occurring in the absence of humoral antibody. Recently, it has been suggested that cell-mediated immunity to rubella is depressed in pregnancy and that this may contribute to the dissemination of virus to the fetus.<sup>24</sup>

Once the rubella virus infects the fetus, a chronic, nonlytic infection is established. This was first demonstrated in vitro.<sup>25</sup> Infection of strains of human fibroblasts, once established, persists for weeks or months in stationary cultures. When the cell cultures are placed in fresh vessels under conditions that allow uninfected control cells to divide, mitotic inhibition is observed. Rubella virus carrier cultures derived from congenitally infected infants exhibit decreased cell division rate, and are not susceptible to cure with antibody. They also show resistance to superinfection not mediated by interferon.<sup>26</sup> Crucial evidence was added when the number of cells in fetal organs was measured. There was a 50% decrease in rubella-infected fetuses compared to controls.<sup>27</sup> The possibility that this inhibition of cell division is mediated by a soluble protein was suggested.<sup>28</sup>

Four additional mechanisms of fetal damage by rubella virus remain to be considered. First, it seems certain from histologic examination of the brain and the organ of Corti that much rubella damage is vascular in

origin. Damage to endothelial cells leads to thrombosis of small blood vessels and surrounding tissue necrosis.<sup>11</sup>

Second, some cells, particularly those in the lens of the eye, are probably killed by rubella virus.

Third, study of rubella carrier cell cultures from aborted fetuses shows increased incidence of chromatid breaks. Specific chromosomal anomalies in fetuses with rubella syndrome have been reported or suggested,<sup>12</sup> but the evidence that chromosomal abnormalities are a cause of rubella anomalies is not compelling.

Fourth, there are many parts of the rubella syndrome that are the direct result of persistent infection. Among these are the encephalomeningitis, which often continues during the first year of life<sup>13</sup>; the cataracts, which may grow worse after birth and in which the virus survives for years<sup>14</sup>; the postnatal hepatitis; the thrombocytopenia, which is partly due to megakaryocyte destruction and which eventually resolves after birth; the pneumonias, which occur in the early months of postnatal life; the myocarditis, which may be present at birth<sup>15</sup>; and the osseous lesions.

The relationship between the persistent virus carrier state and function of the immunologic system is difficult to resolve, as the facts are somewhat confusing. It is clear that (1) lymphocytes of normal individuals can be infected in vitro and show decreased phytohemagglutinin (PHA) response after infection<sup>16</sup>; (2) lymphocytes from infants with rubella syndrome often carry virus for long periods after birth<sup>17</sup>; (3) infants with congenital rubella syndrome usually have high titers of rubella antibody,<sup>14</sup> particularly of the IgM type; (4) humoral antibody responses to antigens such as diphtheria toxoid, tetanus toxoid, blood group antigens, and types 1 and 3 poliovirus are decreased in infants with rubella syndrome when they are excreting virus, but not after they stop.

Just recently, absence of cell-mediated immunity to rubella was demonstrated in nine of 12 infants with rubella syndrome.<sup>18</sup>

One can formulate an explanation

for viral persistence in the following way:

1. Antibody-forming cells (B lymphocytes) are only partly damaged in infants with rubella syndrome.

2. Thymus lymphocytes are themselves infected with the virus, do not go into mitosis, and therefore have reduced competence to destroy infected cell clones. The occasional defective PHA response, the relative immune defects, and the slow conversion from IgM to IgG antibody would be explained by damage to lymphocytes.

3. Antibody to rubella, secreted by uninfected B lymphocytes, is stimulated in utero without the development of tolerance. When uninfected clones of T lymphocytes become available, they attach to and destroy infected cells, releasing virus that is then neutralized by the secretions of B lymphocytes. It is difficult, however, to reconcile the absence of cell-mediated immunity to rubella in those infants with rubella syndrome who no longer excrete the virus. Since the interferon response remains intact in infants with rubella syndrome, persistence cannot be explained by failure of this mechanism.

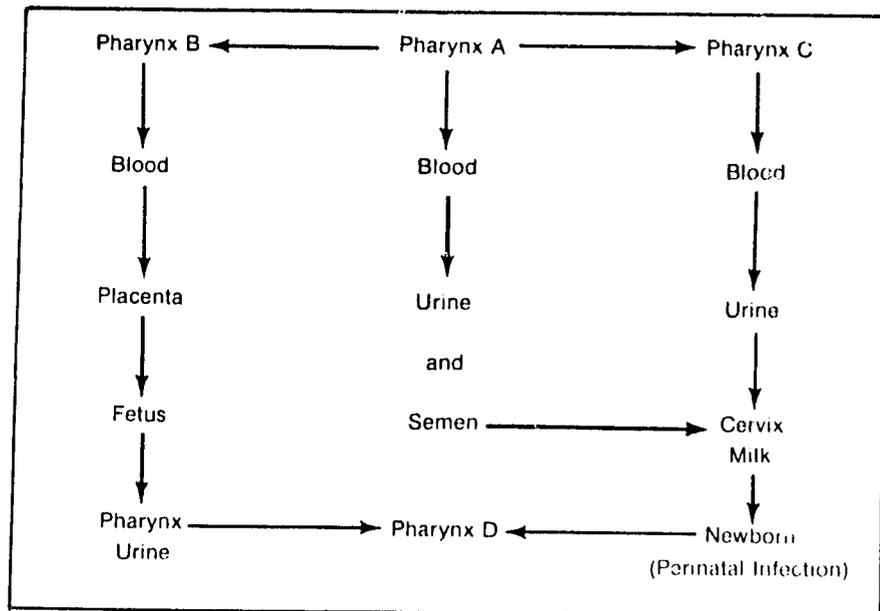
Patterns of acquisition of cytomegalovirus (CMV) antibodies vary from early seropositivity in many developing countries, to slow seroconversion with a high percentage of susceptible child-bearing women in urban centers of industrialized countries. In the United States, Great Britain, and Australia, about 1% of all newborns are infected with CMV at birth, of which about 10% sustain brain damage.<sup>39</sup>

Since acquired CMV infection often becomes latent, and since it has been isolated from the cervix of up to 13% of pregnant women, from the urine of 6%, and from the breast milk of 25%, the problem of distinguishing the effect on the fetus of primary vs recurrent infection is not easy.

The evidence that it is primary infection that is transmitted to the fetus can be stated as follows:

1. Verified primary infection of the mother has resulted in transmission to the fetus.<sup>40</sup>

2. In the industrialized countries it



Routes of cytomegalovirus infection. A = male carrier; B = seronegative pregnant woman; C = seronegative woman who later becomes pregnant; D = seronegative child.

is the younger women who most commonly deliver CMV-infected infants.<sup>41</sup>

3. Mothers who give birth to CMV-infected infants seroconvert during pregnancy and have IgM CMV antibodies.<sup>42</sup>

The sole argument against the above is that recurrent congenital CMV infection has been reported.

Assuming that fetal infections are most often the result of primary maternal infection, it is probable that placental infection precedes invasion of the fetus. Cytomegalovirus has been found more often in the placentas than in the fetuses.<sup>43</sup>

The Figure illustrates the common routes of CMV infection. Carriers can infect pregnant women, who then develop primary infection and transmit it to the fetus. The newborn can be infected by a female carrier after birth by exposure to cervical secretions or milk. Cytomegalovirus probably also can be transmitted venereally, judging from the high rate of infection in genital secretions.<sup>44</sup> Recurrent congenital infection in siblings might be due to passage of maternal white blood cells across the placenta, with cell-to-cell passage of CMV. Transfused blood can cause acquired CMV infection, even though the donors are immune and pass anti-

body to CMV in the same infusion.

The mechanism of fetal damage by CMV is incompletely described. Since CMV is lytic in human fibroblasts, much of the damage may simply be the result of cell destruction. Cytomegalovirus-infected infants have fewer cells than the controls.<sup>45</sup> This is true in the presence or absence of necrosis or inclusion bodies. In fact, the lytic behavior of CMV in human fibroblasts is deceptive: in epithelial cells, infection appears to be much more benign. A CMV strain highly lytic in human fibroblasts inoculated into cultures of fibroblasts that had been transformed to epithelial cells by SV40 infection results in a chronic carrier state, mimicking somewhat the situation in vivo. Cocultivation of trypsinized cells shows that proportionately more cells are carrying CMV than is apparent from disruption of inoculated cultures. In addition, macromolecular metabolism in infected fibroblasts is grossly altered in two opposite directions: cellular DNA synthesis is turned off, but cellular RNA and protein synthesis increases. Whether the new protein is cellular or viral is still unclear, but the net effect is the production of metabolically overactive cells that cannot divide.

Cytomegalovirus is another agent

that persists in the infected infants after birth. Whether postnatal virus replication further damages the infant is unknown.

Mumps is a viremic infection, and although the published reports are not in complete agreement, it appears that mumps in pregnancy increases the abortion rate. More controversial is the proposed association between mumps and endocardial fibroelastosis.<sup>16</sup>

Newborn animals injected intracerebrally with mumps, influenza, or parainfluenza viruses may subsequently develop aqueductal stenosis owing to destruction of the ependymal cells.<sup>17</sup> Gliosis does not develop and it is difficult to diagnose such an infection on pathologic grounds unless one searches by immunofluorescence for the specific viral antigens, or determines antibodies against specific viruses.

Fetal brain damage may also result from selective lysis of dividing cells. The best example of this is provided by the H-1 picornavirus, which destroys the cerebellar granular cells in immature cats or hamsters.<sup>18</sup>

Other mechanisms that have been shown to operate in animals, but not yet in man, are alteration of neural tube closure by influenza virus, or cavitation of the brain through cell destruction in bluetongue disease of sheep.

Recent support for the supposition that influenza A<sub>2</sub> virus is teratogenic was provided by the development of hydrocephalus in monkeys inoculated intracerebrally with this virus during the fetal state.<sup>19</sup> Thus, some viruses may be capable of destroying certain brain cells during fetal development, leaving behind morphological derangement without inflammation.

#### Ascending Infection

If the fetus survives the risks of transplacental infection, it must still face the hazards surrounding parturition. These hazards can be placed in two categories: first, the amniotic infection syndrome<sup>20</sup> in which agents invade the amniotic cavity from below, assisted by premature rupture of membranes or by passage of organisms through intact membranes

when labor is prolonged. Second, the infant may be exposed to pathogenic organisms at birth.

**Bacteria.**—The critical event with regard to the likelihood of bacterial infection is the rupture of membranes. When this occurs more than 24 hours antepartum, the risk of infection increases dramatically. The histologic demonstration of placental inflammation rises from 6% to 22% after 24 hours.<sup>21</sup> Positive amniotic cultures begin to appear as early as six hours after rupture. Neonatal mortality in the premature group rises significantly if delivery does not take place within 24 hours.

Pneumonia is commonly associated with the amniotic infection syndrome, probably resulting from aspiration of infected amniotic fluid. Postnatally acquired pneumonia yielded bacteria 93% of the time, whereas pneumonia present at birth was culture positive in only 63%.<sup>22</sup> Whether the instances of negative bacterial cultures represented viral infection is an unanswered question.

**Fungi.**—*Candida albicans*, a common organism in the vagina, is often acquired by the newborns who later develop thrush. Cases of amniotic infection associated with placentitis do occur, but with surprising infrequency considering the ubiquity of the organism.<sup>7</sup>

**Viruses.**—Herpesvirus hominis (HVH) type 2 is one of the most prevalent venereal infections. In rare cases it crosses the placenta and causes infection of the fetus that is clinically similar to cytomegalic inclusion disease.<sup>23</sup> Infection before the 32nd week of pregnancy results in abortion in a third of cases, probably as a result of direct viral invasion.<sup>24</sup> Maternal genital herpetic lesions between the 32nd and 40th weeks of gestation result in a 10% incidence of disease and prematurity in neonates. If active vulval lesions were present at the time of vaginal delivery, or if at the time of cesarean section the membranes had been ruptured more than four hours, the risk of fetal infection was 40%. Infants delivered by cesarean section before membranes had ruptured were spared. This implies that the herpetic infection as-

cends via the amniotic cavity or results from contamination of the infant during expulsion from the birth canal. The frequency of this occurrence in the United States is about one in 6,000 births.

The mechanism of fetal damage in HVH infection can be ascribed to the direct lytic action of this highly cytopathogenic virus in two organs: the brain and the liver. In the brain, destruction of nerve cells leads to the development of large necrotic areas and cerebral edema, which may sometimes be compatible with life but is certainly incompatible with normal development. In the liver, necrosis of parenchymal cells precipitates liver failure and disseminated intravascular coagulation. Milder forms of neonatal infection are also common.

#### Conclusion

In summary, the mechanisms by which microorganisms damage fetuses are evident clinically as abortion, malformation, growth retardation, prematurity, stillbirth, perinatal death, mental retardation, and more subtle disorders. At the tissue level, the microorganisms interfere with the function of brain, heart, lungs, or liver. At the cellular level, the actions of microorganisms are varied and may be multiple. They may cause cell destruction (eg, CMV in the whole fetus, toxoplasmosis in the brain, and HVH in the liver). The vascular endothelium is particularly susceptible to damage. They may cause metabolic derangement leading even to death, inhibition of cell division (eg, rubella), or specifically invade dividing cells (eg, some myxoviruses in animal model systems). Microorganisms might theoretically cause chromosomal disorders. The inflammatory reaction of the fetus against the organism may result in disease as in the case of syphilis, whereas in rubella it seems likely that part of the disease is immune suppression by direct action on the lymphocytes. Finally, it is possible that certain agents are transmitted vertically, causing neoplastic or autoimmune disease only when some repressor mechanism breaks down later in the life of the individual.

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# Fetal Malnutrition and Postnatal Immunocompetence

Ranjit K. Chandra, MD

The resemblance between fetal growth retardation (FGR) and malnutrition acquired postnatally is striking. The similarities include suboptimal physical development (including retarded bone age), loss of subcutaneous fat, dry skin with reduced turgor, hypoglycemia, hypothermia, frequent and severe infections, and high mortality. In addition, restriction of growth during fetal life is associated with perinatal asphyxia, minimal postnatal weight loss, polycythemia and elevated levels of erythropoietin, and increased incidence of congenital malformations. Increased frequency of infection in such infants is an established clinical observation. Failure or impairment of immune defense mechanisms of infants with FGR is, therefore, suspected as a basis for the susceptibility of such infants to infection. Heretofore, there have been no comprehensive systematic analyses of the effect of fetal malnutrition on postnatal immunocompetence, unlike undernutrition acquired after birth for which considerable data are now available.<sup>1-4</sup> The following is a summary of our recent studies relevant to this problem.

## SUBJECTS AND METHODS

The diagnosis of FGR was made in 26 infants whose birthweights were below the tenth percentile on standard intrauterine growth charts (Fig 1). The length of gestation was assessed by maternal history of

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Table 1.—Lymphocyte DNA Synthesis in Response to Phytohemagglutinin\*

Group	AB Pooled Serum		Autologous Serum	
	Range	Mean	Range	Mean
Healthy newborn (No. = 26)	81-145	110	104-178	143
FGR (No. = 26)	34-99	69	56-101	87

\* Percent increase over unstimulated values.

Table 2.—Percentage of Peripheral Blood T Lymphocytes

Group	Age		
	Newborn (Cord Blood)	1 mo	3 mo
Healthy newborn (No. = 26)	87	80	72
FGR (No. = 26)	58	49	44

Table 3.—Opsonic Activity of Plasma

Group	Concentration of Plasma, %			
	20	10	5	2.5
FGR (No. = 26)	361*	331	190	133
Healthy newborn (No. = 26)	444	458	229	141
Healthy adults (No. = 20)	488	488	415	295

\* Number of yeast particles ingested per 100 PMNs in 30 minutes.

Table 4.—Migration Ability of PMNs

Group	Random Mobility, mm	Chemotaxis	
		PMNs/HPF*	Distance Migrated†
FGR (No. = 26)	7 (4-11)‡	7 (3-11)	33 (22-41)
Healthy newborn (No. = 26)	18 (14-20)	21 (18-29)	87 (56-105)

\* Number per high power field.

† Percent of healthy adult controls.

‡ Mean value, with range in parentheses.

Table 5.—PMN Function

Group	Phagocytosis (Organisms/100 PMNs/30 min)	Bactericidal Capacity, %	NBT Test*
FGR (No. = 26)	455 (210-560)†	31.6 (11-63)	0.07 (0.01-0.23)
Healthy newborn (No. = 26)	429 (180-534)	9.8 (4-25)	0.23 (0.11-0.41)
Healthy adults (No. = 20)	488 (330-575)	4.7 (1-11)	0.32 (0.21-0.50)

\* Change in optic density.

† Mean value, with range in parentheses.

the last menstrual period and by neurological examination of the newborn; it varied from 29 to 42 weeks. Seventeen infants had a gestational maturity of 37 weeks or more. Two infants weighed more than 2,500 gm (5.5 lb), which is the World Health Organization accepted standard of maturity, but were below the tenth percentile of expected weight. All subjects showed clinical features of retarded growth. Full-term infants weighing more than 2,500 gm at birth and healthy adults served as controls for various tests. (Informed consent was obtained from each parent or guardian concerning the investigation of the infants in accordance with institutional policies.)

Serum levels of IgG, IgA, and IgM were estimated immunochemically by the method of single radial diffusion in agar, using monospecific rabbit serum.<sup>7</sup>

#### Development of Humoral Immunity

Tetanus toxoid was injected intramuscularly in a dose of 40 flocculation units and repeated after four weeks. Blood for estimation of antitoxin activity by the tanned red cell hemagglutination method<sup>8</sup> was obtained ten days after each inoculation.

Typhoid vaccine containing  $10^8$  killed *Salmonella typhi* organisms per milliliter was given subcutaneously in a dose of 0.1 ml and repeated after 11 days. Antibodies against O and H antigens were determined.<sup>7</sup>

Trivalent live attenuated poliovirus vaccine was given orally to infants older than 2 months in a single dose containing  $10^6$  TCID<sub>50</sub> of each type of poliovirus. Type 1 poliovirus neutralizing antibody titers were determined one, four, and eight weeks following immunization.

#### Development of Cell-Mediated Immunity

Delayed cutaneous hypersensitivity response was investigated by applying a 10% solution of 2,4-dinitrochlorobenzene (DNCB) in acetone to one forearm, and a challenge dose of 0.2 ml of 0.5% DNCB solution to the opposite forearm one month later. The challenge site was examined after 48 hours and the reaction graded as positive (vesiculation or induration or both) or negative (absence of induration and vesiculation with or without mild erythema).

Lymphocyte response to mitogen was measured by means of tritiated thymidine incorporation into nucleic acid by lymphocytes stimulated by phytohemagglutinin (PHA).<sup>9</sup> Cells were washed twice and duplicate cultures established containing  $2 \times 10^6$  lymphocytes per milliliter in medium 199 plus 15% pooled AB serum or au-

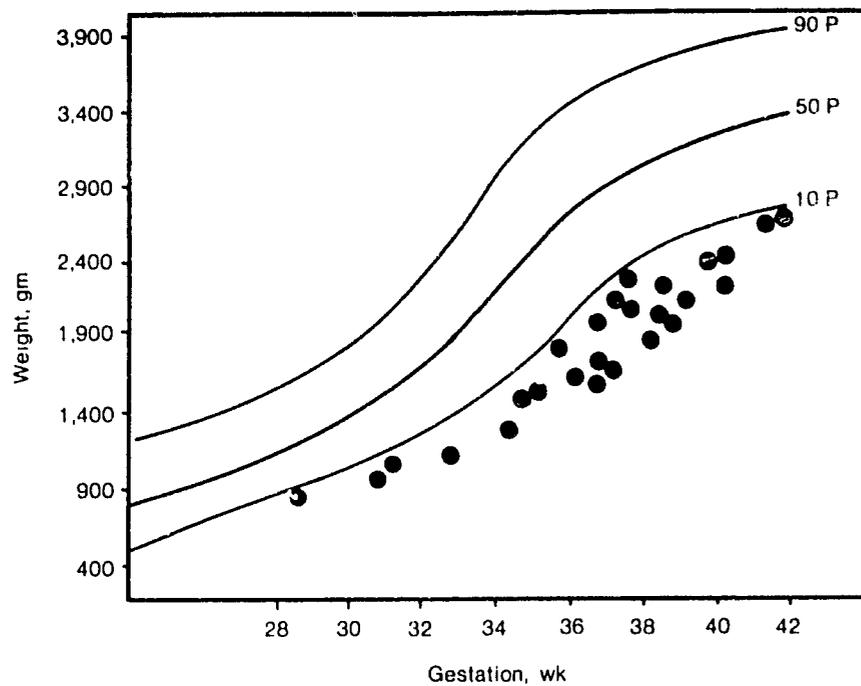


Fig 1.—Birth weight and gestational maturity of 26 infants, plotted on standard percentile (P) chart of fetal growth.

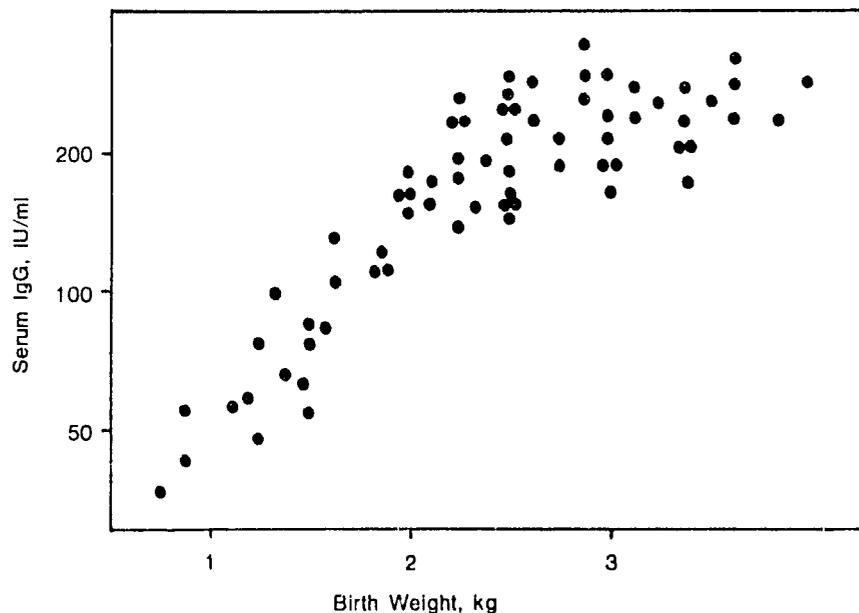


Fig 2.—Serum levels of IgG related to birth weight.

tologous serum. The cells were harvested at 96 hours. Stimulation indices were derived by dividing counts per minute of PHA-stimulated cultures from those of unstimulated controls, and expressed as a percentage.

Thymus-dependent (T) lymphocytes were detected by the method of Jondal et al,<sup>10</sup> except that the leukocyte-sheep red blood cell pellet was fixed with glutaraldehyde before being counted in a cover

slip preparation. Two hundred lymphocytes were examined and all cells binding more than three sheep red blood cells were considered to be both lymphocytes and T cells. Determinations were made on cord blood as well as samples taken one and three months after birth.

Opsonic function of plasma was measured with 0.1 ml of yeast suspension ( $1 \times 10^8$  particles per milliliter) preincubated for 30 minutes in the presence of

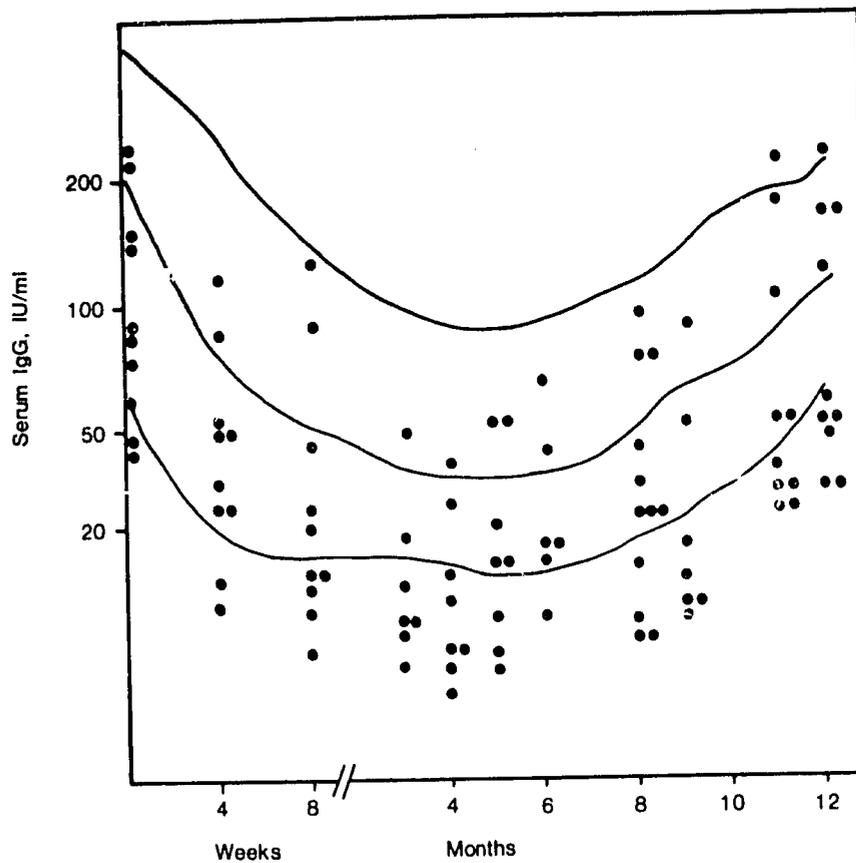
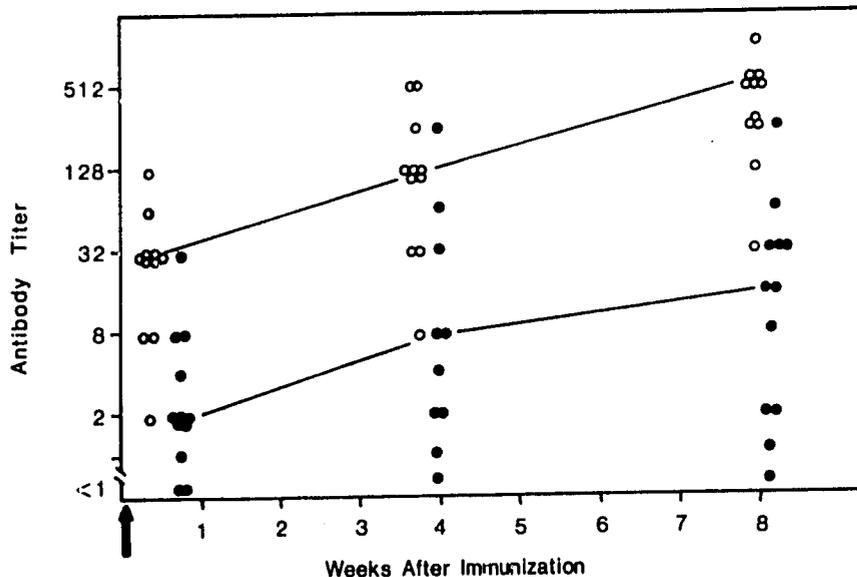


Fig 3.—Longitudinal follow-up of serum IgG levels in ten infants with FGR, plotted on mean and range of values for healthy children.

Fig 4.—Neutralizing antibody titers to poliovirus type 1 in full-term, healthy infants (open circles) and in infants with FGR (closed circles). Mean values are indicated for both groups at one, four, and eight weeks following immunization.



Group	Gluten	Milk	Egg White	Egg Yolk
FGR (No. = 15)	6*	9	5	7
Healthy infants (No. = 15)	0	1	0	0

\* Number positive.

specified dilutions of plasma mixed with 0.2 ml of polymorphonuclear leukocyte (PMN) suspension containing  $5 \times 10^6$  cells per milliliter. The mixture was kept in a water bath at 37 C for half an hour under constant rotation. Wright-stained smears were examined and the number of yeast particles ingested by 100 PMNs was counted.

Random mobility of PMNs was determined in a suspension containing  $5 \times 10^6$  cells per milliliter in which the cells were packed into vertically placed microhematocrit tubes. The distance traveled by the advancing front of cells in six hours was measured with an ocular micrometer.

Chemotaxis was measured by modification of the Boyden assay<sup>10</sup> as described by Wilkinson.<sup>11</sup> Chemotactic factor was generated by incubation of equal parts of serum with standardized suspensions of *Escherichia coli* from 18-hour broth cultures for five minutes at 37 C. Two end points were established: one, the number of cells that had migrated to the lower surface of the  $3\mu$  pore filter in three hours and two, the distance traveled through such a filter by the leading front of at least four cells, and expressed as percentage of similar performance by cells from healthy adults.

Phagocytosis was measured by the same method used to measure the opsonic function of plasma, except that the discriminatory variation was the PMN suspension from either the study subject or the healthy control, using 20% plasma from healthy adult donors in the incubation mixture.

Intracellular bacterial killing was measured by the bactericidal test of Quie et al,<sup>12</sup> using *Staphylococcus aureus* and surface counting of colonies on nutrient agar plates. The intracellular bactericidal capacity was calculated as the ratio of the number of viable intracellular bacteria at 140 minutes to the number at 20 minutes, using control plasma from healthy adult donors in the culture medium.<sup>7</sup>

Reduction of nitro blue tetrazolium (NBT) by the PMNs was measured by the

method of Baehner and Nathan.<sup>11</sup>

Precipitating antibodies to food such as gluten, whole milk, egg white, and egg yolk were detected by the double diffusion in agarose method.

## RESULTS

There was a significant correlation between cord blood IgG level and birth weight ( $r = .765$ ) as shown in Fig 2. The levels reached a plateau at the birth weight of 2,250 gm (5 lb), with no further increment with increase in weight.

There was no difference between serum IgA and IgM levels in healthy infants and those in infants with FGR.

Results of a follow-up study of serum IgG concentration in ten infants with fetal malnutrition are shown in Fig 3. There was a wide scatter of values, but most of the estimations were in the lower end of the normal range or below it. At the period of 3 to 5 months when there is a physiologic decrease in the IgG level, the levels in infants with FGR were extremely low. This was associated with frequent bacterial infections suggestive of antibody deficiency syndrome. Subsequently, the IgG concentration tended to increase.

### Development of Humoral Immunity

There was no significant difference in *t. tanus* antitoxin levels of study subjects with FGR and those of healthy controls. Agglutinins against *S typhi* O antigen were conspicuously absent ( $\leq 1:16$ ) in both groups of neonates. There was no difference between antflagellar antibody titers achieved in full-term newborns and those in infants with FGR. Adult controls showed a much higher response to this antigen, as well as a substantial rise in anti-O antibody (1:1,024). Seroconversion was achieved in eight out of 12 infants with FGR given trivalent live poliovirus vaccine, but the antibody titers were significantly lower than those in healthy, full-term controls (Fig 4).

### Development of Cell-Mediated Immunity

Sensitization with DNCB failed in 12 of 15 infants with FGR compared

to seven of 15 healthy newborns and only one of ten healthy children 1 year old. There was a significant reduction ( $P < .01$ ) in DNA synthesis by lymphocytes in response to PHA by cells from infants with FGR (Table 1). There was also a significant reduction ( $P < .01$ ) in the number of circulating T cells in infants with FGR (Table 2).

There was a slight reduction in plasma opsonic function of infants with FGR when the test was run with 20% serum. When the plasma concentration was 10% or lower, the difference was statistically significant ( $P < .01$ ). Compared to adult controls, healthy newborns showed a similar trend at lower ( $\leq 5\%$ ) concentrations of plasma (Table 3).

Random mobility, as well as chemotactic activity of PMNs from infants with FGR were both significantly reduced (Table 4).

There was no significant difference in phagocytosis by cells from adults, healthy newborns, and infants with FGR (Table 5). The PMNs from healthy newborns were able to kill ingested bacteria almost as effectively as cells from adult subjects. However, PMNs from infants with FGR showed a severe reduction in bactericidal capacity. Similarly, phagocytosis-stimulated NBT reduction was severely impaired in PMNs from infants with FGR. Healthy newborns showed only slight impairment ( $.05 > P < .1$ ) compared with adult controls.

Precipitating antibodies to several food antigens were commonly observed in children with FGR, but not in healthy infants (Table 6).

## COMMENT

Our study has demonstrated a variety of defects in immunocompetence of infants with FGR, many of which parallel observations in older infants and in children with malnutrition acquired postnatally.<sup>12</sup> Additionally, there was a significant reduction in maternal-fetal transfer of IgG, which must reflect placental dysfunction since the time period available for such a transfer was adequate in the majority of infants studied. Preliminary data suggest that interference

with placental transfer is greatest for IgG 1.<sup>13</sup>

The presence of circulating antibodies against some common food antigens in infants with FGR is interesting. This conceivably might reflect more frequent and easier access of these antigens to immunologically competent tissue through increased absorption of large molecular proteins through atrophied gut mucosa, as in postnatal malnutrition (R. K. Chandra, MD, unpublished data). An additional possible factor may be defective "scavenger function" of the liver, as seen in patients with cirrhosis. Liver cell dysfunction is a well-established complication of severe undernutrition.

The failure of infants with FGR to develop delayed hypersensitivity to DNCB and impaired DNA synthesis in response to PHA stimulation of lymphocytes indicates a depression of cell-mediated immunity. This may well be the result of selective reduction in the numbers of T cells. The number of rosette-forming cells is decreased in patients with recognized defects in cellular immunity,<sup>14</sup> as well as in those with malnutrition.<sup>15</sup>

Most previous studies of PMN function in the newborn have been done within the first few hours after birth, when adverse perinatal metabolic factors may alter the cellular response. Moreover, no distinction was made between truly premature and small-for-date infants. We have observed that PMNs of infants with FGR exhibit metabolic and functional defects that persist well beyond the neonatal period. Infants with evidence of fetal malnutrition show reduction in PMN mobility, both random and directed, resembling the "lazy leukocyte" syndrome. Such a defect could substantially impair the inflammatory response in vivo and predispose to recurrent infections. The significant reduction in bactericidal capacity of PMNs as well as impaired metabolic response to phagocytosis as judged by the NBT test serve to compound the defect. On the basis of our observations in children with undernutrition,<sup>16</sup> it is likely that these latter abnormalities are reversible. It remains to be shown whether one or

more nutrients such as iron<sup>1</sup> are specifically involved in the causation of PMN dysfunction.

There was little difference in opsonic activity of plasma in various study groups, using 20% serum. At lower serum concentrations, opsonic function was reduced in samples from neonates, and particularly in those with FGR. This suggests a quantitative rather than a qualitative abnormality. Of various factors that promote opsonization, heat-labile complement is important and is not transferred across the placenta. At birth, C3 levels are only one half of adult values, which may explain some of the above findings.

Obviously, the relevance of such abnormalities in immune function in vitro to biological activity in vivo must be considered with caution. The correction of defects after nutritional rehabilitation and "catch-up growth" would support the causal role of fetal malnutrition in producing secondary immunodeficiency.

Our observations raise some further questions. How long does impaired immune competence in FGR persist? What are the possible long-term effects in terms of susceptibility to infection, autoimmune disease, neoplasia, and aging? Is this a threshold phenomenon, or is there a gradient of impairment related to the degree of fetal malnutrition from the severe to the subtle? Since different types of low birth weight seem to be associated with different functional outcome, do defects in immune function result from some but not other varieties of fetal malnutrition? Finally, what critical etiologic role does the deficiency of specific nutrients play?

### Conclusion

Low birth weight associated with term pregnancy produces a syndrome closely resembling malnutrition acquired postnatally. There was a positive correlation between cord blood IgG level and birth weight up to 2,250 gm. Physiologic decrease in IgG level between 3 and 5 months of age was more pronounced in these infants. Antibody response to tetanus toxoid and *S typhi* was comparable to healthy neonates, but antibody response to poliovirus vaccine was impaired. There was a significant reduction in the number of peripheral T lymphocytes, which persisted for at least three months after birth. Cell-mediated immunity, assessed by sensitization to DNCB and lymphocyte response to PHA, was significantly impaired. Opsonic function of plasma was reduced, partly because of lower levels of C3. Phagocytosis by PMNs was normal. There was a severe defect in bactericidal capacity and oxidative metabolism of PMNs following phagocytosis, and in mobility, both random and chemotactic.

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# Diagnosis of Chronic Perinatal Infections

Charles A. Alford, Jr., MD; Sergio Stagno, MD; David W. Reynolds, MD

Clinical diagnosis of perinatal infections in newborns has long been a vexing problem. The causative maternal infections are often asymptomatic (cytomegalovirus [CMV] and *Toxoplasma* infections), or are so mild as to escape notice (rubella, syphilis, herpesvirus, and enterovirus infections).<sup>1</sup> These, in turn, can result in a wide spectrum of disease in the affected infants. Certainly, some neonates have severe disorders with multiple organ system damage and a fulminant course; however, even in this group, where clinical clues are obvious, similarities among symptoms make specific diagnosis difficult.<sup>2</sup> To compound the problem further, it has been recognized in recent years that most infections acquired in utero are asymptomatic or too subtle to be recognized in early life.<sup>3-10</sup> In spite of their apparent innocuous nature in infancy, many of them result in serious and irreparable damage not noticeable until long after birth.<sup>3-6, 11-17</sup> This is especially true with respect to central nervous system (CNS) and perceptual involvement by *Toxoplasma gondii* and rubella virus, and, perhaps to a lesser extent, by CMV, *Treponema pallidum*, herpesvirus hominis (HVV), and other agents as well.<sup>18</sup> For these reasons, the true incidence and the medical and social importance of perinatal infections, particularly the "chronic" forms, have very likely been grossly underestimated.

Laboratory diagnosis of such infections also presents problems. The most direct means is isolation of the responsible pathogens. These are not

always readily recoverable, however, and the techniques required are varied, often tedious, and costly. Their use is usually limited, therefore, to attempted diagnosis in suspect patients with severe disease rather than in those with the more commonly occurring milder forms of infections that may not be recognized by clinical means. Large-scale screening studies, employing isolation methods, have been successfully used to ascertain the incidence and natural histories of individual types of infections, but similar large-scale studies to determine the general significance of perinatal infections as a group are not yet feasible.<sup>6, 7, 19, 20</sup>

Antibody determinations should be of greater diagnostic usefulness because they can be carried out more easily and rapidly than isolation methods. However, interpretation of results obtained from serum samples collected soon after delivery is often complicated by the presence of IgG antibodies that are transmitted from the mother to the fetus.<sup>12</sup> The demonstration of persistent or increasing amounts of antibody during the early months of life can, however, be of diagnostic import, but the necessity for repeated bleedings is a serious problem in large-scale studies to identify newborns with subclinical infections. In addition, there is a delay of diagnosis in individual suspect cases.

Current indications are that the human fetus can muster an immune response when it is appropriately stimulated.<sup>12, 20, 21</sup> Many types of intrauterine infections, both subclinical and symptomatic, can provide the necessary antigenic stimulus.<sup>19, 20, 22-28</sup> Since many, if not most, infants infected in utero, other than those with bacterial infections, are asymptomatic at delivery, it seems clear now

that the developing immunologic mechanism of the fetus, viewed in its entirety, is quite capable of confining the predominantly intracellular infections, particularly when they are acquired late in gestation.

At least part of the fetal antibody can be identified from the large pool of maternally transferred antibodies by two approaches: detection of IgM fetal antibody specific for a given pathogen, primarily by fluorescence microscopy,<sup>10, 19, 20, 21, 22</sup> and the demonstration of elevated IgM levels as a nonspecific monitor to characterize newborns with high risk of intrauterine infection.<sup>23</sup> The latter has been studied more extensively because of the availability of easy methods for quantitating serum immunoglobulin and because it can be used to monitor many infections instead of just one. This approach, however, must be coupled with more specific methods to identify the infection, such as the IgM-specific fluorescent antibody techniques.<sup>21</sup>

The purpose of this review is to discuss various diagnostic facets of perinatal infections in light of their natural histories. Diagnostic findings will be contrasted among individual infections, and the discussion, of necessity, will be limited to "chronic" perinatal infections. However, the principles cited can also be applied to diagnosis of acute, self-limited perinatal infections with modifications based on differences in the natural histories of the latter. Perinatal infections as used here will refer only to those acquired in utero at any time during the birth process (natal). By this definition almost any organism may be involved, especially if maternal bloodstream (primary) infection occurs late in gestation when the initial processes of labor cause gross breaks in

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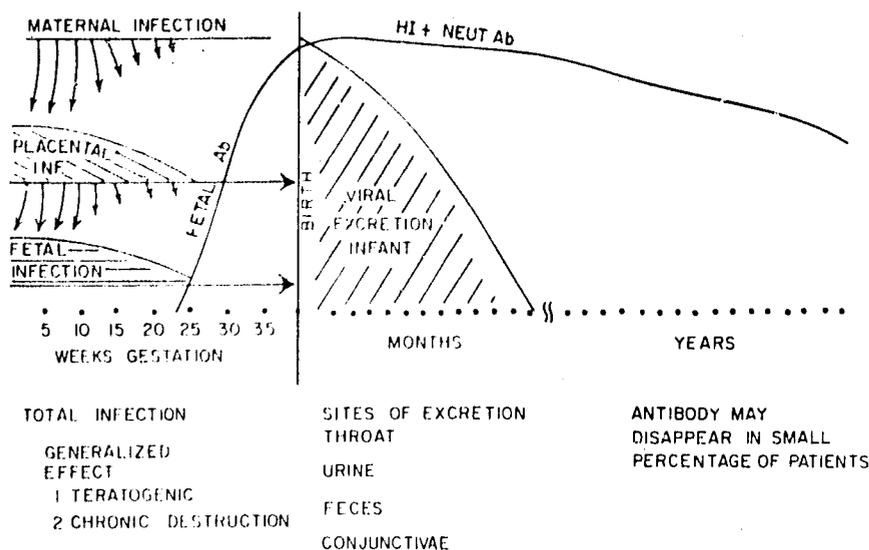


Fig 1.—Pertinent data concerning natural history of congenital rubella. Throat is most common site to demonstrate excretion of virus. (From Alford<sup>11</sup>)

Infection	Mother*	Fetus†
Cytomegalovirus	40 to 150	5 to 20
Rubella		
Epidemic	20 to 40	4 to 30
Interepidemic	1	0.5
Toxoplasma	1.5 to 6.4	0.75 to 1.3
HVH	10 to 15	?
Syphilis	0.2	0.1
Cumulative total (excluding epidemic rubella)	5.3 to 173	6.3 to 22

\* Number per 1,000 pregnancies.

† Number per 1,000 live births.

the "placental barrier." Of primary interest are those in which the infecting agent possesses a special capacity to traverse the intact placenta and produce persistent infections of the conceptus, such as the perinatal infections caused by rubella, CMV, HVH, *To gondii*, and *Tr pallidum*.

As determined by serologic surveys, approximately 10%, 40%, 70%, and greater than 99% of women in the child-bearing age group are susceptible to rubella, CMV, toxoplasmosis, and syphilis, respectively.<sup>29-31</sup> The major variable influencing susceptibility is age, where an inverse relation pertains. Other factors include socioeconomic status, geographic location, sexual promiscuity, and feeding habits. In developing countries, incidence of these infec-

tions is predictably higher than in the United States (Table 1). The general incidence of active maternal infection during pregnancy, approximately 14%, is singularly impressive.

Incidence of fetal infection, though alarmingly high (0.6% to 2.2%), only partially reflects that observed in mothers for each infection, and this observation has led to the concept of the "placental barrier." It should be noted, however, that fetal risk subsequent to active maternal infection remains ill defined.

#### Maternal Infections

Diagnosis of maternal infection is the ideal prerequisite in suspecting potential fetal or newborn involvement. But, unfortunately, clinically recognized disease is the exception

rather than the rule. Important features to be determined whenever possible include type of infection (primary, reinfection, or reactivation), location of the infectious agent (bloodstream or genital tract), and gestational age at the time of occurrence.

**Rubella.**—At present, rubella is considered a threat to the fetus mostly with primary maternal infections during the first 20 weeks of gestation. However, reinfection does occur in a small proportion of women with natural immunity and in a much larger percentage of those with vaccine immunity.<sup>32</sup> The exact role of reinfection in the production of fetal involvement is unknown.

The ratio of inapparent to apparent rubella infection in adult women is approximately 1:1. Therefore, a history of exposure to rubella or the occurrence of a rash demands serologic investigation. Important diagnostic features of the natural history of rubella (Fig 1) resemble the type of response that is seen in primary infections with *Toxoplasma* and *Treponema*.<sup>32</sup> Antibody may appear as early as 14 days after exposure, irrespective of clinical illness.<sup>33</sup> With reinfection, a booster antibody response occurs, accompanied by a brief period of virus replication in the pharynx but usually without symptoms. Thus, it is exceedingly important to obtain the first serum for antibody determination as soon as possible after a presumed exposure. The presence of hemagglutination-inhibition (HI) antibody in serum collected within 13 days of the earliest possible exposure date means prior immunity, in which case protection is thought to be complete. A negative result must be followed by a subsequent HI determination 28 days after exposure; the initial serum specimen should be followed by a convalescent sample in 14 to 21 days. If evidence of seroconversion is found, primary infection is assumed to have occurred. If stable or rising HI titers are found, a complement fixation test (CFT) or IgM rubella antibody determination may be performed to establish whether primary infection has occurred as opposed to

previous disease or reinfection, respectively.

**Toxoplasmosis.**—As in rubella, primary infection is thought to be the major prerequisite to fetal involvement with *Toxoplasma* infection.<sup>14</sup> Fetal invasion can occur at any stage of gestation. There is increasing tendency to fetal involvement with advancing gestation, but throughout pregnancy this occurs in only 40% of the primary maternal infections. The severity of fetal disease is inversely related to the age of conceptus at the time of maternal infection. Maternal toxoplasmosis is almost always asymptomatic or nondescript.

The serologic response to the primary infection, measured by indirect fluorescent antibody (IFA) or the Sabin-Feldman dye test, is similar to that shown for rubella (Fig 1). However, a rise in the IgM *Toxoplasma* response must be shown in order to document primary infection since IgM antibody persists for months or years in a high percentage of previously infected individuals.

**Cytomegalovirus.**—Even with primary infection with CMV, most women have subclinical involvement or a nonspecific illness; however, a heterophile-negative infectious mononucleosis syndrome should alert the physician to consider this infection. The CFT is commonly employed for diagnosis of CMV. Convalescent serum specimens should be collected four and eight weeks after the acute infection to insure documentation of the antibody rise.

The very high incidence of active CMV infection in young pregnant women is due mostly to localized cervical or urinary tract infections, rather than to a generalized primary involvement.<sup>35-37</sup> Naturally transmitted CMV infections occur in about 50% of cases with cervical involvement. Like congenitally acquired infection, these are chronic and, therefore, potentially dangerous in spite of their relatively innocuous nature in early infancy.<sup>35</sup> Natal infections are approximately five to ten times more common than intrauterine CMV infections, and if they prove to be hazardous, even in a minimal way, they may represent the most common form of CMV infection

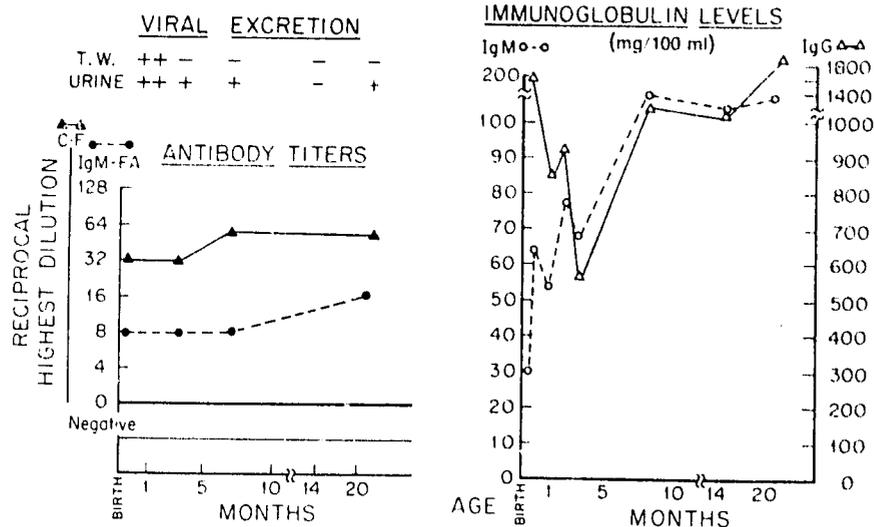


Fig 2.—Virological, serologic, and immunologic findings in asymptomatic form of congenital CMV infection. Development of IgM and IgG is even more accelerated than depicted here when the congenital infection is symptomatic. T. W. signifies throat washings. (From Alford<sup>14</sup>)

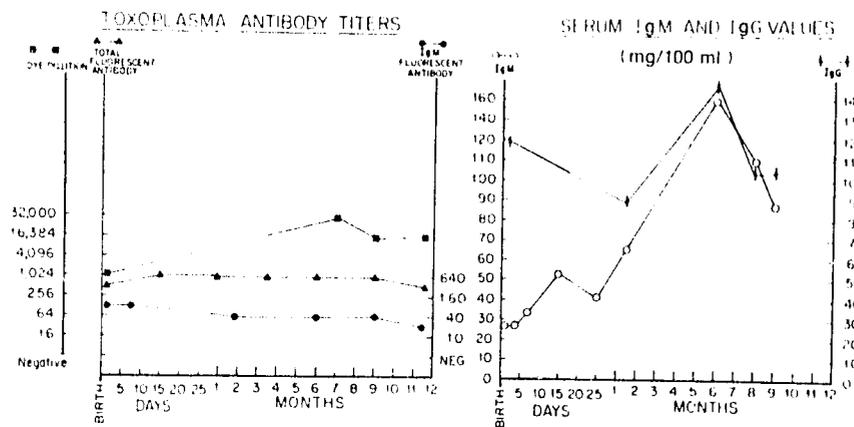


Fig 3.—Serologic and Ig response that accompanies asymptomatic congenital toxoplasmosis. Total fluorescent antibody measures IgG antibody almost exclusively. Maternal specific antibody response may be very similar to that shown here for infant, including persistence of IgM specific antibody. Development of IgM and IgG is even more accelerated than depicted here when congenital infection is symptomatic. (From Alford et al<sup>14</sup>)

in human populations. Unfortunately, cervical CMV infections are clinically silent, are not accompanied by demonstrable antibody changes, usually occur in women with preexisting CMV antibody, and are detectable only by surveys employing virological culture techniques.

**Herpesvirus Hominis.**—Though transplacental infection can occur as a result of primary infection with HVH, it is apparently rare, since most women become immune to HVH before reaching child-bearing age.<sup>16</sup>

The major problem in newborn infection then is one of natal transmission of HVH through recurrent type 2 infections of the maternal genital tract. These infections are most frequent after gonorrhea and CMV in young pregnant women (Table 1), and must always be considered dangerous if present at or near term.

Genital HVH infection is diagnosable clinically by the presence of typical vesicular lesions in only about one third of cases.<sup>18</sup> Confirming the diagnosis by cytologic or virological

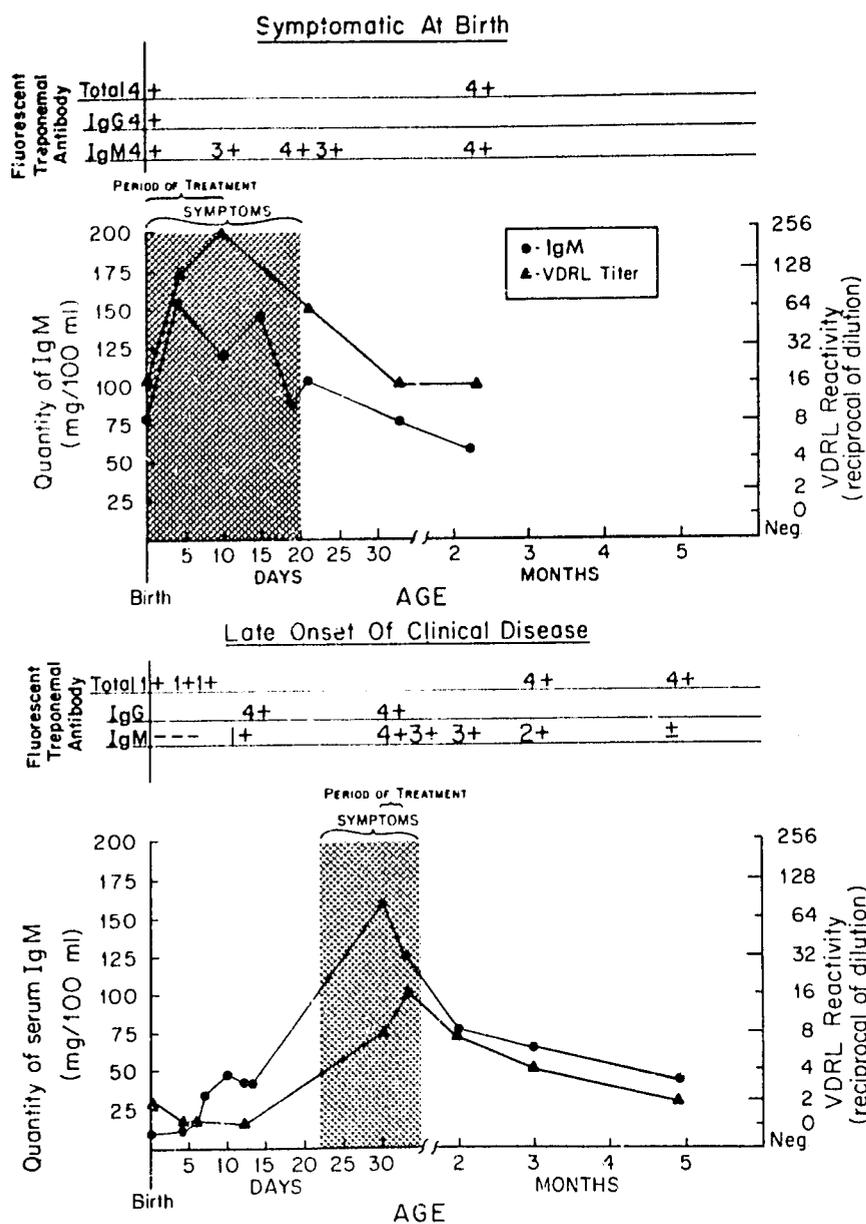


Fig 4.—Serologic and Ig responses in two forms of congenital syphilis. Note delay in infant's VDRL, fluorescent treponemal antibody, and Ig response with late onset form. This response is observed when infection occurs late in gestation or with inadequate maternal therapy. Apparent incubation period is caused by slow replication time of *Treponema*. Naturally acquired infections would have similar delay in serologic response following incubation periods of four to eight weeks for CMV and 1 to 14 days for HHV. (From Alford et al<sup>10</sup>)

means is particularly important in this group, especially when the infection is primary, since there is a high risk, approximately 40%, to the infant delivered vaginally.<sup>30</sup> In about 50% of the women, infections are asymptomatic, whereas the remainder show nonspecific signs and symptoms such as pelvic pain and cervical inflammation.

**Syphilis.**—A chancre in the gravida frequently goes undetected because of its location in the vagina or on the cervix. In addition, extragenital chancres in such diverse sites as the nipple, anus, and tongue often are not suspected of being syphilitic. Therefore, any ulcerative lesion appearing de novo during pregnancy in a site likely involved in sexual activity

should be examined by darkfield microscopy. Primary, secondary, or reinfection syphilis are all dangerous to the fetus; the first two are far more common than the last, except in highly promiscuous populations. There is inherent protection from fetal involvement with syphilis in the first 20 weeks of gestation. Tendency for invasion increases as gestation advances, just as in toxoplasmosis. In untreated primary or secondary syphilis, after the 20th week, infection of the fetus is inevitable even when it is acquired late in gestation.

Reagin antibody, namely that tested by VDRL, appears one to three weeks after the chancre, reaches a maximal titer two to three months later, and persists for an indefinite period in untreated patients. In general, high VDRL titers<sup>31</sup> reflect active infections. Reagin antibody may, however, occur in a variety of conditions other than syphilis. In contrast, the absorbed fluorescent treponemal antibody (FTA-ABS) test is specific for *Tr pallidum* infection. This antibody apparently persists for life in untreated patients.

#### Infants and Children

**Clinical Evaluation.**—The great majority of infants born with chronic perinatal infection will be missed during the early months of life because they lack signs or symptoms. Well over 95% of newborns with congenital CMV infections are asymptomatic; figures for the other chronic infections are as follows: toxoplasmosis, 75%; rubella, 65%; and syphilis, 50%.<sup>32,33,34,35</sup> Some of these infants will have hearing defects that cannot be detected in the neonatal period or even for a few years thereafter. Eye and CNS diseases, even though persistent, most often have no overt manifestations in the newborn and, therefore, pass undetected, although they may express themselves as subtle abnormalities much later. Even gross anomalies such as cardiac and renal defects, cataracts, and immunodeficiency may be functionally compensated during the early months only to decompensate thereafter.

Clinical manifestations of newborn infections not only overlap but also

may mimic many other severe disease states. Prematurity, intrauterine growth retardation, adenopathies, and other abnormalities accompany chronic intrauterine infection but are less specific.

Certain signs have a reasonable degree of diagnostic specificity in infected neonates. For congenital rubella, these include anomalies of the heart, cataracts, glaucoma, pigmented retina, bone lesions, immunologic defects other than IgA deficiency, and the "blueberry muffin syndrome." In congenital syphilis, they are mucocutaneous lesions, especially the eczematoid variety, and osteochondritis. For natively acquired HHV infection, these include vesicles on the skin and mucous membranes, and keratoconjunctivitis. Microcephaly with or without periventricular calcifications occurs most commonly with congenital CMV, while congenital toxoplasmosis, a very similar clinical entity, can cause hydrocephalus with generalized calcifications. One finding usually resulting from the active CNS involvement with *Toxoplasma* or rubella is a disproportionate elevation of cerebrospinal fluid (CSF) protein concentration with respect to pleocytosis.<sup>5,11,44</sup> All suspect infants must be followed up closely and carefully in the first months of life for evidence of missed or decompensating lesions.

Since the great majority of the infected infants are asymptomatic at birth and seem to remain so for years, the critical question currently is what proportion of these are in jeopardy. This question cannot yet be answered precisely. There is preliminary evidence from our studies that asymptomatic toxoplasmosis is almost always accompanied by chronic brain involvement, which can impair intelligence if untreated in the neonatal period.<sup>3,45</sup> Hearing defects of varying severity can occur in a large percentage of children born with congenitally acquired rubella and CMV who are otherwise asymptomatic.<sup>13,32,46</sup> These, if unrecognized, can lead to communication handicaps. Immunologic defects, especially IgA deficiencies, may develop in asymptomatic rubella and toxoplasmosis and will be

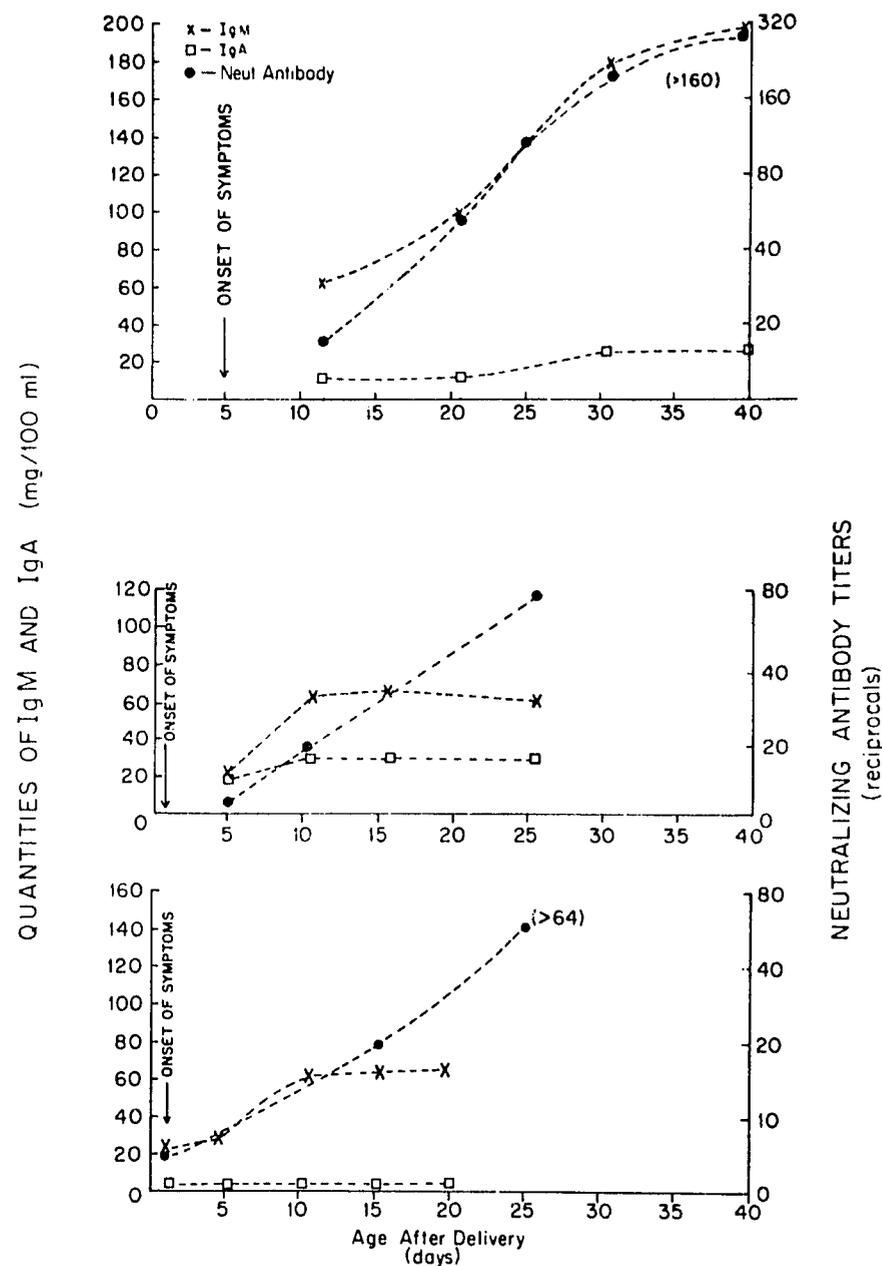


Fig 5.—Serologic and Ig responses in patients with natively acquired HHV infection (top and middle) and in patient with late intrauterine acquisition of enterovirus (acute perinatal) (bottom). Latter patient had silent, self-limited, aseptic meningitis, with limited period of viral excretion in throat and feces. Neonates infected by HHV were symptomatic, one with severe systemic infection involving CNS (top), and the other with only vesicular lesions on forehead (middle). The former of these two exhibited more striking IgM response associated with larger viral load and more severe disease. Specific IgM antibody response might be expected to parallel IgM response in all.

missed unless specifically monitored.<sup>5,47</sup> Mild degrees of prematurity, intrauterine growth retardation, or both may occur without other evidence of infection. Long-term antigen production with CMV, toxoplasmosis, and untreated syphilis

infections and overstimulation of the humoral immune system, as expressed by excess production of IgG,<sup>48</sup> suggest that slowly progressive immune complex disease is a reasonable possibility in these conditions.<sup>49</sup>

**Laboratory Evaluation.**—Pertinent

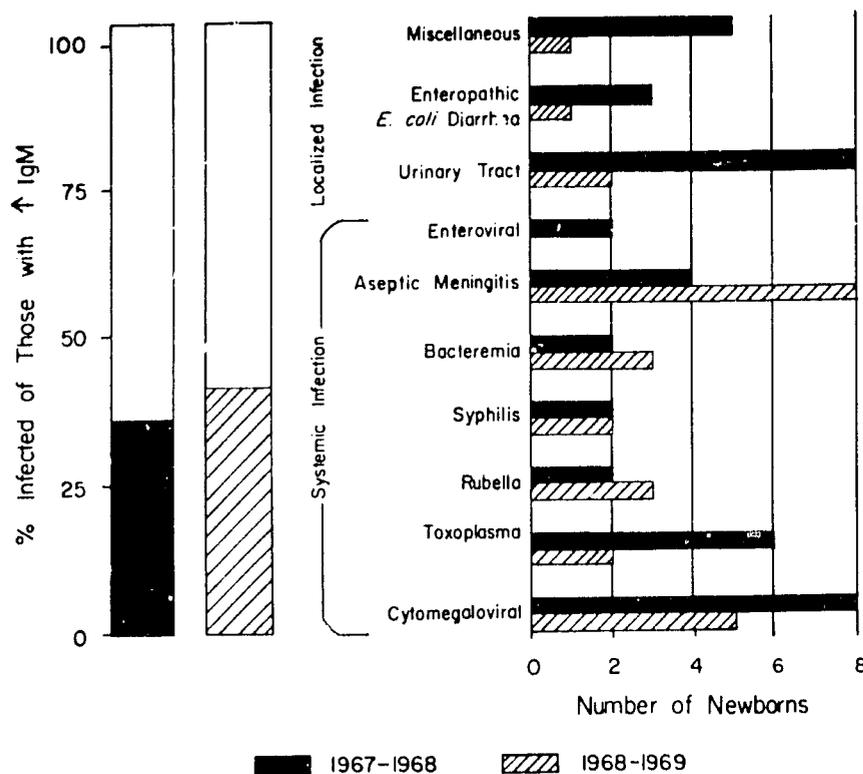


Fig 6.—Infections in infants born with elevated IgM levels. (From Alford<sup>21</sup>)

Type of Infection	No. of Infected Infants	Incidence Among Infants Born With Elevated IgM Level	Incidence Among 7,500 Deliveries*
CMV	17	1/13	1/400
Toxoplasma	11	1/20	1/682
Rubella	8	1/28	1/938
Syphilis	6	1/37	1/1,250
<b>Total</b>	<b>42</b>	<b>1/5</b>	<b>1/178</b>

\* Data gathered during 2½-year period.

microbiologic, serologic, and immunologic findings in certain individual perinatal infections are depicted in Fig 1 through 5.<sup>10,11,47,50,51</sup> These reflect average cases that, as stated, are mainly subclinical in nature. Figures 4 and 5 depict serologic responses that accompany late intrauterine acquisition of syphilis and enteroviral infections, as well as responses accompanying natal acquisition of HVH.

Once the offending organism has been introduced and has become established as an intrauterine infection, it may persist throughout pregnancy

variably distributed in fetal tissues, and may be isolated from materials collected from the newborn infant (Fig 1). Virus is excreted for months or years in the throat and urine of infants with congenitally acquired CMV (Fig 2).<sup>12</sup> For diagnostic specificity as regards intrauterine acquisition, isolation must be attempted in the neonatal period since viral excretion after that time may represent natively acquired infection.<sup>13</sup> In congenital rubella, virus is consistently excreted in the throat and occasionally can be detected in urine, conjunctival secretions, feces, CSF, bone

marrow, and white blood cells (Fig 1). But isolation attempts in infants with suspected congenital rubella should be performed as soon as possible after delivery, since the period of virus shedding is variable among patients. After four months, only 33% of infected infants will excrete virus, and after eight months only 11% will.<sup>14</sup> Virus persists much longer in sequestered areas such as the eye and the CNS. The best site from which to recover HVH in infants is the cutaneous vesicles; HVH can also be recovered from the throat and urine and may be detected in the eye or CSF when disease is located there.<sup>15</sup> Typical giant cells with intranuclear inclusions can also be seen in biopsies of the skin lesions of HVH or in cells scraped from the base of the vesicles. Cells with type A Cowdry intranuclear inclusions can occasionally be seen in urine specimens collected from infants with symptomatic CMV infections. Concentration of urinary sediment by millipore filtration before staining, and repeated examinations will increase the yield; but this maneuver, no matter how well done, is less efficient than virus isolation. If there are open lesions in congenital syphilis, the *Treponema* can be identified in the involved tissues including the placenta if a careful search is made<sup>16</sup>; the viruses can all be recovered from the diseased organs for many hours after death.

Specific antibodies are produced in utero and for long periods postnatally in all chronic intrauterine infections (Fig 1 through 5).<sup>20,21</sup> They are also manufactured after an appropriate incubation period after natively acquired infections (Fig 5). Serologic tests are of greater diagnostic usefulness because of their greater availability and the fact that they can be performed with more ease and speed than virological methods. However, interpretation of results obtained on serum collected soon after delivery is complicated by the presence of antibodies that are transmitted from the mother to the fetus. After delivery, maternal antibodies decrease and finally disappear from the serum of uninfected infants at variable intervals during the first year of life. In contrast, in

infected infants, antibody levels comparable to those in the maternal serum persist for long periods of time. Consequently, the demonstration of persistent or increasing titers during early months of life can be diagnostically important in neonates with suspected chronic perinatal infection (Fig 1 through 5). Occasionally, as a result of the intensity of the stimulus in utero, antibody levels are sufficiently higher (eightfold or more) in the cord serum than in the maternal serum to be diagnostic. This is more common in congenital syphilis than in the other infections and serves to emphasize the need to compare results of serologic tests obtained in mothers and infants in the neonatal period. Serial determinations of regular antibody levels is required, preferably at monthly or at least three-month intervals in most cases, to prove the presence of infection. The most commonly available serologic tests are HI for rubella, CFT for CMV and HVH, IFA for toxoplasmosis, and FTA-ABS and the VDRL for syphilis. Recent data from our laboratory indicate that IgG FA or HI is better for proving congenital CMV than are CF determinations. The CMV CF antibody often disappears in the early months of life even with continued viral excretion, whereas antibodies measured in the other tests do not. The repeated bleedings, necessary when regular serologic procedures are used for diagnosis, inevitably delay the diagnosis, but provide specificity that clinical findings often lack. The latter are extremely important if proper follow-up care and prognosis are to be given.

Since the infected fetus usually has its own specific antibody in addition to that placentally transferred, detection of fetal antibody is theoretically the most practical way of diagnosing an intrauterine infection. Most methods for detection of fetal antibody in umbilical cord or neonatal serum are based on the concept that since IgM antibody, in contrast to IgG, cannot be transferred by the placenta, its presence must represent synthesis by the fetus. Agents that produce chronic perinatal infections stimulate synthesis of specific IgM antibody, which

can be measured by IFA methods (Fig 2 through 4).<sup>10,19,21-25</sup> These techniques are rapid and economical but are not yet sufficiently developed to assure complete specificity and sensitivity. The treponemal and *Toxoplasma* IgM FA procedures are presently better perfected for general use than the others.

When in utero infection causes overt disease in the newborn, the antigenic stimulus is usually sufficient to induce elevation of total IgM level along with the production of specific IgM antibody. Therefore, demonstration of elevated levels of IgM (above 19.5 mg/100 ml) in cord or neonatal serum is taken to indicate excessive intrauterine antigenic stimulation and, in this limited sense, serves as a nonspecific monitor of intrauterine infection.<sup>21</sup> The IgM responses that occur in specific infections are represented in Fig 2 through 5. When the IgM level is increased in infants thought to be infected, definitive laboratory diagnosis should be sought immediately.

#### Use of Screening Tests for Increased IgM Level

In congenitally infected infants in whom symptoms are absent, some laboratory monitor is needed for initial identification. Some six years ago, our group instituted a prospective program to evaluate the diagnostic use of increased cord IgM levels as a screening procedure. The IgM level was measured by immunodiffusion in cord serum obtained from every infant born at our hospital. After exclusion of specimens contaminated by maternal blood, infants who showed a level above 19.5 mg/100 ml were subjected to a series of tests to determine the reason for the IgM level elevation. The tests were performed irrespective of clinical findings. Procedures used to define infection have been detailed elsewhere.<sup>12,21</sup> In addition to classical diagnostic methods, specific IgM FA tests for syphilis, CMV, and *Toxoplasma* infections were evaluated as means of rapid detection of subclinical infections.

As noted in Fig 6, a total of 123 infants with increased IgM levels were found among 2,916 examined in the

first year of the study. Infections were detected in 42 (34%) infants born with elevated IgM levels. An additional 69 infants had elevated IgM levels in the second year of the study. In 27 (39%), we detected evidence of infection. The infection rate among infants with IgM levels above 19.5 mg/100 ml was 42 times greater than that of infants with lower values. When calculated on the basis of total deliveries, the incidence of chronic systemic congenital infections determined by the IgM screening was one in 178 (Table 2). Cytomegalovirus was the commonest cause of infection.

Virtually all infected neonates were asymptomatic at birth, indicating that increased IgM levels can be used in the search for "silent" infections. More than half of these infants later exhibited elevated CSF protein values and lymphocytosis that persisted up to 5 months of age.<sup>21</sup>

This phenomenon was particularly striking in patients with congenital toxoplasmosis, but was also seen in at least 50% of neonates congenitally infected by rubella, even though other evidence of infection was not readily apparent. Asymptomatic CMV infections were seldom accompanied by such changes.<sup>21</sup> In patients with acute aseptic meningitis due to such agents as enteroviruses that produce acute, self-limited infections, cell counts were disproportionately elevated in relation to protein level. In addition, the pleocytosis persisted for only a few days. The most disturbing feature of the CSF changes in chronic perinatal infections was the disproportionate elevation of protein levels. It suggests to us a greater degree of cellular breakdown and correlates well with reduced intellectual capacity observed in older children who were born with congenital toxoplasmosis but who were asymptomatic in early life.<sup>13</sup>

Symptomatic neonates with more severe forms of chronic intrauterine infections have higher levels of IgM in their cord sera (30 mg/100 ml) and, in some at birth, the IgM level may even have reached adult levels or higher.<sup>12</sup> Determination of IgM level is particularly useful as a means of selecting chronically infected neo-

nates among a group of sick infants in whom cause is not readily apparent. Undoubtedly, among asymptomatic infants, some are born with IgM levels within normal range and represent false negatives. How often this occurs is unknown; however, until better methods are developed, it remains a practical approach to the diagnosis of asymptomatic intrauterine infections.

Theoretically, detection of specific IgM antibody in cord and early neonatal serum is of greater diagnostic importance than screening for non-specific IgM level elevations. All infants in our study who were infected

with *Toxoplasma* or CMV and the ones symptomatic with treponemal infections had specific cord IgM antibodies detected by the FA technique. A specific diagnosis was thus obtained within three to seven days in such cases. Whether the use of the FA procedures as a screening aid would have substantially increased our yield is unknown, but a few infants born with IgM levels within the normal range did have specific IgM FA antibodies.

Neither of these approaches is suitable for detection of infections acquired too late to elicit a serologic response evident at the time of delivery

(Fig 4 and 5). Diagnosis of maternal infection would be the ideal approach to detection of perinatal infections in the newborn, and greater efforts in this direction should be made in the future. After all, diagnosis of congenitally acquired infections is really a retrospective maneuver that leads to a substantial delay in coping with the real problem, namely, that of the fetal involvement.

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## Summarized Discussion of Session II

Dr. Stiehm supported Dr. Chandra's study with some details about his own investigations in Ghana. He pointed out that there is a decrease of T lymphocytes in kwashiorkor and marasmus and that these cells seem unusually susceptible to nutritional insult. Dr. Katz asked whether it was the diminished survival or diminished production of the T cells that was responsible for this decrease. Dr. Stiehm expressed the view that it was almost certainly diminished production, since malnourished children have small tonsils and are lymphopenic. However, he added that turnover studies have not been done.

A discussion then followed among Drs. Béhar, Stiehm, Lechtig, and Mata regarding the meaning of elevated IgM level in cord serum. It was suggested that not all instances of IgM level elevation are due to infection, but some may be caused by another stimulus, such as damage to placental vessels. Moreover, if samples of cord blood are collected by inexperienced personnel, they may inadvertently become contaminated by maternal blood and thus have falsely high IgM levels. In Guatemalan villages, Dr. Mata pointed out, some 15% of newborn infants have an elevated IgM level when cord blood is collected properly. Dr. Alford concurred that the main cause of contamination is improper collection of the specimens, and he cautioned against invoking the existence of placental leaks, or lakes of blood in vivo. In measurement of immunoglobulins by quantitative techniques, in which the values are expressed as milligrams per 100 milliliters, addition of a small amount of maternal blood from in vivo contamination would not substantially raise IgM levels. On the other hand, if one uses the fluorescent anti-

body technique, which detects nanograms of immunoglobulins, even a minute contamination may alter the results of measurements in cord serum. Thus, false positive results may be reported in cases of those infections in which maternal IgM antibodies tend to persist, for example, in CMV, but it would not happen in cases of rubella where maternal IgM lasts for only several weeks.

Dr. Rush inquired whether the state of immunologic development or immunologic maturity can be used as a marker of the stage of development of the infant. He wished to distinguish the pathologic state that may lead to an abnormal immunologic pattern in the infant from the basic physiologic immaturity appropriate to age. Dr. Chandra commented that this would indeed be possible in the future, but that given the present state of knowledge, one could not define infants' maturity in terms of immunologic measurements. The discussants then considered the various categories of definition of maturity, and many participants agreed that it would be useful to base maturity on as many different criteria as possible. Dr. Luckey suggested that perhaps a scale of 20 points might be developed that would include not only growth, etc, but also such things as sphingomyelin/lecithin ratio in the lungs, quantity of phospholipids, and so forth. Dr. Stiehm supported this view, but added that with respect to cellular immunity, or phagocytic capacity, for example, there was little difference between infants born after 33 weeks' gestation and those born after 40. However, he thought that alpha fetoprotein level might be useful in this context, since it reaches a maximum of 30 mg/100 ml at 20 weeks of gestation, and

then falls as gestational age increases, dropping about 5 mg/100 ml at term. Dr. Chandra interjected that hepatitis may perhaps elevate alpha fetoprotein level and thus create a spuriously high measurement. Dr. Sever added that IgM levels can also be helpful in this assessment of maturity, because they are very low in the low-birth-weight, short-gestation infant, but they approach normal levels in low-birth-weight, long-gestation infants. Dr. Luckey then asked about the function of the macrophage defense mechanism of the fetus, noting that the quantity of macrophages in the fetus were quite high. Dr. Faulk indicated that there was no evidence of any maternal macrophages acting in the fetus. The effector, the macrophage-like cell, in the fetus is found in a very high concentration in the fetal spleen, but its concentration is relatively lower in the neonatal spleen, and very much lower in the adolescent spleen.

Another aspect of this discussion dealt with the difficulties in assigning the specific blame for low-birth-weight infants to either malnutrition or to infection. Some discussion dealt with the problem that one may be observing very small numerical variations and, therefore, as was pointed out by Dr. Rush, very large populations would be required for the statistically acceptable determination of the validity of such observations. Dr. Mata added that he was unaware of any evidence that either nutritional intervention or improvement of hygiene alone has ever been shown to effect a dramatic change in the problem of low birth weight.

(To be concluded in the May issue.)



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# Malnutrition and Infection During Pregnancy: Determinants of Growth and Development of the Child (Conclusion)

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## Session III: The Problem of Low Birth Weight

# Nutritional Influences in Industrial Societies

John C. Sinclair, MD, Saroj Saigal, MD

Evidence that maternal undernutrition contributes to the problem of low birth weight in industrial societies will be discussed in the following categories: (1) epidemiological associations, (2) clinical studies of growth-retarded babies, (3) maternal dietary studies, and (4) pathologic findings in perinatal deaths.

### Epidemiological Associations

**Mean Birth Weight.**—As summarized by Rosa and Turshen,<sup>1</sup> quite large differences between populations occur with respect to mean birth weight. In contrast, differences in mean length of gestation are minor. Thus, between-population variance in mean birth weight reflects principally differences in rate of fetal growth. It is a central problem of perinatal medicine to know the relative contributions of heredity and environment to the variance in fetal growth rate, and, among the environmental factors, to know the effect of maternal undernutrition.

Within industrial societies, similar though less obvious differences are seen in the mean birth weight. For example, mean weight at term for male fetuses is 3,650 gm (8.04 lb) in Norway<sup>2</sup>; 3,630 gm (8.00 lb) in Sweden<sup>3</sup>; 3,560 gm (7.85 lb) in Aberdeen, Scotland<sup>4</sup>; 3,495 gm (7.70 lb) in a British national sample<sup>5(p133)</sup>; and 3,290

gm (7.25 lb) in the study of Lubchenco et al in Denver.<sup>6</sup> Another American study, in a predominantly black population, gave a mean fetal weight at term for boys of 3,210 gm (7.07 lb),<sup>7</sup> and in the US *Collaborative Perinatal Study*, the value (for blacks, sexes combined) was 3,163 gm (6.97 lb).<sup>8</sup> At the Harlem Hospital in New York City, which serves a poor, black, urban community, mean birth weight at term is below the American national average, and further reduction in the mean birth weight occurs in association with maternal preconception weight below 50 kg (110 lb), low maternal weight gain during pregnancy, or the history of a previous low-birth-weight baby.<sup>9,10</sup>

These associations suggest the hypothesis that the adequacy of maternal nutritional status, either during or before pregnancy, may contribute to the variation in fetal growth rates, and may contribute to differences, both between-population and within-population, in mean birth weight at term.

**Incidence of Low Birth Weight.**—The incidence of birth weights < 2,500 gm (5.5 lb) is generally highest in those countries where the mean birth weight is lowest, and varies from about 5% to 25%.<sup>1</sup> Similar, though less severe, differences occur within industrial societies. For example, the incidence of low birth weight in the US *Collaborative Perinatal Study* was 7.1% for whites and 13.4% for blacks, and this difference was not accounted for by a small difference in the distribution of gestational ages. In the British perinatal mortality survey,<sup>5(p133)</sup> the incidence of low birth weight was 6.7%. One third of low-

birth-weight babies were born at 38 weeks or later. Thus, the problem of low birth weight is twofold: preterm delivery of normally grown fetuses (the majority) and growth retardation among fetuses usually born at term (a sizable minority). The increased incidence of low birth weight in populations with low mean birth weight is usually due to an increased incidence of fetal growth retardation. Again, these observations suggest a nutritional hypothesis.

**Maternal and Other Associations With Fetal Growth Retardation.**—Epidemiological studies have identified a number of maternal and other associations with fetal growth retardation as it occurs in industrial societies. These associations are reflected in the approach that we use to classify our growth-retarded infants according to presumed cause:

#### Maternal factors

1. Low maternal preconception weight (< 50 kg), with no detectable pathologic process in mother, placenta, or fetus
2. Maternal hypertension, with or without maternal proteinuria, edema, or kidney disease (> 140/90 mm Hg); (a) before 20 weeks, or (b) after 20 weeks
3. Maternal nonhypertensive cardiorespiratory disease, or other maternal disease
4. Heavy maternal cigarette smoking (> 20/day)
5. Other maternal pathologic process (drug use, uterine anomaly, etc)

#### Fetal factors

6. Major congenital malformation of the fetus, with or without chromosomal anomaly
7. Chronic fetal infection
8. Multiple pregnancy

#### Unknown factors

9. None of the above

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The absence of "maternal under-nutrition" from this list reflects our lack of knowledge about the calorie, protein, and other nutrient intake of our mothers, rather than a lack of interest in and concern for this measurement. Presumably, maternal undernutrition may play a primary role in some otherwise unexplained cases of fetal growth retardation (group 9), and a contributory role in groups 1 through 8.

We have recently reviewed our experience with fetal growth retardation at a local Hamilton, Ontario, community hospital delivering fetuses of unselected pregnancies. Our object was to determine the frequency of association with fetal growth retardation of the various factors listed above. We included in the review all babies weighing under 2,500 gm at birth who were products of a 32-week or more gestation, and whose birth weights fell below the fifth percentile for gestational age on the Aberdeen fetal growth standard.<sup>4</sup> (Adjustments were made for fetal sex

and birth order, but not for maternal height and weight.) The babies were born during the period July 1971 to November 1972. In each case, we reviewed the hospital chart and, if possible, conducted a maternal interview retrospectively. During the maternal interview, detailed information on cigarette smoking during pregnancy was sought.

The sample selected is given in Table 1. Eighty-nine cases were selected (about 2.1% of total births), and 24 cases were excluded because gestational age was uncertain. There were 26 boys and 63 girls, a ratio reflecting, at least in part, a sampling bias introduced at gestational ages over 38 weeks because the 2,500-gm limit was imposed regardless of fetal sex. (Since the fifth percentile on the Aberdeen curve lies above 2,500 gm after 38 weeks, and farther above for boys than for girls, more boys than girls are excluded by the 2,500-gm limit.)

As compared with the pregnant general population, mothers who deliver growth-retarded babies in Hamilton have a higher incidence of short stature, low preconception weight, hypertension (both early and late in pregnancy), other illness, and heavy smoking habit (Table 2). Twin pregnancy and babies with major congenital malformation are both greatly overrepresented in the growth-retarded group. We find none of the associations listed in the growth retardation classification list in only 10% of

our growth-retarded cases. This is in striking contrast to our frequent failure to find a pathogenetic association in cases of preterm delivery of a normally grown fetus.

The frequent identification of a pathologic association in our cases of fetal growth retardation does not rule out a contributory effect of maternal undernutrition. Moreover, those cases in whom small maternal size is the sole association noted may indicate a nutritional effect. However, it appears unlikely, in the Hamilton experience, that maternal undernutrition is often the sole cause of a degree of fetal growth retardation that is sufficient to result in low birth weight.

#### Clinical Studies of Growth-Retarded Infants

Clinical studies of growth-retarded newborn infants demonstrate a higher incidence of unfavorable outcomes among this group. Thus, in industrial societies, the baby who is small for his gestational age is often suffering from some pathologic process that has impaired his fetal growth below its genetic potential. Such infants cannot usually be regarded as genetically small but otherwise normal.

**Perinatal and Neonatal Mortality.**—The total contribution of fetal growth retardation to perinatal mortality can be estimated from Usher's Montreal experience of 26,453 consecutive live births of infants weighing over 1,000 gm (2.2 lb).<sup>11</sup> Among the 438 perinatal deaths in this sample, 68 (15.5%) occurred among infants whose birth weight was below the third percentile for gestational age. These included 35 stillbirths and 33 neonatal deaths. About 40% of perinatal deaths among growth-retarded infants are caused by major malformation.<sup>11-12</sup> Among the remaining deaths, the most important causes are fetal and intrapartum asphyxia, meconium aspiration with its complications, hypoglycemia, and pulmonary hemorrhage. Growth-retarded babies are overrepresented approximately tenfold among both stillbirths and neonatal deaths from asphyxia.<sup>13(15)</sup> Many such infants have meconium aspiration at birth, sometimes complicated by pneumomediastinum, pneumothorax, cere-

Table 1.—Composition of Fetal Growth Retardation Sample\*

Gestational Age, Weeks	No. of Babies		
	Singleton	Twin	Total
< 38	19	10	29
38-42	56	4	60
≥ 43	0	0	0
Total	75	14	89

\* Data collected in Hamilton, Ontario, in 1971 and 1972.

Table 2.—Pathogenetic Associations With Fetal Growth Retardation

Factors	Sample Incidence, %	Population Incidence, %*
<b>Maternal</b>		
Height ≤ 153 cm (60 in)	22	10
Preconception weight < 50 kg (110 lb)	28	16
Hypertension		
< 20 wk	11	1-3
> 20 wk	27	2-5
Antepartum hospital admission	12	...
Other maternal illness	10	3
Heavy cigarette smoking (> 20/day)	19	7
<b>Fetal</b>		
Major congenital malformation	10	...
Chronic fetal infection	1	...
Twin pregnancy	16	...
None of above, where known	10	...

\* Data taken from US Collaborative Perinatal Study and represent a white population.

bral edema, and inappropriate anti-diuretic hormone secretion. The British perinatal mortality survey reported a 43% incidence of fetal growth retardation in autopsies performed on infants who died in the first week of life with pulmonary hemorrhage.<sup>10,11</sup> The mechanism of the association is unclear, and there is a suggestion that it is not characteristic of current practice, which includes more careful avoidance of cold stress.

In the Hamilton experience, there were four neonatal deaths among the 89 cases of fetal growth retardation. All were due to major malformation.

**Perinatal Morbidity.**—Congenital anomalies are an important cause of perinatal morbidity among growth-retarded infants. In van der Berg and Yerushalmy's analysis<sup>11</sup> of the Oakland (Calif) Child Health and Development Studies' experience, neonates weighing between 1,501 and 2,500 gm (3.3 and 5.5 lb) were separated into four quartiles that were identical in birth weight distribution but differed greatly in the gestation. Among the quartile experiencing very slow rate of fetal growth, they found a high incidence of congenital anomalies, particularly congenital heart disease. However, those infants in this quartile of very slow fetal growth who were without congenital anomalies experienced certain advantages as compared with the younger infants of identical weight—a shorter duration of hospitalization and a shorter duration of incubator care. Undoubtedly, these latter observations reflect a relative freedom from respiratory distress syndrome, recurrent apneic spells, and hyperbilirubinemia. Feeding regimens can be instituted earlier, and they are able to ingest adequate calories for weight gain soon after birth. Hypocalcemia is less frequent than it is in preterm infants.<sup>11</sup>

The growth-retarded infants are particularly vulnerable to hypoglycemia. Pildes and co-workers<sup>12</sup> reported a 5.7% incidence of neonatal hypoglycemia in infants whose birth weight was less than 2,500 gm. Hypoglycemia was defined as two consecutive values of blood glucose below

Group & No.	% With Low Protein Intake (gm/Day)		% With Low Serum Protein Level (gm/100 ml)	
	34-48	< 34	5.5-6.0	< 5.5
General 894	8.5	3.3	25.5	5.0
Indians 59	17.0	9.7	12.5	0

\* Data from 1973 Nutrition Canada National Survey<sup>20</sup> of pregnant women in their third trimester.

20 mg/100 ml. A high percentage (27%) of infants in the survey were below the tenth percentile weight for gestational age, by the fetal growth standard of Lubchenco et al.<sup>13</sup> From the data of Pildes and associates, it can be deduced that the incidence of hypoglycemia in growth-retarded infants is approximately 8%. This figure is lower than more recent estimates, for example, 12%<sup>14</sup> and 21%.<sup>15</sup>

Growth-retarded babies have high hemoglobin concentration, hematocrit value, and red blood cell mass for age and weight.<sup>12,16,17</sup> The increased hematocrit level has been attributed to chronic hypoxia in utero. The association of polycythemia with respiratory and neurological symptoms has received increasing attention.<sup>12,18,19</sup>

We recently reviewed the early neonatal consequences of fetal growth retardation, and found that some of these resemble findings of postnatal malnutrition.<sup>21</sup> Among the similarities are high extracellular fluid volume for body weight<sup>22,23</sup> and a high metabolic rate for body weight after refeeding.<sup>24,25</sup> Studies of Lindblad et al.<sup>26</sup> provide a model for the study of correlation between fetal and postnatal malnutrition. They determined that free amino acid levels in maternal plasma fall during normal pregnancy. At term, fetal levels are higher than maternal levels, and the same is true at the time of delivery after short gestation. However, in hypertensive pregnancy associated with fetal growth retardation, the fetal/maternal ratio for essential amino acids is lower than normal because of higher maternal plasma levels for these amino acids (especially valine, isoleucine, and leucine). Moreover, fetal glycine concentration is considerably higher in these latter fetuses at delivery, and the glycine/valine ratio is higher than it is in both

normal preterm and full-term fetuses. The glycine/valine ratio is not, however, elevated in the hypertensive mothers. In contrast, groups of mothers in Pakistan of low socioeconomic class giving birth to babies of low birth weight show elevated glycine/valine ratios, as do their infants.

#### Maternal Dietary Studies

Maternal nutrient intake during pregnancy has been extensively reviewed by Hytten and Leitch<sup>27</sup> and by Bergner and Sussler.<sup>28</sup> Recent studies of nutrient intake during pregnancy have focussed on groups at high risk for undernutrition and low birth weight. The *Nutrition Canada National Survey*,<sup>20</sup> incompletely analyzed and reported at present, included a sample of pregnant women in their third trimester (unfortunately, not randomly selected), with whites and Indians separately tabulated. Twenty-four-hour dietary recall histories were obtained. The lower tail of the distribution of protein intake is given in Table 3. Low protein intakes occurred frequently, especially among Indians. However, total serum protein concentrations among Indians were not low. Other studies have demonstrated a high mean birth weight among American and Canadian Indians. Thus, the role of nutrition in this group is unclear and may be obscured by genetic or other factors influencing fetal growth.

King and co-workers<sup>29</sup> studied nutrient intake in 18 pregnant San Francisco teen-agers, using three-day food intake records. They found no nutrient that was adequately supplied to all the girls, even when prescribed prenatal supplements were included in the tabulation. Nutrients most deficient in the diets were calcium, iron, vitamin A, and energy.

Protein was the most nearly adequate of the nutrients studied.

Rush and co-workers<sup>9</sup> obtained 24-hour recall estimates of nutrient intake prior to enrollment in a randomized, controlled trial of nutritional supplementation in pregnancy. They found that the median daily protein intake reported by women in early pregnancy is about 55 gm in a poor American black population.

Aside from socioeconomic limitations on calorie and protein nutrition, industrial societies contain at-risk groups whose nutrient intake is limited by fad diets, misguided attempts at weight reduction, or a persisting prejudice in obstetric practice that dictates that weight gain in pregnancy should be kept below 9.1 kg (20 lb).

#### Pathologic Studies

Fetal body, organ, and cellular growth, as studied at autopsy, provides a special opportunity in the case of perinatal deaths for a study of the effects of maternal nutrition on the human fetus. Naeye and co-workers<sup>11</sup> have recently reported a pronounced effect of maternal nutritional status on fetal body, organ, and cellular growth in a series of perinatal deaths studied at Babies Hospital, New York City. They excluded major congenital anomalies, chromosomal disorders, chronic congenital infections, major placental lesions, multiple births,

infants of diabetic mothers, and gestations with hydramnios, oligohydramnios, Rh disease, maternal hypertension, or other evidences of toxemia of pregnancy. Included in their study are the offspring of women who were placed on diets offering as little as 1,200 calories per day, and for as long as six months or more, in an effort to reduce weight gain or total body weight. Maternal nutritional categories were defined on the basis of prepregnancy weight for height (above or below average) and pregnancy weight gain (above or below average). A progressive decrease in newborn body and organ measurements took place in passing from nutritional category 1 (overweight mothers with high pregnancy weight gain) to category 4 (underweight with low weight gain). The major effect was seen in infants born after 33 weeks. In all nutritional categories, many newborn body and organ values were smaller for mothers placed on a specific low-calorie diet than for mothers who received general dietary advice.

#### Conclusion

There are a number of clues that suggest that maternal undernutrition contributes to the problem of low birth weight in industrial societies, but the evidence is not complete and the prevalence and magnitude of the nutritional effect are both unknown

at present.

Although slow fetal growth is strongly associated with small maternal size and low pregnancy weight gain, the majority of low-weight-for-date babies are born in a setting of other pathologic associations—maternal hypertension or other systemic disease, heavy maternal cigarette smoking, multiple pregnancy, or fetal malformation.

Fetally growth-retarded babies are usually not "small but normal," but show a variety of clinical features that suggest deprivation, and a reduction of growth below genetic potential. Similarly, pathologic studies show a correlation between indices of poor maternal nutritional status and evidence of deficient fetal organ and cellular growth.

Dietary studies, although often lacking in precision, suggest that maternal intake of calories or protein or other nutrients may be deficient during pregnancy, particularly in certain at-risk subgroups within industrial societies.

It is apparent that firm evidence establishing maternal undernutrition as a cause of low birth weight in industrial societies depends on the completion of a properly controlled randomized trial of nutritional supplementation in such at-risk subgroups.

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# Maternal Nutrition and Fetal Growth in Developing Countries

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The incidence of low birth weight ( $\leq 2.5$  kg [5.5 lb]) is excessive in rural and urban low socioeconomic groups in preindustrialized countries (Fig 1). Since the large part of the population in these countries is in this socioeconomic stratum, low-birth-weight infants constitute a large proportion of the total newborn population. About 3 million infants born in Latin America alone during 1973 were of low birth weight. Since most of them presumably were full-term newborns, their low weights reflected fetal growth retardation.

Low-birth-weight infants have a higher mortality during the first year of life than do infants of normal birth weight.<sup>1,2</sup> In addition, they show impaired mental development.<sup>3,4</sup> It is possible that this impairment influences their ability to develop into functioning adults.

## Influence of Maternal Nutrition on the Proportion of Low-Birth-Weight Babies

Experiments in animals have shown that severe caloric or protein malnutrition in the mother delays fe-

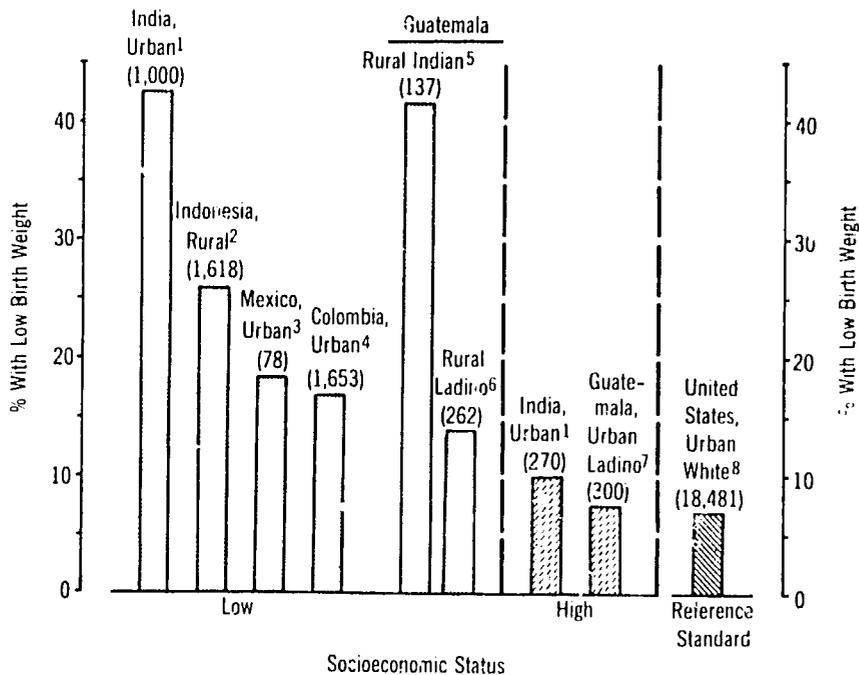


Fig 1.—Influence of maternal nutrition on fetal growth in preindustrialized countries. Number of cases given in parentheses. Data taken from following sources: (1) Udani<sup>22</sup>; (2) Shattock<sup>23</sup>; (3) Cravioto et al<sup>24</sup>; (4) Oberndorfer et al<sup>25</sup>; (5) Mata et al<sup>26</sup>; (6) Lechtig et al<sup>27</sup>; (7) Hurtado<sup>28</sup>; and (8) Niswander and Gordon.<sup>29</sup>

tal growth and changes the relative size of several organs. These effects may be irreversible in organs in which the nutritional insult has affected the rate of cell division.<sup>6</sup>

In humans, an effect of maternal nutrition on birth weight has been

demonstrated in cases of acute starvation. Infants born during famine periods show consistently lower birth weights than those born in the same country during times of adequate food supplies.<sup>7-9</sup>

Studies of the influence of chronic

From the Institute of Nutrition of Central America and Panama, Guatemala City. Reprints not available.

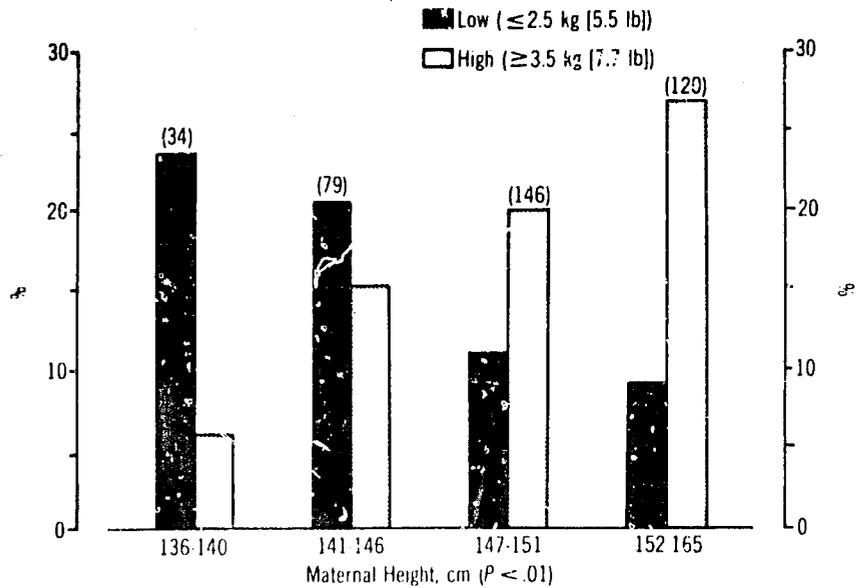


Fig 2.—Relationship between maternal height and birth weight. Number of cases given in parentheses.

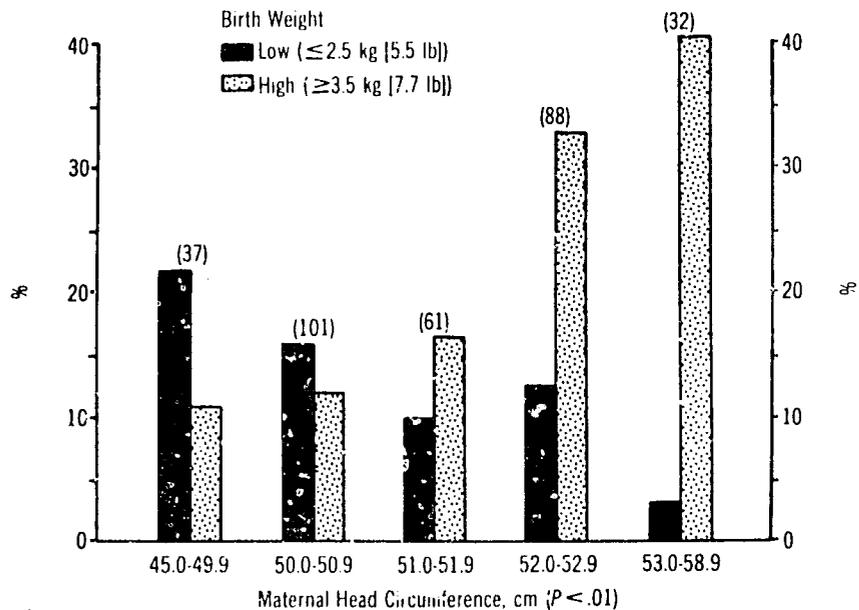


Fig 3.—Relationship between maternal head circumference and birth weight. Number of cases given in parentheses.

moderate malnutrition on fetal growth have yielded results somewhat more difficult to interpret. Malnutrition prior to pregnancy is usually evaluated by anthropometric measurements. The difference in mean height between adult women from low socioeconomic groups in Guatemala and a sample of white population from the United States is approximately 12 cm (4.7 in). Most of this difference is accounted for by growth retardation during the first

seven years of age<sup>2,10,11</sup> (C. Yarbrough, PhD, et al, unpublished data).

In contrast, height of 7-year-old children from high socioeconomic groups in developing countries is similar to that in the industrialized countries and is greater than the height of children of low socioeconomic status from the same ethnic group.<sup>11-15</sup>

A consistent association has been found between height of the mother and the birth weight (Fig 2) in four rural villages in Guatemala studied

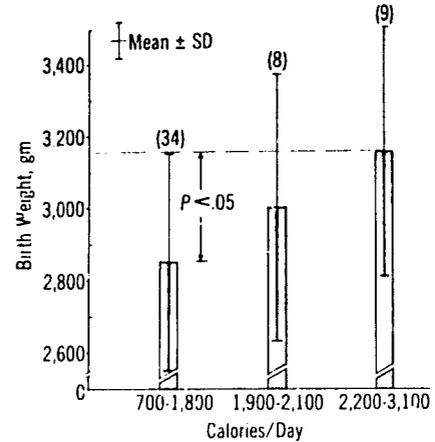


Fig 4.—Relationship between maternal dietary intake and birth weight. Number of cases given in parentheses.

prospectively. Prepregnancy weight has also shown consistent relationship to birth weight among mothers of the same height (C. Yarbrough, PhD, et al, unpublished data).

It is generally accepted that differences in head circumference of adult populations are accounted for by differences in the rate of growth of the head during the first two years of life.<sup>16</sup> Figure 3 depicts the relationship between head circumference of the mother and the proportion of infants with low and high birth weights. The relationship is significant even after controlling for maternal height and weight, and, therefore, may reflect the specific influence of the very early nutritional experience of the mother on fetal growth.

The indicators most frequently used to estimate maternal nutritional status during pregnancy are weight gain during pregnancy and dietary intake.

Weight gain during pregnancy is strongly correlated with birth weight in both industrialized and developing societies.<sup>16,17</sup> Most studies in industrialized countries fail to find an association between nutrient intake during pregnancy and birth weight.<sup>17,18</sup> This may be attributable to the poor reliability of the data or to the fact that most of the women in the samples were relatively well nourished. In the developing countries, several dietary and interventional studies have shown an association between maternal intake and birth weight. However,

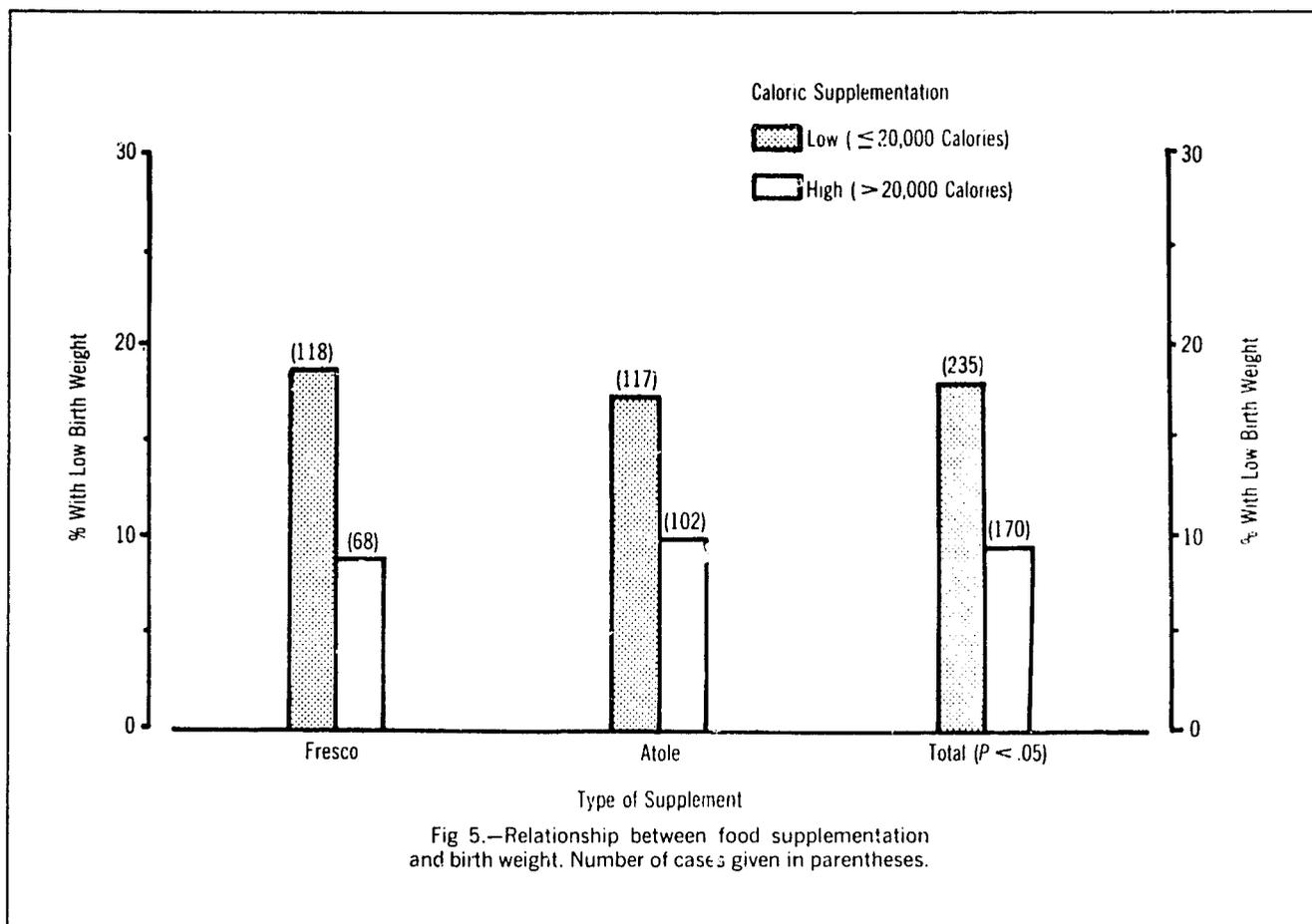


Fig 5.—Relationship between food supplementation and birth weight. Number of cases given in parentheses.

other variables such as infectious diseases and medical care have not been explicitly controlled.<sup>19</sup> We have analyzed dietary data of 51 pregnant women from rural villages not receiving food supplements. Average birth weight increased progressively as dietary intake increased (Fig 4). This relationship is still evident when weight of the newborns is corrected for such influences as height, parity, duration of illness during pregnancy, and sex of the newborn.<sup>20</sup> Data from a longitudinal nutrition intervention project in Guatemala on the effects of mild to moderate malnutrition on physical growth and mental development support this relationship.<sup>21</sup> Two types of supplement, *atole* (a gruel commonly made with corn, which supplies proteins and calories), and *fresco* (Spanish for "refreshing, cool drink," which supplies only calories), which contain protein and calories, respectively, or calories alone, are provided on a voluntary basis. A wide range of

supplement intake during pregnancy was observed; therefore, the amount of supplement ingested, physical growth, morbidity, home diet, and family sociocultural characteristics were monitored.

Figure 5 shows the percentage of low-birth-weight infants in the high and low supplemented groups. There were significantly fewer low-weight newborns in the high supplemented group than in the low supplemented one. These data indicate an association between caloric supplementation during pregnancy and birth weight, with the risk of low birth weight among the high supplemented mothers being roughly half that observed among the low supplemented mothers.

We have investigated nearly 50 maternal variables including home diet, height, parity, morbidity, and sociocultural characteristics that might account for these results. None of them could explain the observed

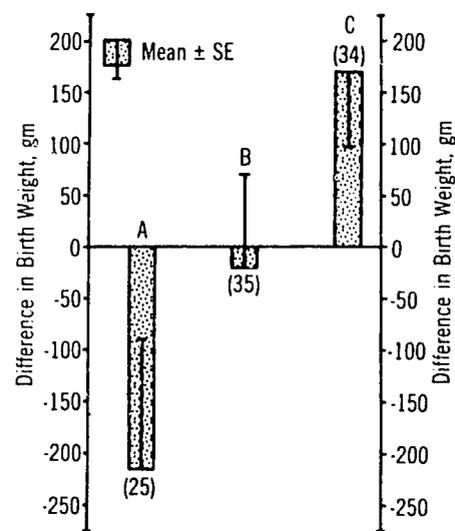


Fig 6.—Relationship between food supplementation and birth weight in consecutive pregnancies. Difference in caloric supplementation: A = from -40,000 to zero calories; B = from 100 to 20,000 calories; C = from 20,000 to 120,000 calories. Number of pairs of pregnancies given in parentheses. Difference between groups A and C:  $P < .01$ .

Magnitude of Dose Relationships		
Relationship	Slope*	SE
Caloric supplementation during pregnancy & birth weight (No. = 405)		
Before controlling for suspected confounding factors (in simple correlation)	29†	10.6
After controlling for suspected confounding factors‡ (in multiple correlation)	30†	10.6
Home diet and birth weight		
Total caloric intake during pregnancy provided by home diet	25	16.4

\* Birth weight (gm)/10,000 calories.

†  $P < .01$ .

‡ Home diet, height, head and arm circumference, weight, parity, gestational age, anorexia, and diarrhea.

association between caloric supplementation during pregnancy and birth weight (Table). The effect of home diet calories and birth weight is similar to that observed between supplemented calories and birthweight.

To investigate self-selection as a possible explanation for the association observed between caloric supplementation during pregnancy and birth weight, we studied differences in birth weight between consecutive

siblings of the same mother in order to explore the possibility that a third factor induced both high consumption of food supplement during pregnancy and heavier newborns (Fig 6). Three groups were defined by differences in level of caloric supplementation of the mother for two consecutive pregnancies. When caloric supplementation during the later pregnancy was lower than during the preceding pregnancy (bar A), the birth weight

of the later infant was also lower than the birth weight of the preceding one. When the caloric supplementation during the later pregnancy was higher than during the preceding pregnancy (bar C), the later newborn was also heavier than the preceding one ( $r = .295$ , No. = 82,  $P < .01$ ). Thus, the relationship of supplemented calories to birth weight is consistent in the whole population studied and between siblings of the same mother.

### Conclusion

Our review of the available pertinent data convinces us that an improvement in nutritional status during pregnancy leads to a substantial decrease in the incidence of low-birth-weight infants in preindustrialized societies. Simple caloric supplementation is an important positive factor. Improved fetal growth may, in turn, contribute to a reduction in the high infant mortality in these countries.

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# Infection and Low Birth Weight in an Industrialized Society

John L. Sever, MD, PhD; David A. Fuccillo, PhD; Jonas Ellenberg, PhD; Mary Ruth Gilkeson

The importance of infections of the pregnant woman as causes of low birth weight of infants in industrialized societies has been indicated in a number of studies. These infections have included upper-respiratory tract and systemic infections, bacterial infections of the urinary tract, and genital mycoplasmal infections.<sup>1,2</sup>

Specific perinatal infections such as rubella, cytomegalovirus, and *Toxoplasma* are known to be associated with higher rates of low-birth-weight infants.

## MATERIALS AND METHODS

The Collaborative Perinatal Study was a prospective investigation of approximately 60,000 pregnancies at 12 university-affiliated institutions throughout the United States. It consisted of detailed examinations of mothers during pregnancies, observation of the children from birth through 7 to 8 years of age, and collection of cord serum and serial serum specimens from the mothers from the time of registration until six weeks postpartum.

Serum specimens from women who delivered low-birth-weight infants and from matched controls, selected on the basis of race, age, last menstrual period, and dates of the specimens, were selected for detailed examination.

The complement fixation method was used for determining antibody levels to seven viruses: coxsackievirus B3, coxsackievirus B4, influenza A, mumps, rubella, rubeola, and varicella. The methods of preparation of the antigens and performance of the tests have been reported previously.<sup>3</sup>

The indirect hemagglutination method was used to determine antibody levels to

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cytomegalovirus, *Toxoplasma*, herpesvirus hominis (HVH) type 1, HVH type 2. The methods for these tests have been published previously.<sup>4,5</sup>

## RESULTS

Table 1 lists the data for 130 women who delivered children weighing 1,001 to 1,500 gm (2.2 to 3.3 lb) and for 130 matched controls. A total

of seven seroconversions and eight rises in antibody titer were found in the first group and five seroconversions and 17 rises in titer in the second. Table 2 summarizes the results for 391 women who had children weighing 1,501 to 2,000 gm (3.3 to 4.4 lb) and for 391 matched controls. In these two groups there were a few who either underwent seroconver-

Table 1.—Results of Serologic Tests of Mothers Whose Babies Had Birth Weights of 1,001 to 1,500 gm

	Mothers of Abnormal Infants (No. = 130)		Control Mothers (No. = 130)	
	Seroconversions	Fourfold Rise	Seroconversions	Fourfold Rise
Complement fixation				
Coxsackievirus B3	1	0	1	3
Coxsackievirus B4	1	0	0	3
Influenza A	1	2	1	5
Mumps	1	1	0	2
Rubella	0	0	1	0
Rubeola	1	0	0	0
Varicella	0	1	0	1
Seroconversions    Eightfold Rise    Seroconversions    Eightfold Rise				
Indirect hemagglutination				
Cytomegalovirus	1	3	1	1
<i>Toxoplasma</i>	0	0	0	0
HVH type 1	0	0	1	1
HVH type 2	0	1	0	1

Table 2.—Results of Serologic Tests of Mothers Whose Babies Had Birth Weights of 1,501 to 2,000 gm

	Mothers of Abnormal Infants (No. = 391)		Control Mothers (No. = 391)	
	Seroconversions	Fourfold Rise	Seroconversions	Fourfold Rise
Complement fixation				
Coxsackievirus B3	1	2	1	2
Coxsackievirus B4	0	2	3	2
Influenza A	1	11	0	9
Mumps	1	2	0	5
Rubella	2	2	2	1
Rubeola	0	1	1	0
Varicella	1	1	0	2
Seroconversions    Eightfold Rise    Seroconversions    Eightfold Rise				
Indirect hemagglutination				
Cytomegalovirus	1	10	1	5
<i>Toxoplasma</i>	2	0	1	0
HVH type 1	0	1	0	1
HVH type 2	0	0	0	0

sions or had a rise in antibody titers. There was no significant increase in infections among the low-birth-weight groups.

#### COMMENT

We have reported previously that low birth weights and other severe abnormalities are associated with congenital rubella.<sup>2</sup> We have also noted an increase in low birth weights and birth defects among black children born to women who had toxoplasmosis during pregnancy. Clearly, low birth weight must be considered among the abnormal findings observed following certain perinatal infections.

In the present study, when we analyzed the possible effect of 11 infectious agents in relation to low birth weight alone or to a combination of abnormalities and low birth weight, no significant association was found. We therefore conclude that these infections are not a major factor in the cause of low-birth-weight infants in this study population.

The consideration of the possible role of bacterial urinary tract infections and genital *Mycoplasma* infec-

tions in relation to low birth weight in the United States and other countries appears to be particularly important at this time. In our own studies we have found low rates of mycoplasmal infections among pregnant women from middle class populations when compared to lower socioeconomic groups (D. L. Madden et al, unpublished data). Similarly, the frequency of low-birth-weight infants is lower in the middle and upper class group. Further intensive studies of these infections and the use of antibiotics for infected pregnant women are certainly needed.

#### Conclusion

Perinatal infections with rubella, *Toxoplasma*, and other microorganisms are known to result in increased rates of low-birth-weight babies and birth defects. When low birth weight was considered alone, however, no significant association was found for 11 perinatal infections, using tests based on maternal seroconversions and antibody titer rises. More than 500 women in this study group and a similar number of matched controls were studied. Uri-

nary tract bacterial infections and genital mycoplasmal infections of the mothers may be of considerable importance in relation to low birth weight and should be investigated in detail.

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# Infection and Low Birth Weight in a Developing Country

## A Study in an Indian Village of Guatemala

Juan J. Urrutia, MD; Leonardo J. Mata, ScD; Frederick Trent, MS; José R. Cruz, MS; Elba Villatoro; Russell E. Alexander, MD

**T**he causes of fetal growth retardation and premature delivery are not well defined. Although diet,

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smoking, maternal weight and height, and social class can be correlated with the size of the fetus, the relative contribution of other factors to fetal growth has not been clarified. Among such factors in developing countries, the highly prevalent infectious diseases occupy a prominent place. This report summarizes studies of the frequency of infections in pregnant women and discusses their possible influence on intrauterine growth.

#### PATIENTS AND METHODS

The present data were derived from a long-term prospective investigation of the interactions of nutrition and infection and the outcome of pregnancy in a highland Indian village.<sup>1-3</sup> Since 1972, all women of child-bearing age have been visited every month and interviewed regarding their menses. A urine gonadotrophin test was used for the diagnosis of pregnancy in women reporting amenorrhea. Each pregnant woman underwent anthropometric

and obstetric examinations at 6 to 7, 12 to 14, 26 to 28, 36, and 38 weeks of pregnancy and, furthermore, was visited in her home every week by a physician who assessed her health. When an acute infection was found, visits were repeated at three-day intervals to determine the duration and clinical characteristics of the disease. Urinary tract infections were diagnosed exclusively by demonstration of significant bacteriuria, ie, counts of enteric Gram-negative bacteria in excess of 100,000 colonies per milliliter of urine. Since most women refused pelvic examination, vaginal inflammation was assessed by cytologic evaluation of the urine sediment by the Papanicolaou method.<sup>4</sup> Serial blood specimens were obtained from the mothers at 6 to 7, 26 to 28, 36, and 38 weeks of gestation; these were tested for seroconversion rates to *Toxoplasma gondii* by the fluorescent antibody technique,<sup>5</sup> cytomegalovirus and herpes simplex by the complement-fixation method,<sup>6</sup> rubella by hemagglutination inhibition,<sup>7</sup> and *Treponema pallidum* by VDRL. A fourfold rise of serum antibodies was considered significant for cytomegalovirus (CMV), herpesvirus hominis (HVH), and *Toxoplasma* infections. Umbilical cord blood was collected from most infants, and levels of IgM, IgG, and IgA in cord serum were determined by radial immunodiffusion.<sup>8</sup> Each infant was weighed by a nurse within one hour of delivery.

## RESULTS

### Incidence of Infectious Diseases During Pregnancy

Table 1 shows the incidence of infectious diseases during pregnancy among 82 women observed from conception to delivery. Respiratory infections were the most common, and upper respiratory tract was affected four times more frequently than lower. Diarrhea and dysentery were second in frequency, followed closely by urinary tract infections, which occurred with the same frequency in each trimester of pregnancy, whereas respiratory and intestinal infections were most prevalent in the last trimester.

Other illnesses including conjunctivitis, otitis media, stomatitis, and skin infections were also seen. The last included deep abscesses that were serious health problems because of their long duration and accompanying systemic manifestations such as chills and fever.

The average duration of illness was

Trimester of Pregnancy	Respiratory Tract Infection		Diarrhea and Dysentery	Urinary Tract Bacterial Infection†	Other Illnesses‡
	Upper	Lower			
1	37 (45)§	5 (6)	7 (9)	8 (10)	7 (9)
2	26 (32)	6 (7)	9 (11)	8 (10)	5 (6)
3	41 (50)	14 (17)	13 (16)	6 (7)	8 (10)
Incidence per 100 pregnancies	104 (127)	25 (30)	29 (36)	22 (27)	20 (25)

\* Data obtained from 82 pregnant women observed prospectively from conception to delivery in Santa Maria Cauque, Guatemala, 1972 and 1973.

† > 100,000 colony-forming units per milliliter of urine.

‡ Conjunctivitis, otitis media, stomatitis, skin infection.

§ Number of episodes (rounded percentage).

Trimester of Pregnancy	Respiratory Tract Infection		Diarrhea and Dysentery	Urinary Tract Bacterial Infection†	Other Illnesses‡
	Upper	Lower			
1	3.2§	1.2	0.3	0.9	0.8
2	2.7	1.1	0.6	0.5	0.8
3	4.8	2.4	1.0	1.1	0.9
Mean	3.6	1.6	0.7	0.8	0.8

\* Data obtained from 82 pregnant women observed prospectively from conception to delivery in Santa Maria Cauque, Guatemala, 1972 and 1973.

† An average duration of 273 days (39 weeks) was assumed for every pregnancy.

‡ Conjunctivitis, otitis media, stomatitis, skin infection.

§ Mean days of illness per 100 days.

Agent	No. (%) of Women With Agent
<i>Trichomonas vaginalis</i> , alone	12 (23)
<i>Candida albicans</i> , alone	11 (21)
<i>T vaginalis</i> & <i>C albicans</i>	9 (17)
No agent	21 (40)

\* Data obtained from 53 pregnant women observed prospectively through pregnancy in Santa Maria Cauque, Guatemala, 1972 and 1973.

7.5 days per 100 days of pregnancy (Table 2), which is equivalent to 21 days out of the 280 days of gestation. Diseases during the third trimester lasted longer than those during the first and second trimesters.

### Frequency of Vaginal Inflammation and Infection During Pregnancy

Vaginal inflammation was studied in 57 women from the first to the last trimester of pregnancy. Cellular changes indicative of inflammation were found in 48% of women at the beginning of pregnancy, and in 79%

Agent, Test‡	No. of Subjects	No. (%) Positive
Cytomegaloviruses, CF	51§	3 (5.9)
Herpesvirus hominis, CF	60§	3 (5.0)
Rubella virus, HI	61	0
<i>Treponema pallidum</i> , VDRL	61	0
<i>Toxoplasma gondii</i> , FA	61	1 (1.6)
Total	61	7 (11.5)

\* Fourfold or higher rise of titer for cytomegalovirus, herpesvirus hominis, and *Toxoplasma*.

† Data obtained from 61 women in Santa Maria Cauque, Guatemala, 1972 and 1973.

‡ Tests include the following: complement-fixation (CF), hemagglutination inhibition (HI), and fluorescent antibody (FA).

§ Anticomplementary sera excluded.

by the end of gestation.

Nonspecific inflammation (ie, that in which no potential causative agent was found) occurred in 40% of the women (Table 3). *Trichomonas vaginalis* and *Candida albicans* were found in 23% and 21% of the women, respectively, and 17% had both. No at-

Localities	No. of Infants	No. (%) of Cases† With IgM Level > 0.20 mg/ml
Santa Maria Cauque, Guatemala‡	263	111 (42)
Santo Domingo Xenacoj, Guatemala§	211	80 (38)
Four ladino villages in Guatemala	132	18 (14)

\* Umbilical cord serum IgM level measured in Guatemalan newborns from rural communities, 1964 to 1973.

† Specimens with values of IgA > 0.10 mg were excluded from tabulation.

‡ Blood collected by traditional folk midwives.

§ Blood collected by a trained midwife.

|| Blood collected by a physician.

tempts were made to isolate or characterize other organisms reportedly associated with perinatal infections such as group B streptococci or T-strain mycoplasmas.

At the beginning of the study, a limited number of vaginal swabs collected by the pregnant women themselves were examined. Among these, three cases with evidence of CMV infection and one with HVH infection were detected.

#### Silent Infections During Pregnancy

The frequency of silent or asymptomatic infections is much higher. In a preceding survey of pregnant women from the village, about 14% were found to harbor *Shigella* or *Salmonella*, 54% *Entamoeba histolytica*, and 25% enteroviruses.<sup>2</sup> Reactivated infections with CMV and HVH occurred in six (10.9%) of 61 pregnant women (Table 4). A single infection with *To gondii* (1.6%) was identified. There was no infections with rubella or *Tre pallidum*. In all, of 61 pregnant women, seven (11.5%) showed evidence of having been affected by at least one of these agents.

#### Infection and Outcome of Pregnancy

Forty-two percent of newborns had elevated IgM levels in cord blood, a finding similar to that in another highland village. No correlation was found between cord serum IgM level and birth weight.

Some of these serum samples may have had admixtures of maternal blood, which would give spuriously high levels of IgM. However, when such collections are done most precisely, as was true for blood collected by experienced physicians in four ladino villages, no less than 14% of

specimens had substantial elevations of IgM, reflecting a high rate of intrauterine exposure to infectious agents (Table 5).

No relationship was noted between incidence of infectious disease during pregnancy and birth weight by contingency table or similar types of analyses. However, step-wise multiple regression analyses suggested that morbidity during pregnancy could explain part of the variance of birth weight (J. J. Urrutia, MD, and L. J. Mata, ScD, unpublished data).

Diseases affecting lower-respiratory and urinary tracts were more frequent in the group of mothers who delivered low-birth-weight infants; these differences, however, were not statistically significant. No differences in concentration of cord IgM were found between infants of mothers who had fewer than three episodes of illness during pregnancy and infants of mothers who had more.

#### COMMENT

Our investigation showed very high rates of infection during pregnancy in an Indian village in Guatemala. There was also evidence that the newborn infants had a high incidence of elevated specific antibody titers, suggesting intrauterine exposure to infectious agents.

Attack rates of infection among these rural women are well in excess of the rates for industrial nations.<sup>8-10</sup> The precise methods used in this prospective study permitted detection of the majority of illnesses experienced by the women, and thus the observed rates we report very likely reflect true incidence of infectious diseases. This incidence exceeds considerably the previous estimates for this popu-

lation, based on a retrospective analysis.<sup>10</sup> Since most information about morbidity during pregnancy throughout the world is based on data derived retrospectively, it is reasonable to conclude that true incidence of illness is higher than has been suspected heretofore.

Infection of the pregnant women may affect fetal growth indirectly through interference with the woman's nutritional status, which in turn may result in diminished fetal nutrition. In addition, there may be a direct effect on the fetus by infection across the placental barrier.<sup>10</sup> However, not enough is known about the effects of specific maternal infections. The best available information relates to urinary tract infections and indicates a correlation between such infections in the pregnant woman and low birth weight and neonatal mortality throughout the world.<sup>11</sup> It does not seem to be affected by the mother's social class. Likewise, vaginal colonization with T-strain of *Mycoplasma*, *Listeria monocytogenes*, HVH type 2, and CMV has been associated with low birth weight and fetal wastage.<sup>10-13</sup> On the other hand, data relating such common diseases as upper-respiratory tract infections and diarrhea, which are highly prevalent among the rural women we studied, and fetal survival and well being are lacking. However, in view of the high incidence of stillbirths, prematurity, and fetal growth retardation in the Indian village we studied,<sup>2</sup> it is reasonable to suspect causal influence of the highly prevalent maternal infections. There is a great need for a precise assessment of this problem. Our prospective study, designed to answer this question, is in progress.

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# Survival and Physical Growth in Infancy and Early Childhood

## Study of Birth Weight and Gestational Age in a Guatemalan Indian Village

Leonardo J. Mata, ScD; Juan J. Urrutia, MD; Richard A. Kronmal, PhD; Claire Joplin, MS

Many factors contribute independently or jointly to the cause and pathogenesis of low birth weight. Attempts to identify these factors in a given population, however, are usually unsuccessful, and conclusions are equivocal. Among the variables related to fetal growth, socioeconomic status and size of the mother consistently show positive correlations. Thus, incidence of low birth weight, defined as less than 2,501 gm (5.5 lb),<sup>1</sup> is lowest in the nations with the highest standard of living.<sup>2</sup> Although the United States is among the most developed nations, its incidence of low birth weight is higher than that of some European countries,<sup>3</sup> primarily because of the high incidence of low

birth weight among its population groups of low socioeconomic class.

The problem is more serious in developing nations, but it is extremely difficult to assess there because of inadequacy or lack of statistical data. Data on birth weight in these countries are usually derived from hospital records that, aside from their inaccuracy, are not representative of the rural and peripheral urban population. Nevertheless, even such lim-

ited reports from Latin America, Asia, and Africa<sup>4-6</sup> indicate low birth weight rates ranging from 16% to 26%.

The magnitude of the problem of low birth weight can only be assessed by prospective observation of communities that are representative of larger areas or regions. One such study has been underway since 1963 in a typical Guatemalan Indian village, Santa Maria Cauque. When the

Table 1.—Birth Weight and Height of Live Singletons by Cohort\*

Year	No. of Infants	Mean Weight $\pm$ SD, gm	Range, gm	% < 2,501 gm
1964	37†	2,595 $\pm$ 360	1,510-3,313	35
1965	45	2,573 $\pm$ 376	1,635-3,267	42
1966	46	2,506 $\pm$ 321	1,344-3,135	46
1967	59	2,580 $\pm$ 389	1,710-3,374	41
1968	57	2,510 $\pm$ 422	1,357-3,903	44
1969	53	2,526 $\pm$ 448	1,194-3,387	38
1970	67	2,558 $\pm$ 412	1,225-3,562	36
1971	60	2,564 $\pm$ 328	1,745-3,310	48

\* Data obtained from subjects in Santa Maria Cauque, Guatemala, 1964 through 1971.

† The study began Feb 11, 1964; infants born before this date are not included.

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Reprint requests to University of Costa Rica, Guadalupe, Costa Rica (Dr. Mata).

Cohort	Age, mo			
	Birth	3	6	12
Weight, gm				
1964	2,595 ± 118†	5,288 ± 224	6,501 ± 251	7,341 ± 269
1967	2,580 ± 101	5,149 ± 187	6,326 ± 190	7,113 ± 264
1970	2,558 ± 101	4,978 ± 205	6,227 ± 222	6,931 ± 247
Height, cm				
1964	46.5 ± 0.7	56.4 ± 0.8	61.5 ± 0.8	67.6 ± 1.0
1967	45.7 ± 0.6	55.8 ± 1.4	61.6 ± 1.5	67.5 ± 2.3
1970	45.7 ± 0.5	54.8 ± 0.9	60.3 ± 0.7	65.8 ± 0.8

\* Data obtained from subjects in Santa Maria Cauque, Guatemala, 1964 to 1972.

† Mean ± SD.

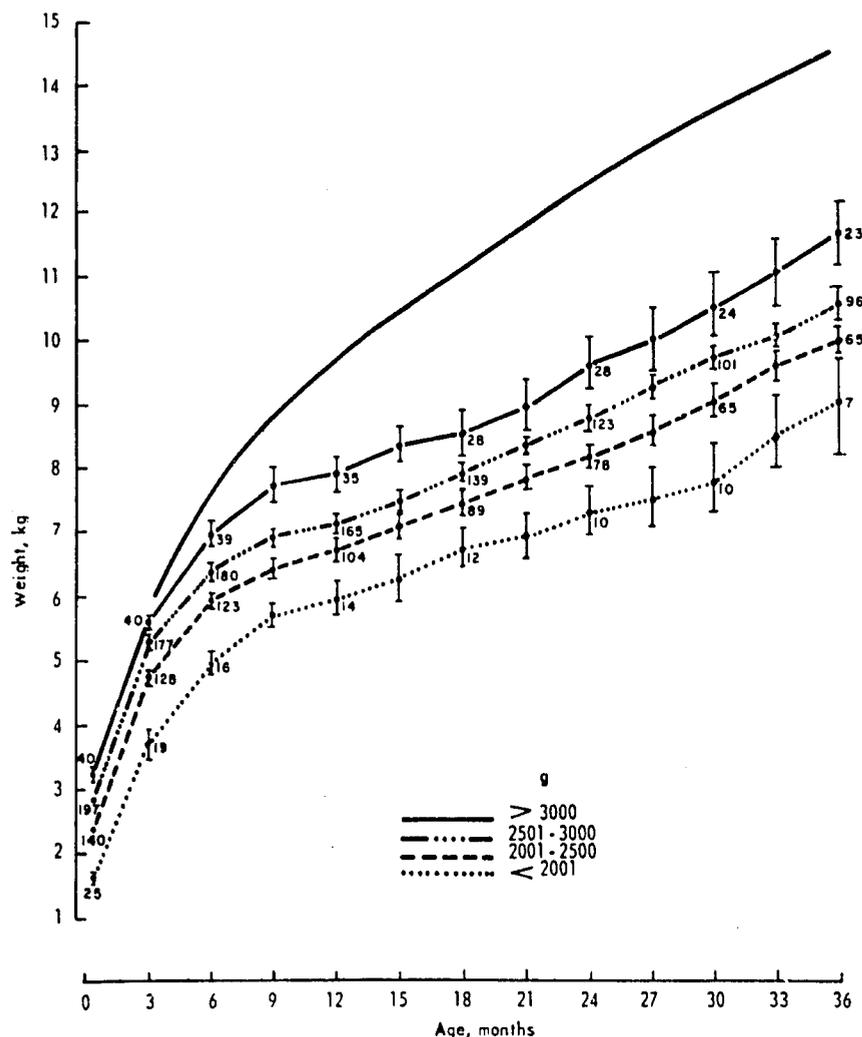


Fig 1.—Weight curves (means ± 2 SE) of cohorts of children defined by birth weight, Santa Maria Cauque, Guatemala, 1964 to 1972, in comparison with INCAP standard.<sup>12</sup> Numbers in curves denote children measured.

study began, this community, near Guatemala City at an altitude of 1,890 meters (6,200 ft), had a population of 1,071. By 1971, when observations reported here were com-

pleted, it had grown to 1,370 people, and it has been growing at a rate of 3% per year with minimal migration.<sup>12</sup> The birth rate has been approximately 50 per 1,000 population,

with an infant mortality of about 90 per 1,000 livebirths. Deliveries take place at home according to tradition and custom. Breast feeding is begun shortly after birth, and the total lactation period is one to four years. Chronic protein-calorie malnutrition and a high rate of infection are prevalent in people of all ages.<sup>12</sup>

## SUBJECTS AND METHODS

Early in 1963, a health center, staffed by a team of health workers, established a firm association with villagers that permitted observations on virtually the whole population. The center provides care and serves as a base of operations. Services consist of treatment of illnesses and injuries. However, immunization programs were deficient and no large-scale nutritional or health intervention was implemented during the period of observation (1963 to 1972).

The key factor responsible for the completeness and high accuracy of the collected data was an early acquaintance with the village authorities, leaders, women, and folk midwives. Deliveries were reported when they took place. Auxillary public health nurses, posted in the village around the clock (including weekends), visited the homes within one hour of an infant's birth, measuring the newborn and collecting pertinent information about the mother and the infant and their immediate environment.

There were 465 deliveries during the study period, resulting in 460 singletons and ten twins. Among the singletons, 446 were born alive; birth weight was obtained on 430 (96%) and gestational age on 416 (93%). All infants remained under observation and were weighed and measured periodically.<sup>12-14</sup>

## RESULTS

There was a remarkable constancy in the pattern of fetal growth, infant mortality, and postnatal growth during the study period. The mean and standard deviation of birth weight were similar during the individual years of the study, as was the incidence of low-birth-weight infants (Table 1). Likewise, the mean weight and height at various ages, exhibited by the yearly cohorts, were quite stable (Table 2). Although some environmental and social characteristics changed during the study period (for example, the average area of land for

Fig 2.—Mean head circumference curves of cohorts of children defined by birth weight, Santa Maria Cauque, Guatemala, 1964 to 1972. Numbers in curves denote children measured.

cultivation per family decreased by 20%, more men became landless laborers, the water supply was improved, electric current became available, and there was a slight decrease in the illiteracy rate), such changes apparently did not result in alterations of behavior of important biological variables used to measure fetal and postnatal growth. This constancy of biological measurements permits the following analysis and interpretations.

### Survival

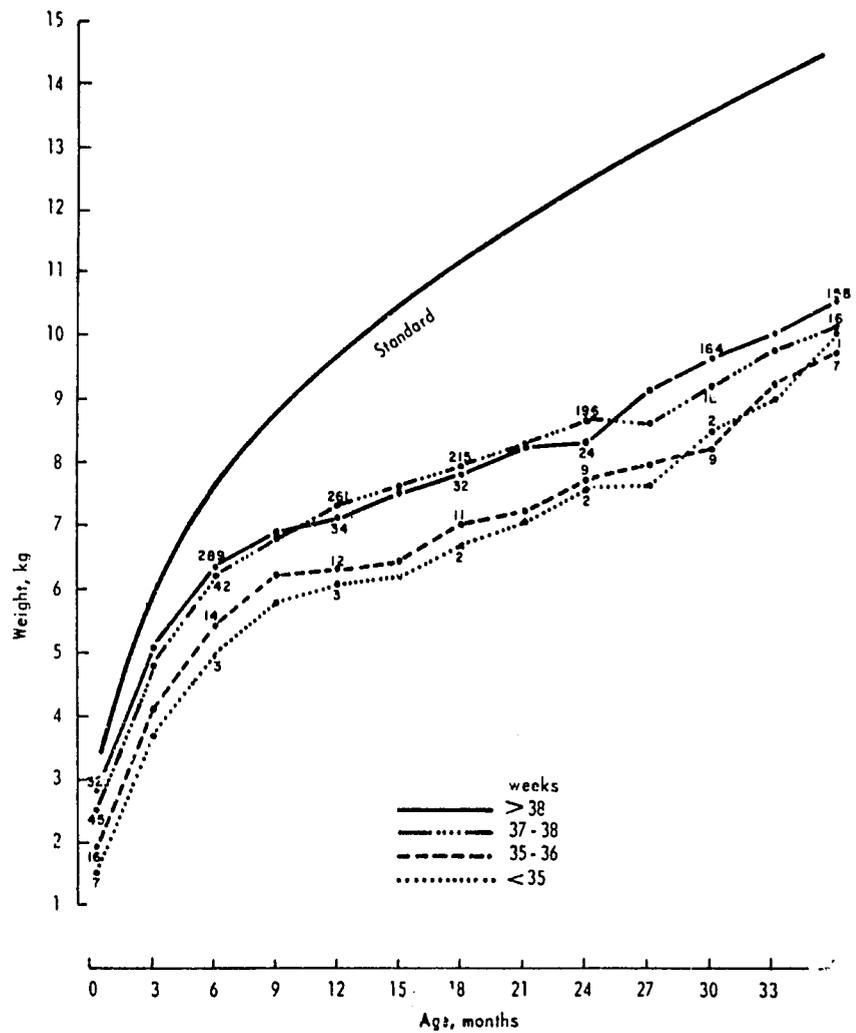
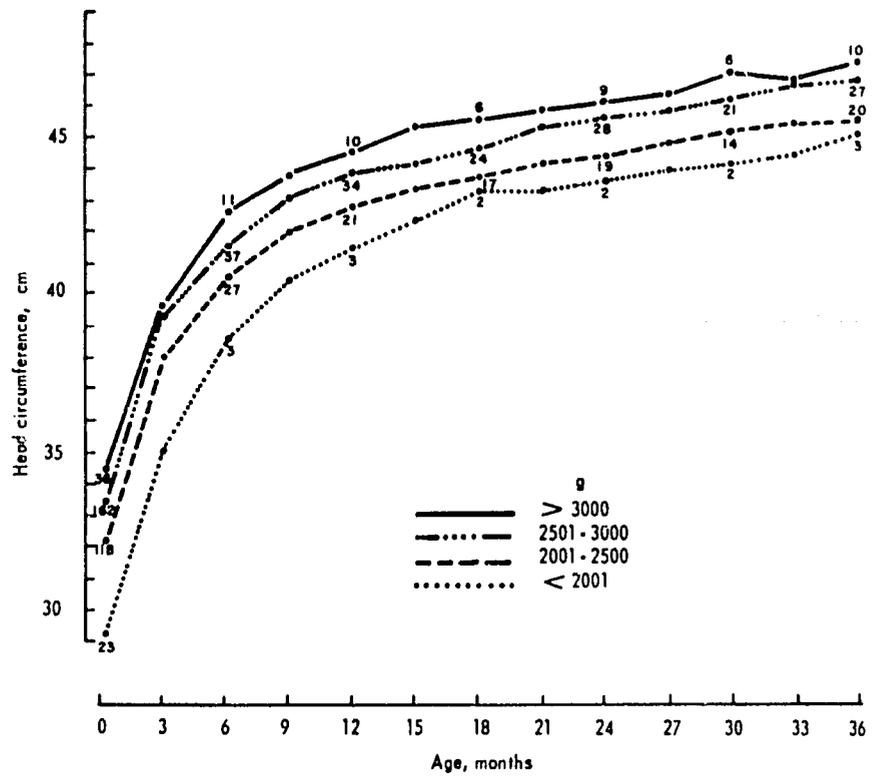
A direct correlation between gestational age and survival was also noted. This association, however, was not as clear-cut, although products of gestation of less than 37 weeks had a higher mortality than term newborns (Table 1). Survival of term infants was almost always associated with large birth weight. Since, under field conditions, birth weight can be determined better than gestational age, it becomes a good predictor of survival in the neonatal and postneonatal period.

The relationship of fetal maturity (defined by the combination of birth weight and gestational age) to survival is described elsewhere.<sup>11</sup> Pre-term infants died more often than was expected. The small-for-date infants born at term had a high mortality in the first two years of life. Term infants adequate for gestational age fared the best.

### Postnatal Growth

All of the 430 singletons with known birth weight and all of the 416 with known gestational age were observed prospectively. Seven children were lost to follow-up because of migration. Other attrition in numbers was due to the fact that the data are

Fig 3.—Mean weight curves of cohorts of children defined by gestational age, Santa Maria Cauque, Guatemala, 1964 to 1972, in comparison with INCAP standard.<sup>12</sup> Numbers in curves denote children measured.



Birth Weight, gm	No. of Infants	Age	
		< 29 Days	29 Days-5 mo
< 1,501	5	3 (600)†	1 (200)
1,501-1,750	11	2 (182)	3 (273)
1,751-2,000	17	4 (235)	4 (235)
2,001-2,250	47	2 (43)	2 (43)
2,251-2,500	99	3 (30)	0
2,501-2,750	125	2 (16)	3 (24)
2,751-3,000	82	0	2 (24)
3,001-3,250	32	0	0
3,251-3,500	11	0	0
> 3,500	1	0	0
<b>Total</b>	<b>430</b>	<b>16 (37)</b>	<b>15 (35)</b>

\* Data obtained from 430 singleton infants in Santa Maria Cauque, Guatemala, 1964 to 1973.

† Deaths, and in parentheses, rate per 1,000 live births of that birth weight category.

based on cohorts whose numbers become fewer with progressive age, and to death, which was more frequent during the first two years of life (L. J. Mata, ScD, et al, unpublished data).

Weight curves were determined for children within categories of birth weight, computing the means ( $\pm 2$  SE) of weight values at three-month intervals (Fig 1). When a measurement was not available, the closest value within approximately two weeks was used. It is evident that the proportionate differences in weight observed at birth are maintained during the first years of life. Unpublished observations of the 1964 and 1965 cohorts indicate that children tend to remain within their birth weight categories during the first eight years of life. A similar tendency is noted for head circumference (Fig 2) and height, but less so for chest circumference. Measurement of the last carries a much greater risk of inherent error.

For all variables, the lowest curves correspond to the very small infants (less than 2,001 gm [4.4 lb]) who were preterm by gestational age. The next lowest curves represent children with birth weights of 2,001 to 2,500 gm, most of whom had 37 or more weeks of gestation. A few of these were preterm by gestational age and as a group behaved differently from those small for gestational age in that their growth curve was very close to that of

full-term infants with birth weights of 2,501 to 3,000 gm (6.6 lb).<sup>11</sup> Infants with birth weights above 2,500 gm exhibited different growth patterns (Fig 1 and 2) if they were subdivided into two birth weight groups.

Weight curves as a function of gestational age tended to show only two distinct groups, the preterm and the term infants (Fig 3). The same applies to height and head and chest circumferences. Head circumference correlated well with gestational age during the first 15 months of life; thereafter, differences were less noticeable. It should be stressed that head circumference and gestational age correlated well during the period of head growth, and particularly in the first months of life.

The growth pattern as a function of fetal maturity (defined by birth weight and gestational age) is described elsewhere.<sup>11</sup> Preterm infants with very low birth weights had the worst growth curves. Small-for-date infants born at term were next, and term infants adequate for gestational age grew best.

#### COMMENT

In the region from which these data were derived, there is considerable biological stability in host measurements. Whatever changes were detected in certain host and environmental variables in the eight-year span did not appear to influence biological measurements such as birth weight, infant mortality, and physical growth. Among three dozen variables relating to ethnic composition, family size, family organization, literacy and schooling, land and home ownership, type of agricultural crops, quality of housing and environmental sanitation analyzed between 1959 and 1971 at four-year intervals, only a few showed substantial change. A similar stability has been noted about food habits and prevalence of infection.

Whereas stable preindustrial societies are known to exist,<sup>13</sup> certain evolutionary changes are detected even under conditions of isolation. Guatemalan Indian and non-Indian villages show considerable proclivity toward change at the moment, but

the remarkable constancy of certain host and environmental factors offers a unique opportunity for observing associations between antenatal and postnatal events, as illustrated above.

An extremely high incidence of low birth weight occurs in this village.<sup>10,11</sup> Studies from Latin America, Africa, and Asia<sup>14</sup> indicate the universality of the problem, which is not yet recognized because adequate statistics are generally lacking.

In the Indian village, most neonatal and postneonatal infant deaths occurred among low-birth-weight infants, supporting the classical concept of the relationship between low birth weight and poor survival established in urban industrial populations.<sup>15-17</sup> A well-trained pediatrician and two public health auxiliary nurses closely attended most ill village infants, administering antibiotics, hydration, and advice whenever necessary. These measures decreased the infant mortality by 40% from the preexisted level (J. L. Mata, ScD, et al, unpublished data), but failed to lower it below 90 per 1,000 live births.

The association between low birth weight and survival was so striking that infant mortality stands out as an indicator of fetal growth and maternal health. On the basis of the data presented, it can be assumed that an infant mortality of 100 per 1,000 in similar regions where infants are breast fed indicates a 30% to 40% incidence of low birth weight, providing tetanus neonatorum is not a problem in the area. This concept, however, cannot be generalized to all situations. For example, a high infant mortality may occur despite a low incidence of low birth weight if infants are improperly weaned at an early age, as presently occurs in large urban centers of developing nations. An international investigation of childhood mortality<sup>18</sup> has shown that the interaction of poverty, low birth weight, improper weaning, and infectious disease accounts for most premature deaths throughout large urban areas of Latin America.

Observations reported here show that fetal growth is correlated with postnatal physical growth. Infants

born with deficient weight (or pre-term) had a tendency to remain in the lower growth tracks, whether the variable measured was weight, height, or head and chest circumferences. This applied throughout the length of the study, ie, seven years. Differences of weight became accentuated with time; those of head circumference were greater during the first 15 months of life, and particularly in the first month.

The relationship between birth weight and postnatal physical growth has been the concern of many workers who found positive correlations by retrospective analysis.<sup>20-22</sup> Prospective studies have been done all too infrequently. For instance, two studies have shown that premature and small-for-date infants grow abnormally,<sup>23,24</sup> despite the provision of an adequate environment. The comprehensive study of the 1958 cohort of British infants disclosed that birth weight and gestational age were positively correlated with postnatal growth and development.<sup>25</sup>

Little of this type of information is available from developing countries.

One study of Nigerian infants whose birth weights were below the tenth percentile for the region showed that they had a poorer weight gain than children with larger birth weight.<sup>27</sup> A similar observation was recorded for Gambian newborns observed prospectively in their rural environment.<sup>28</sup>

The relationship of birth weight and postnatal physical growth is important because psychomotor retardation, intellectual impairment, and lower survival are the sequelae of suboptimal fetal and postnatal growth and development.<sup>29-31</sup> Thus, birth weight is important as a predictor not only of survival, but also of physical and intellectual development, particularly in preindustrial societies which have a very high incidence of low birth weight.

The importance of the present study lies in the fact that it is an eight-year prospective field observation of virtually the whole population of newborns in a typical Guatemalan village under natural conditions and without a variability imposed by intervention. We still need to learn more about the cause and pathogen-

esis of low birth weight in developing countries in order to devise some type of control and achieve prevention. The role played by maternal nutrition cannot be denied, and measures to improve it must be undertaken. However, more emphasis should be given to assessing the contribution of certain pathologic processes in the mother that are susceptible to treatment or prevention. Infectious diseases are an example of such a process because they are a direct or a contributing cause of maternal malnutrition, as well as a cause of fetal growth retardation, abnormal development, and premature delivery. Although our knowledge of the factors responsible for the high rates of low birth weight in whole communities is still incomplete, application of what is already known can be an exciting challenge to those concerned with the solution of this problem.

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# Birth Weight and Psychomotor Performance in Rural Guatemala

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Previous studies suggest that low birth weight (<2.5 kg [5.5 lb]) is associated with deficits in mental performance.<sup>1,2</sup> Although potentially confounding factors are often ignored, consideration of other variables does not deny an association between low birth weight and poor mental performance.<sup>1,3</sup>

The problem of whether low birth weight is associated with deficits in mental performance in a developing country must be considered. It is difficult to draw inferences from one population to another since cause of low birth weight may not be the same in the two populations. Different types of low birth weight appear associated with different functional outcomes, such as different morbidity, mortality, and prevalence and type of behavioral abnormalities. Some low-birth-weight newborns may be relatively more impaired mentally than others. Harper and Wiener<sup>4</sup> reported slightly lower intelligence quotients in low-birth-weight twins and triplets than in low-birth-weight singletons.

Most studies reporting a relationship between low birth weight and mental development have been con-

ducted in industrialized nations, and the results are often generalized to developing countries. Therefore, the intent of our study was to examine rural Guatemalan infants and to determine whether the association between low birth weight and mental performance indicated in industrialized nations can also be demonstrated in a developing society.

The association between birth weight and mental performance is usually treated as a threshold phenomenon. Birth weights of 2.5 kg or less have been considered to be associated with deficits in mental performance; whereas those above 2.5 kg generally have not. As has been emphasized, this demarcation may not be reasonable.<sup>1</sup> There may exist a gradient of impairment that extends from severe dysfunction of very low-birth-weight infants to more subtle mental deficits as the weight of the newborn increases. Therefore, we have explored the range of birth weight within which there is an association with psychomotor performance.

## SUBJECTS AND METHODS

The sample consisted of infants born from Jan 1, 1969, to March 1, 1973, in four rural ladino Guatemalan villages and studied from birth to 6 months of age. In these villages, free medical care and nutritional

supplements are offered to all residents. A number of physical and mental assessments are made regularly.

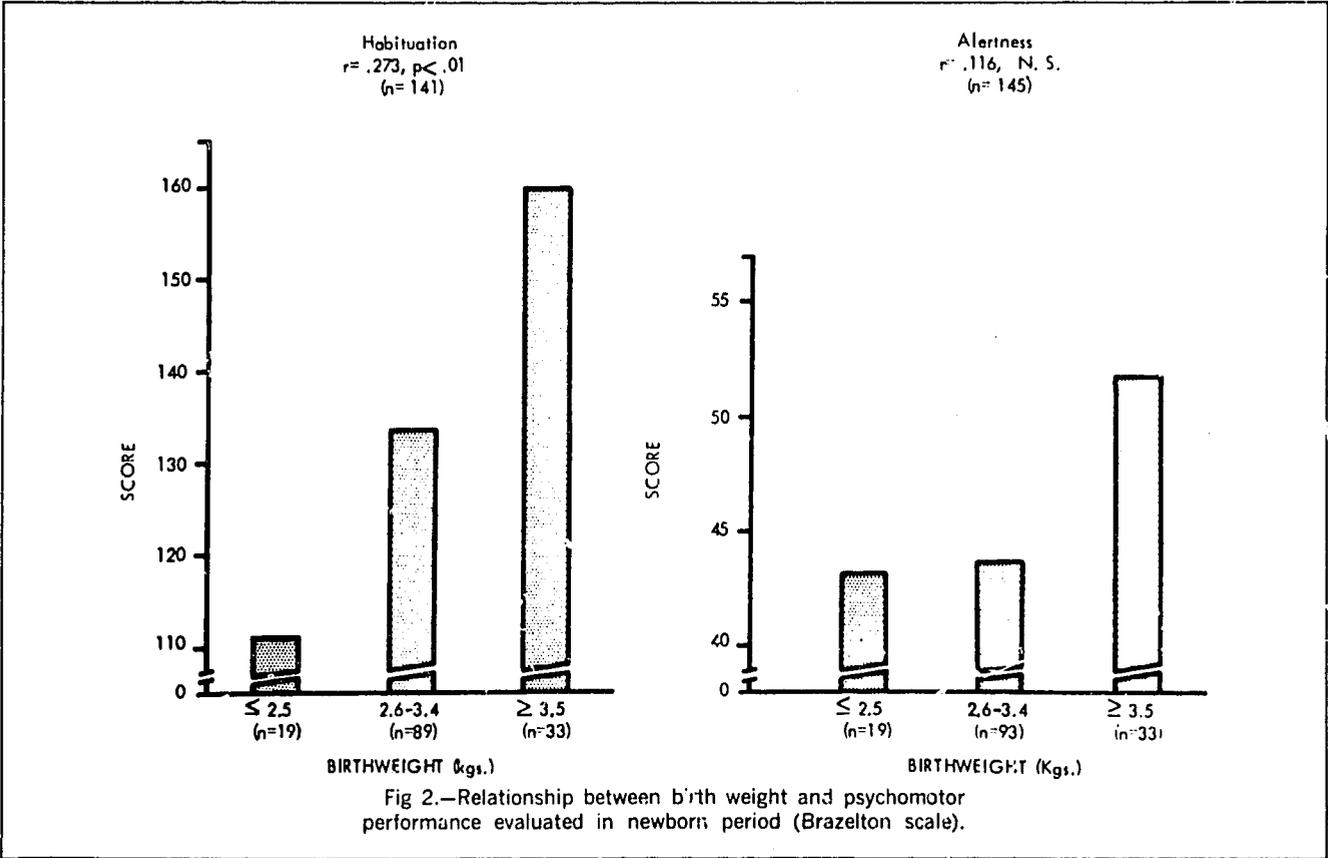
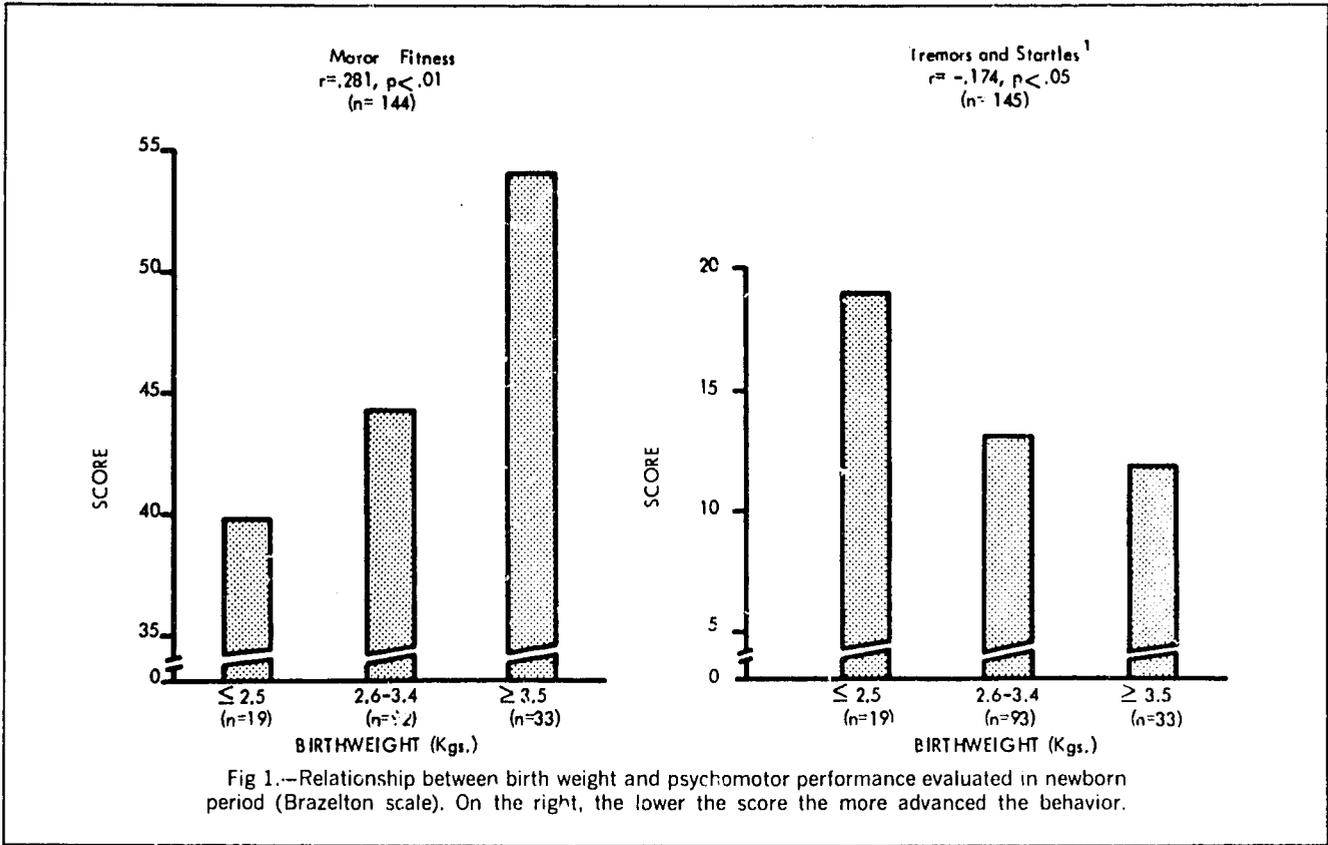
Mean birth weight in 405 live newborns was 3.05 kg (6.7 lb) (range, 1.8 to 4.8 kg [4.0 to 10.6 lb]) excluding nine pairs of twins and two clearly abnormal cases. Of these, 145 were administered the Brazelton Assessment Scale within the first two weeks of life, testing arousal state, irritability, motor capabilities, reaction to external stimuli, and neonatal reflexes. These items were grouped into 11 summary variables of which the four with significant test-retest reliability were analyzed. The Composite Infant Scale (CIS), which consists of 91 items drawn from four widely used scales assessing psychomotor development in infancy, was administered to 352 infants. The CIS was grouped into two subscales, mental and motor, that had high test-retest reliability.

## RESULTS

As seen in Fig 1 and 2, birth weight is clearly associated with performance on three of the four Brazelton variables—habituation, motor fitness, and tremors and startles. The correlation between birth weight and the motor subscale of the CIS is also significant. These results suggest that in these villages birth weight and psychomotor development are associated.

The mean scores on the four Brazelton variables for newborns grouped into three categories of birth weight ( $\leq 2.5$  kg, 2.6 to 3.4 kg, and  $\geq 3.5$  kg

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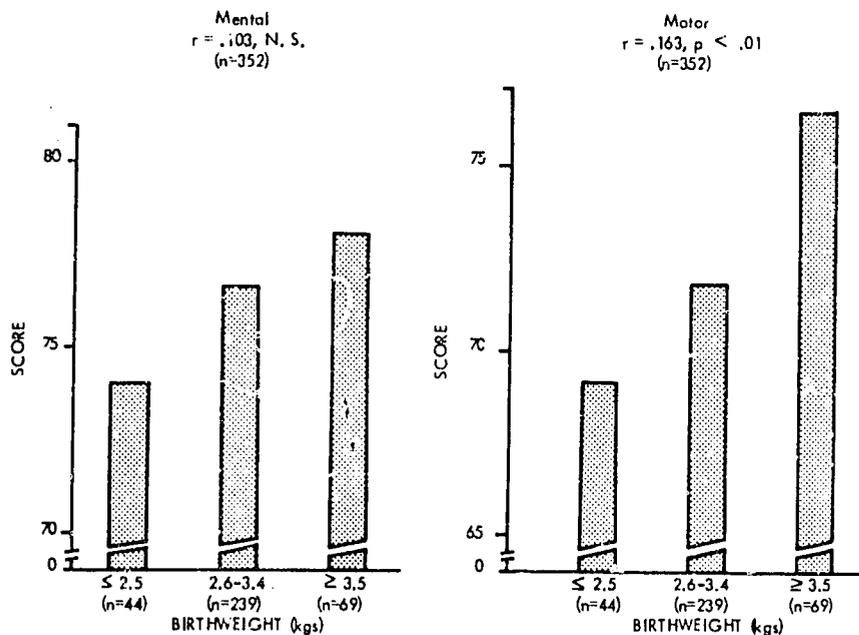
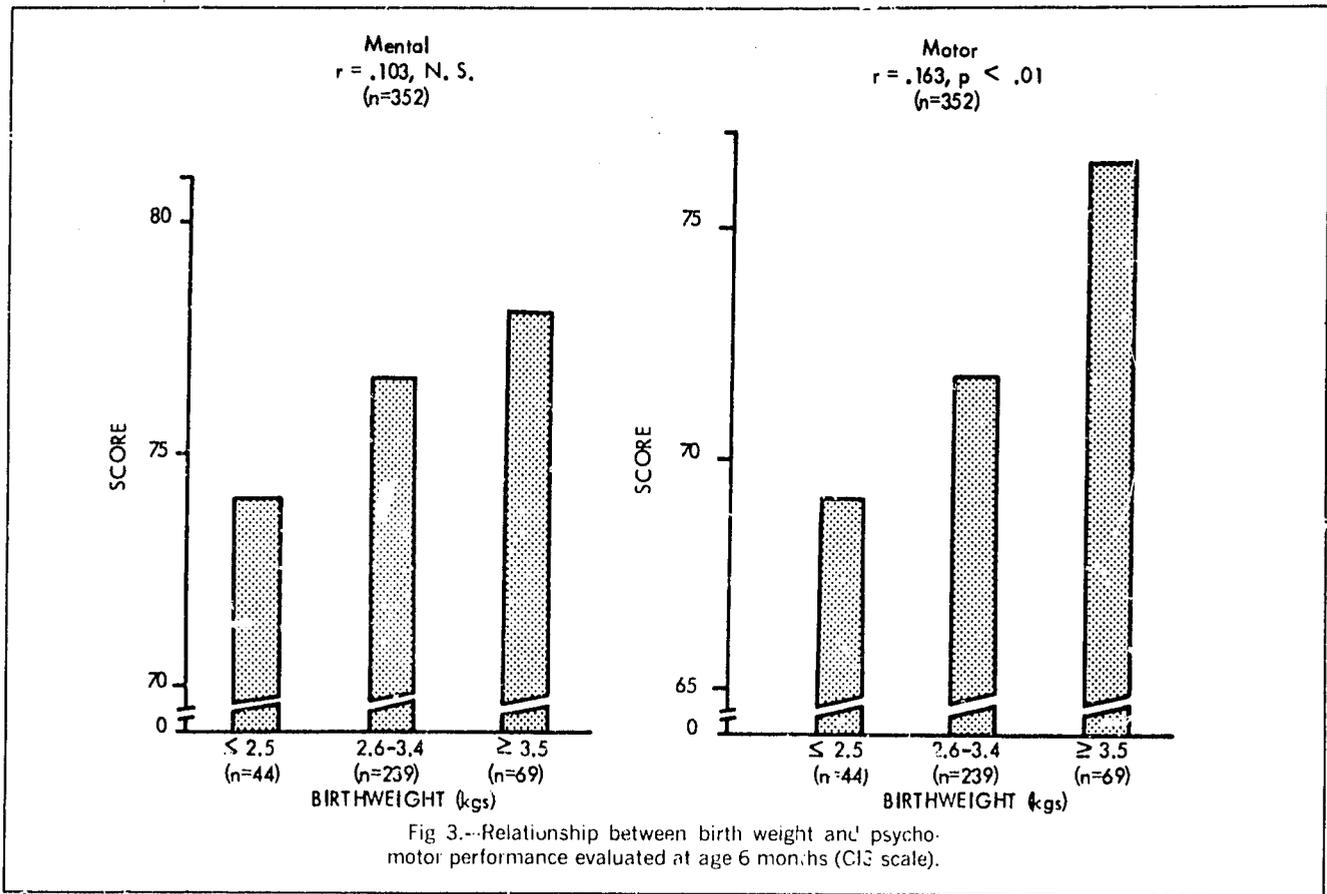


Fig 4.—Relationship between birth weight and CIS performance at 6 months.

[ $\leq 5.5$  lb, 5.6-7.5 lb, and  $\geq 7.6$  lb]). The low birth weight category is consistently related to lower psychomotor performance. Since there are no

newborns weighing less than 1.8 kg (4.0 lb), this result cannot be due to infants of very low birth weight. Furthermore, the performance of new-

borns weighing between 2.6 and 3.4 kg was between the low and the high birth weight categories. Figure 3 demonstrates a similar pattern of results with the 6-month-old infants on the CIS.

In order to determine if there is a relationship between birth weight and psychomotor performance in infants who weigh more than 2.5 kg at birth, the low-birth-weight infants were excluded. Within this restricted range of birth weights, there is a significant correlation between birth weight and the habituation and motor fitness scores. The correlation between birth weight and the motor subscale of the CIS approached significance. Therefore, in our population the association between psychomotor performance and birth weight probably cannot be entirely traced to low birth weight. In fact, this association seems to persist among newborns weighing more than 2.5 kg.

We have measured approximately

Variables Significantly Related to Sixth-Month CIS Performance		
Variables	Subscales	
	Mental	Motor
Mother's head circumference (No. = 351)	.13*†	.13†
Mother's third trimester weight (No. = 205)	.16†	.06
Parity (No. = 351)	-.05	-.13†
Birth interval (9-37 mo) (No. = 256)	.18†	.19†
IgM level (No. = 155)	-.15	-.17†
Morbidity of mother during pregnancy (No. = 214)	-.12	-.14†
Months lactating during pregnancy (No. = 141)	-.22†	-.02
Caloric supplementation during pregnancy (No. = 351)	.11†	.12†

\* Correlation coefficient.

†  $P < .05$ .

50 factors that could be confounding or causal in the observed association between birth weight and performance. After controlling for each of these variables, the association between birth weight and CIS motor performance remained significant.

We also explored differences between siblings in order to determine whether the relationship between birth weight and psychomotor performance is due to differences between mothers related to both birth weight and performance of their infants. In our sample we have 65 pairs of consecutive siblings who have both birth weight and six-month CIS data. The significant relationship observed in the entire sample between birth weight and performance on the motor subscale was replicated with this sibling sample ( $r = .266$ ,  $P < .05$ ). Therefore, the association between birth weight and psychomotor performance is consistent in the whole population and between siblings.

The birth weight appears related to psychomotor development during the first half year of life in these villages. In our populations, very low scores on the CIS seem to be indicative of extremely impaired functioning. This is more predictive of later intellectual functioning than an average or high score.<sup>11</sup> Infants who died in the first three years of life had significantly lower scores on the mental ( $\chi^2 = 33.51$ ,  $df = 20$ ;  $P < .05$ ) and motor ( $\chi^2 = 39.73$ ,  $df = 20$ ;  $P < .01$ ) subscales at 6 months of age than did infants who survived. Figure 4 presents the percentage of

infants falling in the first and fourth quartiles of CIS scores within three categories of birth weight. In comparison to infants weighing 3.5 kg or more at birth, almost three times as many low-birth-weight infants scored in the lowest quartile of the motor subscale. Lower birth weight infants in our population seem to be at higher risk psychologically than heavier ones.

Finally, it is of interest that some variables we have measured are significantly associated with CIS performance independently of birth weight. These variables and the magnitudes of their associations with CIS performance are listed in the Table. In addition to birth weight, these variables seem to be important in understanding the behavioral capacities of 6-month-old infants in these rural villages.

#### COMMENT

In rural Guatemala, birth weight and psychomotor performance during the first six months of life seem to be associated. These results imply that deficits in behavioral development are related to birth weight in developing nations, where the magnitude of the problem is probably greater than in industrialized countries where low-birth-weight infants are less common.

Furthermore, although behavioral deficits are most evident in low-birth-weight infants ( $\leq 2.5$  kg), deficits associated with birth weight also exist with respect to infants generally con-

sidered to be full-sized at birth. Our results indicate that behavioral deficits may be associated with infants who are neither of low birth weight nor obviously impaired.

A final point seems worth mentioning. In addition to low birth weight, a number of factors such as maternal head circumference, parity, birth interval, disease and nutrition during pregnancy, third trimester weight, and lactation are associated with psychomotor performance in rural Guatemala. Because these factors are highly prevalent in the poor segments of the world's population, they may contribute to the inequity of opportunity that is part of the definition of poverty.

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### Summarized Discussion of Session III

The discussion began with a comment by Dr. Rush, who wondered whether calories ingested by the mother could be transported directly to the fetus without first being stored as fat in the mother. This question was prompted by data presented by Dr. Lechtig showing that there was a relationship between caloric intake and birth weight that remained significant even when it was controlled for the third trimester maternal weight. Dr. Kass agreed that maternal weight gain is the best predictor of infant weight, but questioned whether effect of food supplement could be interpreted unless prepregnant weight of the mother was considered. Dr. Lechtig pointed to the differences between mothers in the industrialized countries and those in Guatemala, where the average weight gain during pregnancy is 7 kg (15 lb) and the mean birth weight 3 kg (6.6 lb), and in whom it is unlikely there would be any storage of fat.

The question was then raised as to whether it was possible for the fetus to gain direct energy from maternal food intake. This, Dr. Rush pointed out, would be testable, since calories seem effective whether they are gained in the first or third trimester. It is unlikely that first trimester caloric supplementation could affect fetal growth directly, whereas third trimester caloric intake could very well do so. Dr. Rosenberg asked whether glucose could act through a protein sparing effect in the mother or directly influence growth and protein metabolism in the fetus. To this Dr. Lechtig replied that the limiting factor in the home diet is calories, and that the caloric intake and not protein intake correlates with birth weight in villages where diets are unsupplemented. Association between morbidity and birth weight disappears when home caloric intake is controlled. It appears that the human fetus can adapt better to protein deficiency than to caloric deficiency. Even with low levels of amino acids in the maternal blood, the

placenta is able to keep high levels in the fetal blood. This clinical observation is supported by data from animal experiments. Dr. Behrman interjected that epidemiological data could not be equated with physiologic mechanisms because there was an enormous margin of safety in the placenta with some fiftyfold advantage to the fetus. This was at least true for oxygen consumption. Dr. Rush disagreed and pointed out that at the small level of fetal weight gain, say 100 gm (3.5 oz), one cannot measure safety margin for the fetus. Dr. Behrman came back to his original point and supported it by the statement that glucose followed a diffusion gradient and that one could predict the fetal blood glucose level from that of the mother's blood and, more important, that altering uterine blood flow by 25% did not alter quantitatively transported glucose or oxygen consumption of the fetus. Dr. Rush's final point was that this represented an experimental situation in an animal and could only be considered a hypothesis in the case of the human fetus.

Dr. Sinclair then took up this subject and stated that the reserves of the fetus in relation to the transplacental uptake of glucose were not unlimited, and that, furthermore, in fetal lamb studies in starved ewes, glucose uptake fell much more than fetal oxygen consumption. Again, in these experimental studies, maternal hypoglycemia altered stoichiometric relationship between glucose uptake and oxygen consumption. At this point Dr. Beisel asked whether it was correct to assume that the fetus was always anabolic without considering simultaneously the catabolic processes. If glucose level fell, then one would expect glucagon to be secreted and to cause deamination of amino acids for energy rather than growth. This certainly does occur in the newborn. Dr. Rush made the point that only 2% of variance in birth weight was accounted for by the supplementation, which was not attenuated by

the weight gain. Analytical procedures to determine pathways are very difficult for such small changes since one must remember that 100 gm of birth weight change may halve mortality of the infants. Small changes in terms of effect may have enormous public health influence. Dr. Kass supported this by pointing out that this was precisely the reason why one looked at the infection, since small changes of a few percent were more likely to be due to external agents than to nutrition of the pregnant women. His laboratory has been examining the relationship between vascular lesions in the cord and *Mycoplasma hominis* infection. A 5% to 7% change in the flux of glucose could well be accounted for by minimal vascular changes in the placenta, and many nutrients may in fact affect vascular changes through physiologic means.

Dr. Mata brought up the question of infectious burden of the mother. The older idea that many of the pregnant women were quite healthy is probably incorrect, since in the rural areas of developing nations they suffer upper-respiratory tract infections, pneumonia, hepatitis, and diarrheal and dysenteric episodes. Thus, the nutrition of the mother may not depend on food access alone, and since nutrition of the fetus is clearly related to that of the mother, one would have to examine the other factors that may prevent the mother from being properly nourished, such as infection. Dr. Sinclair raised the issue of nurture of the small baby after delivery, in view of the data presented earlier that the growth curve during the first 1½ years of life is low if birth weight is low. He wondered whether there might be some subtle maternal attitude changes that may increase the risk factor after birth or first weeks of life. For example, low birth weight, illness, etc. may be picked up by the parents and responded to in a very pessimistic way.

# Synergistic Effects of Maternal Malnutrition and Infection on the Infant

## Recommendations for Prospective Studies in Man

William R. Beisel, MD

Certain infections in the pregnant woman may be accompanied by deleterious effects on the fetus, regardless of the presence of fetal infection. Severe maternal infection can lead to fetal death in utero or to premature labor; less severe infections in the mother seem to be involved in the pathogenesis of fetal growth retardation.

The Figure is a set-theory diagram to illustrate a basic mechanistic concept, that various normal nutritional, biochemical, metabolic, or endocrine processes in the mother may be altered during states of infection or pregnancy. Individual maternal processes (illustrated by capital letters in the several sets) may be enhanced or suppressed as a typical physiologic response to pregnancy (lower middle set) or may remain unaltered. Infection (top middle set) initiates many specific responses in the host. In a pregnant woman, individual maternal processes may remain unchanged or may exhibit synergistic, antagonistic, or noncorrelating responses to the combination of both stresses in the same individual. These changes may be detrimental to the fetus. Research is needed to identify specific maternal effects that may cause fetal growth retardation.

Similar set-theory diagrams can be prepared to illustrate theoretical mechanisms that would explain the following: (a) the deleterious effects on the fetus of transplacental infec-

tion; (b) the effects of maternal malnutrition per se; and (c) the more complex interrelationships that appear to develop when infection occurs in an already malnourished pregnant woman.

### Goals for Future Research

Concepts such as those illustrated in the Figure can serve as fundamental null hypotheses for designing clinical and basic studies to investigate such interactions. As a major goal for future research, it will be necessary to identify with certainty the key pathogenic mechanisms that could lead to fetal growth retardation, since there is evidence that harmful effects of infection<sup>1,2</sup> and malnutrition<sup>3,4</sup> may be synergistic during pregnancy.

A broad research approach can logically be subdivided along several distinct lines of clinical investigation to gather important descriptive and epidemiological data and to establish correlations among key variables; also, additional pilot-sized trials are needed to evaluate possible prophylactic or therapeutic measures.

The following list summarizes the information required for defining the role of maternal malnutrition or infection, or both, in the pathogenesis of fetal growth retardation in humans.

#### 1. Descriptive studies

- A. Studies specifically related to fetal growth retardation: (1) to develop and standardize better diagnostic indicators of maternal and fetal malnutrition and infection; and (2) to survey the magnitude and extent of fetal growth retardation in a variety of populations

- B. Studies for general background information: (1) to define nutritional needs of pregnancy with greater precision and in terms of variables such as race, parity, and age; and (2) to define nutritional needs during and after various types of infections in both pregnant and nonpregnant individuals

#### 2. Correlative studies

- A. Studies to relate data on maternal nutrition, infection, or both with birth weight
- B. Studies to relate birth weight with future patterns of growth, development and health

#### 3. Intervention-type studies

- A. Studies to evaluate the possible benefits of prophylactic or therapeutic procedures that may be considered for use during pregnancy, the neonatal period, and childhood
- B. Studies to define requirements for achieving "catch-up" growth

Concepts of the role of maternal malnutrition and infection in the pathogenesis of fetal growth retardation have been largely based on correlations between birth of a small-for-gestational-age infant and some maternal abnormality. However, statistically significant correlation coefficients do not necessarily indicate a cause-and-effect relationship between two variables. Nevertheless, correlations are of value because they call attention to the relationship in question and assist in the planning and design of better studies. Additional research is required to correlate the presence of fetal growth retardation or other manifestations of fetal malnutrition with specific identifiable maternal derangements. To do this, better laboratory indicators than those heretofore employed are needed to diagnose ma-

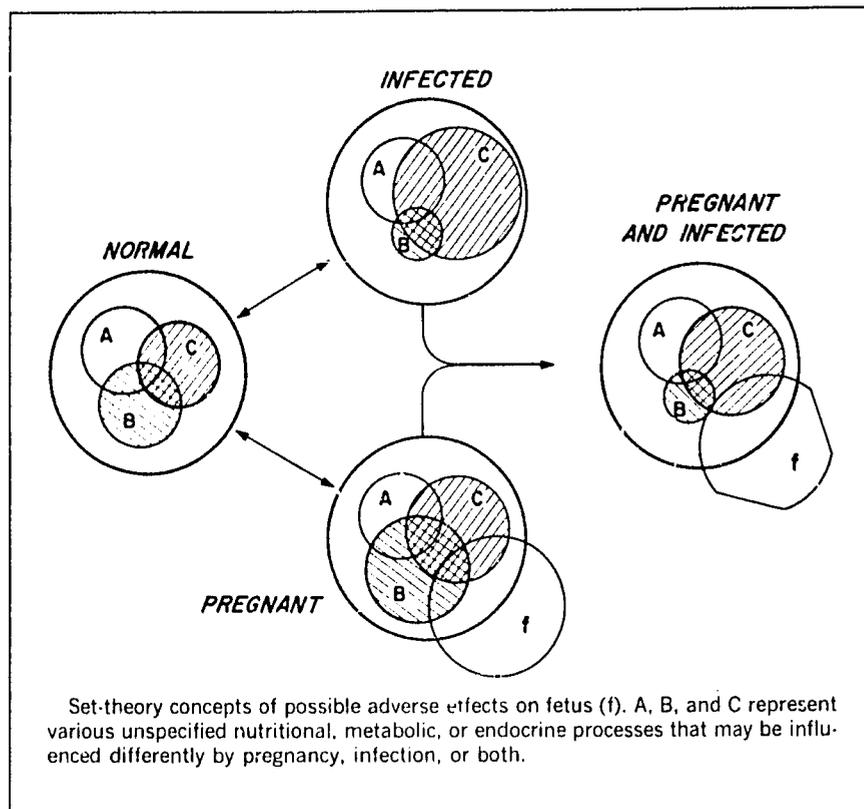
From US Army Medical Research, Institute of Infectious Diseases, Fort Detrick, Frederick, Md. Reprint requests to US Army Medical Research, Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701 (Dr. Beisel).

ternal infection and malnutrition. Such information must be obtained for a variety of populations and different geographical locales.

Key questions that should form the basis for conducting future research are the following: What maternal factors of a nutritional nature are causes of fetal growth retardation? Which nutrients are of principal concern? Is it necessary to consider a variety of maternal infections or only a limited number of specific ones? Is synergism between malnutrition and infection as important as currently suspected? Most of our attention has been directed toward extremes of malnutrition and infection, such as obtain in developing countries. We must now also determine whether relatively trivial infections and minimal malnutrition may affect the fetus.

A second series of closely related studies should concern itself with the timing of an insult. Prolonged famine seems to have its greatest impact if it occurs during the last trimester of fetal maturation.<sup>2</sup> Does a similar relationship hold true for less severe forms of deprivation, or for a deprivation that might involve restriction of total calories, protein precursors, or specific micronutrients? Does maternal infection, in the absence of transplacental fetal infection, have a greater effect on fetal growth if it occurs in the third trimester rather than in the first?

Answers to some of these questions can be gained from long-term prospective studies throughout the duration of pregnancy, using comprehensive diagnostic measures to detect maternal infection or nutritional deficit. Such data can be obtained without experimental manipulation of the study populations. Growth retardation can obviously occur in association with fetal malformation, prematurity, or persistent postnatal infection. More information is needed about a variety of study populations to determine the incidence of intrauterine infections. The data of Lechtig and Mata,<sup>14</sup> based on IgM and C3 values of cord blood, suggest a higher incidence of infection in newborn infants in rural Guatemala or impoverished city dwellers in Peru



Known Effects of Specific in Utero Infections

Organism	Small or Premature Infant	Congenital Defects	Congenital Infection	
			Acute	Persistent Postnatal
Rubella	+	+	+	+
Cytomegalovirus	+	+	+	+
Herpesvirus	-	+	+	+
Varicella	-	-	+	+
Hepatitis	+	-	+	+
Other viruses	-	-	+	-
Syphilis	+	+	+	+
Tuberculosis	+	-	+	+
Mycoplasma	+	-	+	+
Other bacteria	+	-	+	-
Toxoplasmosis	+	+	+	+
Malaria	+	-	+	+

than in higher economic classes from the same countries or from the United States.

How good is the correlation between sensitive diagnostic indicators of in utero infection and the birth weight of individual infants? Would most or all newborn infants experiencing fetal growth retardation show evidence of in utero infection if better diagnostic indicators were employed? Conversely, using the best

available diagnostic indicators, what is the incidence of intrauterine infection in infants whose birth weight is optimal? Too little is known about the types of infection important in the pathogenesis of fetal growth retardation. The Table presents a general summary of the recognized consequences of specific fetal infections. Too little is known about the incidence and causal role, if any, for mild, self-limited, or subclinical intrauter-

ine infections. Are there other specific but unrecognized culprits among the common bacteria and viruses? Can a diagnosis of intrauterine infection be made consistently before birth, and if so, would treatment directed at the unborn infant be able to prevent, ameliorate, or reverse fetal growth retardation? Meaningful clinical studies in this area require careful selection of laboratory assays or clinical measures for data gathering, which would represent another point of attack where new information must be acquired.

#### Indicators of Fetal Growth Retardation

Birth weight has been used most widely as the key variable for correlating data with possible causes of fetal growth retardation. Other data obtained at or soon after birth could be equally useful. Neurological measurements such as alertness, postural tone, and the presence or absence of normal newborn reflexes should be measured systematically to provide data on maturity of the central nervous system. The absorptive capacity of the neonatal gut, estimated even by simple measures such as stool pH, could also be used to estimate fetal maturity. Skinfold thickness in newborns can provide valuable information about their nutritional status.<sup>9</sup> A careful examination of the placenta should also be made, particularly for placental infarcts or other gross lesions that could explain fetal growth retardation.

There is still no consensus regarding indicators that can best differentiate a normal pregnancy from one complicated by maternal malnutrition, infection, or bacterial toxemia. Will detailed measurements of maternal hormone concentrations,<sup>10</sup> individual plasma proteins, lipids, free amino acids, trace elements, and other constituents of plasma or blood also be required?

Several new approaches are being studied to detect the presence of malnutrition during pregnancy. Winick<sup>11</sup> suggests that changes in cell growth during gestation can be evaluated by studying enzymes involved in synthesis or degradation of nucleic acids.

The activity of alkaline RNAase is directly proportional, during growth, to the increase in the cellular content of RNA. In a similar vein, Metcalf et al<sup>12</sup> have attempted to diagnose maternal malnutrition by measuring cellular enzyme activities in peripheral blood leukocytes.

Conventional serologic and microbiologic techniques are available to diagnose specific infections during pregnancy. However, diagnostic indicators are also needed to detect the presence of subclinical infectious processes and determine cause. Studies in our laboratories<sup>13-16</sup> show that many of the nonspecific host metabolic responses to infection have nutritional consequences. These responses can provide two kinds of information about the problem under discussion by indicating both the presence of an infection and the nutrient wastage caused by an infectious process.<sup>14</sup> Although diminished transfer of nutrients from mother to fetus represents a plausible mechanism to account for infection-induced fetal growth retardation, other mechanisms may be involved, including kinins, prostaglandins, lymphotoxins, and other biologically active substances released into the extracellular fluids during states of inflammation or infection.

Better diagnostic methods are needed to detect bacterial toxins that may be involved in the pathogenesis of fetal growth retardation. Diarrhea-producing enterotoxins formed in the maternal gut by noninvasive bacteria may exert indirect harmful effects on the fetus. Enterotoxins may diminish the intestinal absorption of dietary nutrients by the mother, or diminish the rates of blood flow to visceral and pelvic tissues. Bacterial endotoxins also have important vascular effects as well as many effects on intermediary metabolism of the host.<sup>17</sup> The role of enteroviral infections of the mother as a cause of fetal growth retardation remains to be assessed.

More epidemiological information is also needed to define the true importance of fetal growth retardation in terms of its long-range effects. Although "catch-up" growth can be

achieved by some infants, this may be inadequate despite good therapy and a seemingly adequate intake of nutrients. The studies at the Institute of Nutrition of Central America and Panama and elsewhere<sup>18-21</sup> indicate that the small-for-gestational-age infant is at greater than normal risk if it is born in economically deprived societies. Although five-year survival figures are poor for this group, relatively little well-documented information is available about the long-term potential of these infants for achieving full physical and mental development if adequate nutrition is provided for them.

Scientific studies in humans involving the use of dietary manipulation, nutritional additives, or other possible therapeutic measures are among the most difficult to design and interpret. It would seem appropriate to conduct limited specific studies to define the nature and extent of any gains, follow these with large-scale field trials, and finally conclude whether the intervention was effective. Only then could one argue realistically for expenditure of time, money, and effort on large-scale measures to correct fetal growth retardation.<sup>22</sup>

Current trials in lowland ladino Guatemalan villages<sup>18,23</sup> suggest that caloric supplementation of the diet of pregnant women improves the birth weight of their infants. Protein supplements do not seem to produce additional benefits. Studies are needed to determine if the caloric effect occurs only when caloric deficiency is profound.

#### Conclusion

Evidence is accumulating that combined maternal malnutrition and infection can produce synergistic negative effects on fetal growth. Improved diagnostic methods are needed to recognize subtle nutritional inadequacies during pregnancy and the presence of infection in the mother or fetus. Epidemiological data must be obtained to assess the magnitude and extent of the problem in various populations and geographical locations, and to determine the types and severities of various infectious pro-

cesses that could contribute to fetal growth retardation. Correlative data are needed to provide additional information about possible pathogenic mechanisms leading to fetal growth retardation. Finally, carefully controlled trials are necessary to determine efficacy of various prophylactic or therapeutic measures in preventing or diminishing fetal growth retardation.

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## Animal Models for Investigation of Latent Effects of Malnutrition

Paul M. Newberne, DVM, PhD

The selection of an animal model for the collection of biomedical data should be based on a careful analysis of research requirements and the specific characteristics of the different animal species available for use.<sup>1</sup> There are many examples in which a disease does not naturally occur in animals but can be induced by appropriate manipulation. But how does one select the appropriate model? Once it is chosen, how does

one obtain the animals, keep them healthy, and handle them properly in a laboratory environment? An excellent source of information is the *Proceedings of a Workshop on Animal Models for Perinatal Research*, sponsored by the Perinatal Biology and Infant Mortality Branch, National Institute of Child Health and Human Development.<sup>2</sup> I will present models involving maternal nutrient deprivation and its effects on the fetus and young animal.

### Congenital Hydrocephalus

Models for the study of congenital hydrocephalus have been generally unphysiologic and, therefore, somewhat unsatisfactory. In contrast, the

young born to vitamin A-deficient mothers may be hydrocephalic at birth or they may develop hydrocephalus a short time later because of associated elevated cerebrospinal fluid (CSF) pressure.<sup>3-5</sup> Increased CSF pressure is one of the earliest clinical manifestations of vitamin A deficiency, and, when induced in the fetus, it sets in motion a series of events that results in hydrocephalus at the time of birth or shortly thereafter.

Investigations in our laboratory<sup>6</sup> and elsewhere<sup>6</sup> have clearly established that the incidence of congenital hydrocephalus in rabbits increases as maternal vitamin A depletion proceeds; the vitamin A status of the

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mother can be correlated with the incidence of neonatal hydrocephalus (Figure). This model permits one to monitor and control maternal blood concentrations of the vitamin to predict development of fetal hydrocephalus and enables one to study its induction. Young female Dutch Belted rabbits, 6 to 8 months of age, placed on a vitamin A-deficient diet and bred when the serum vitamin A concentration stabilizes at about 20 $\mu$ g to 30 $\mu$ g/100 ml, will litter hydrocephalic young. Permitting serum vitamin A concentration to decrease below about 20 $\mu$ g/100 ml can result in failure to conceive or to carry fetuses to term, whereas serum vitamin A concentrations of 35 $\mu$ g to 50 $\mu$ g/100 ml usually result in clinically normal neonates that develop hydrocephalus later.

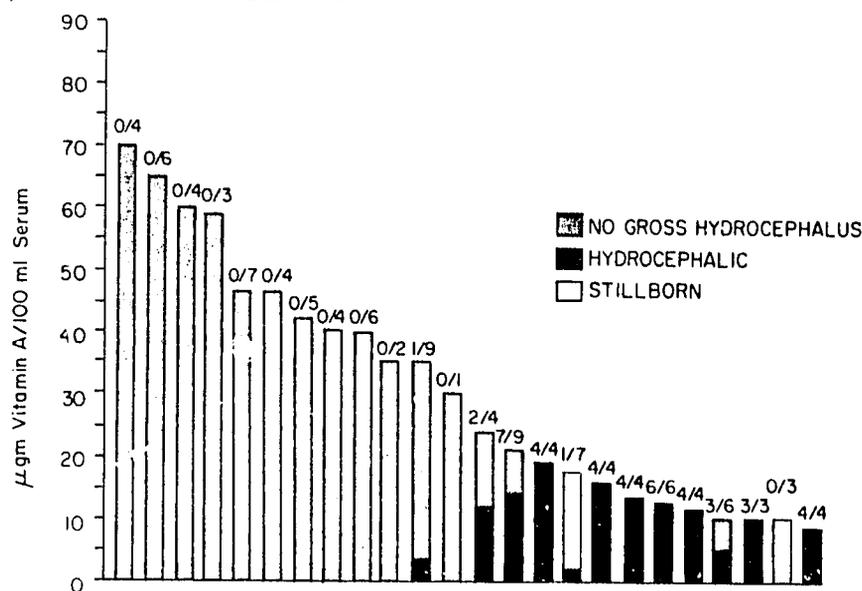
Signs and symptoms of vitamin A deficiency hydrocephalus in the newborn rabbit are essentially the same as those observed in the human infant. There is a distinct bulging over the frontal lobes of the brain and a general distortion of the shape of the head with dilated ventricles, thinned cerebral cortices, and often impaction of brain substance into incisurae. If the newborn appears normal but develops the condition postnatally, the general anatomic alterations develop in a predictable pattern, culminating in characteristic postnatal hydrocephaly. The CSF pressure is increased to a variable degree in any case, and surviving offspring sometimes develop opacity of the cornea and lens of the eye.

During a series of experiments designed to compare animal protein to vegetable protein in rats, a significant incidence of hydrocephaly and other congenital anomalies was found in young born to mothers fed soybean meal as a source of protein. This was not due to the vegetable protein itself, but ultimately was traced to vitamin B<sub>12</sub> deficiency, which interrupted normal growth and differentiation of the fetal central nervous system (CNS) and other tissues.<sup>6</sup> Although substantial numbers of animals were born with gross hydrocephaly, littermates and sometimes entire litters were without grossly de-

tectable hydrocephalus. However, some of these developed the condition postnatally. In many cases, the defect was traced to occlusion of the aqueduct of Sylvius<sup>7,10</sup> and consequent distension of lateral and third ven-

tricles. In others, the aqueduct was intact but gross hydrocephaly was present. An interesting observation in these and in many nonhydrocephalic littermates was a sharp decrease or an absence of material se-

Relationship between maternal blood levels of vitamin A at conception and incidence of gross hydrocephalus in progeny at birth. Fractions represent number of animals affected per total number of animals in litter.



	Protein, gm/kg Body Weight*	
	1.25	3.75
No. of bitches with litters	8	6
Average No. of pups per litter	5.0	6.5
Average birth weight, kg	0.261 ± 0.042	0.307 ± 0.021
Average weight at 6 mo, kg	8.9 ± 0.2	9.3 ± 0.3
No. with paralytic encephalitis	26/35	12/35

\* Protein amounts given to two groups during gestation; recommended amount (3.75 gm) given to both groups during lactation.

Dietary Treatment (Lipotrope Status)		No. of Animals	Av Weight at Infection, gm	Cumulative % Mortality (Days Postinfection)		
Gestation and Lactation	Post-weaning			7	11	30
Deficient	Deficient	17	233 ± 6*	100	100	100
Deficient	Complete	20	240 ± 8	80	100	100
Marginal	Marginal	62	248 ± 5	50	80	91
Marginal	Complete	65	285 ± 7	40	73	90
Moderate	Moderate	70	260 ± 3	47	64	71
Moderate	Complete	60	308 ± 4	20	20	35
Complete	Complete	60	303 ± 4	10	20	25

\* Mean ± SE.

**Table 3.—Phytohemagglutinin (PHA) Responsiveness of Spleen Cells and Peripheral Lymphocytes**

Source	Diet	Trial No.	Tritiated Thymidine Incorporation	
			Control	PHA
Spleen	Normal	1	3,590 ± 234	45,244 ± 1,300
		2	8,425 ± 819	68,812 ± 2,996
Spleen	Marginal lipotrope	1	4,878 ± 266	2,034 ± 330
		2	24,068 ± 206	80,915 ± 895
Blood	Normal	1	1,689 ± 95	100,152 ± 3,602
Blood	Marginal lipotrope	1	1,635 ± 109	33,614 ± 4,486

creted by the subcommissural organ,<sup>11</sup> a specialized area of ependyma located on the anteroinferior surface of the midbrain where the third ventricle continues into the cerebral aqueduct.<sup>12</sup>

#### Susceptibility to Viral Infections

Delayed effects of intrauterine malnutrition have been shown in the progeny of female beagles fed less than the recommended amount of protein during gestation, followed by a normal protein level at the time of whelping and during lactation. Pups 6 months of age challenged with canine distemper virus showed increased susceptibility to viral encephalitis (Table 1). The mechanism for this is, as yet, undefined.

#### Anti-Salmonella Host Defenses

Using diets severely deficient or marginally deficient in lipotropes (choline and methionine), female rats were reared and allowed to breed and deliver offspring. When the offspring reached young adult age, they were infected with a strain of *Salmonella typhimurium* (Table 2). Dietary restriction resulted in diminished host defenses. Paired feeding studies demonstrated that the differences were not a result of reduced food intake after birth but of intrauterine deprivation. Furthermore, the defect was not

entirely relieved by postnatal food supplementation.<sup>13</sup>

The immune response of marginal lipotrope progeny was then investigated.<sup>14</sup> Table 3 illustrates in vitro responsiveness of spleen and peripheral lymphocytes to phytohemagglutinin. In both cases, marginal lipotropes decreased lymphocyte transformation in response to the mitogen. Both marginal lipotrope and marginal protein diets fed to pregnant rats resulted in similar carry-over to the offspring of defects in the thymic-dependent arm of the lymphatic system (Table 4).

#### Demyelination Syndromes

We have also used the rat as a model to investigate the myelination process in the CNS.

When young female rats were placed on a copper-deficient diet,<sup>15,16</sup> reared to a 180- to 200-gm body weight, and bred to normal males, their offspring became copper deficient and exhibited characteristic clinical signs and symptoms as they grew to weaning,<sup>17,18</sup> including tremor, spontaneous body movements, and a loss in aggressive tendencies. Histologically, focal areas of necrosis and decreased myelination in the brain correlated well with symptoms and appeared to develop from prior foci of hemorrhage. Hemorrhagic necrosis of the liver and car-

diac hemorrhage were also present in the newborn. In older deficient animals, ventricular myocardium and septae were noticeably thinned, and there was severe, widespread vascular dilation.

In the brain, substantial decreases in typical myelin lipids, sulfatide and, in particular, galactocerebroside paralleled the deficit in myelin. The composition of gangliosides and phospholipids was unchanged, but galactosylation of ceramide in vitro was depressed in the copper-deficient brain.<sup>16</sup>

The copper-deficient rat may serve as a model in the investigation of poorly understood demyelination encephalitides. In addition, these studies suggest a role for copper in the CNS.

#### Conclusion

There is an important role for animal models in biomedical studies in our continuing efforts to better understand human disease. In each model discussed in this article, the importance of the maternal organism to the welfare of the offspring is central and unmistakable. The fact that unfavorable prenatal and perinatal influences can have far-reaching latent effects on the progeny must be accorded increased attention.

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**Table 4.—Anti-Sheep Red Blood Cell and PHA-Induced Responses of Rats Born to Lipotrope or Protein-Deprived Mothers**

Cell and Serum Source	Antibody-Forming Cells/10 <sup>4</sup> Spleen Cells	Serum Antibody Level		Tritiated Thymidine Incorporation	
		Hemolytic Titer	Hemagglutinating Titer	Control	PHA
Normal offspring	275/13	1:2,048	1:256	5,686 (1,171-9,819)	65,380 (49,573-74,725)
Marginal lipotrope offspring	37/8	1:32	1:8	3,348 (991-5,266)	6,154 (2,368-9,110)
Low protein offspring	58/4	1:4	1:2	4,773 (3,640-5,647)	27,295 (13,450-35,390)

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#### Summarized Discussion of Session IV

The discussion was opened by Dr. Katz who stated that on one hand it was difficult to define malnutrition precisely but not difficult to diagnose it; on the other hand, there were precise means for detecting sepsis, but they could not be implemented in most developing countries with high rates of malnutrition, and therefore it was difficult to diagnose the infection. Dr. Mata responded that one way to determine the function of nutrition is to see if there is correlation between nutritional intervention and an improvement consequent to that intervention in predetermined measurements of health. Dr. Kass agreed, but pointed out that it is important to recognize means of achieving beneficial results without necessarily being able to attack the fundamental cause. He added that a great deal of progress in medicine has been achieved by attacking only secondary causes. For example, he continued, one could set up a controlled pilot study in which antimicrobial agents are added to the diet of pregnant women to determine whether this would improve survival of newborn infants. If one would wait for the establishment of a large-scale improvement in the way of life, the solution may be long in coming, he added. Dr. Béhar agreed that such a project ought to be carried out, but only on a pilot basis, espe-

cially since there are some potential undesirable side effects that would have to be evaluated before routine use could be recommended. Dr. Plotkin reiterated Dr. Kass' point that isolation of specific questions and their systematic analysis may be the best way to approach such complex problems. He added that among single factors that might be studied, one could approach a proper bacterial analysis of stillbirths and their placentas. Dr. Beghin also supported the view of single factor analysis.

Dr. Sinclair raised the point that if perinatal mortality can be halved by the mere raising of birth weight by 150 gm (5.3 oz) on the average, this would both increase a village's population and result in larger individuals. Therefore, food requirements of the village would be significantly affected, and there had been no discussion of agricultural engineering and population control. Without these the quality of life would be severely impaired. Dr. Stiehm followed with a question about the effect of family planning on birthweight, and Dr. Kass responded that if the number of births is reduced, the mean birth weight always rises. Dr. Lechtig countered this by stating that in the situation where birth control was achieved by the hormonal pills, their effect was to interfere with lactation,

which in turn led to the increase in infant mortality. Additionally, Dr. Newberne raised the issue that simple elevation of birth weight alone may not improve survival. He cited evidence from animal husbandry where this could not be achieved. However, Drs. Rush and Béhar pointed out that there was a distinction between perinatal mortality, which is affected by birth weight, and postnatal mortality, which results from a variety of factors and depends on the degree and quality of sanitation, incidence of diarrhea, availability of medical treatment, and other factors.

Dr. Faulk raised the possibility that the survival of low-birth-weight infants may produce an older population with as yet undefined immunologic impairment. He suggested that a group of such surviving children should be studied for the presence of subtle immunologic lesions. Finally, Dr. Mata made a plea that the discussants of this workshop disseminate information to their peers in other professions in order to underscore to the world the serious consequences of malnutrition and infection. He thought that these problems should become a concern not only of those in the health profession, but also of the economists, jurists, educators, and socially aware people in all walks of life.

# Summary of the Workshop Conference

W. Page Faulk, MD

Our conference was opened by Dr. Béhar, who told us that 25 years ago the Institute of Nutrition of Central America and Panama (INCAP) was focusing on the school-age child; interest then shifted to the preschool child, and then to the first two years of life. He reminded us that today the emphasis is on embryonic development. His remarks defined our task: to delineate the measurements of fetal and perinatal growth as a function of environmental, maternal, and placental indices. He aptly pointed out that one cannot undertake such an investigation by studying only the newborn, because a neonate is in fact 9 months old.

Dr. Gordon spoke to us on the question of nutritional individuality. He summarized his comments by saying that nutrition permeates all aspects of life, and that malnutrition is a community problem. I was interested to notice that he also referred to the newborn as being 9 months old, and pointed out that the Chinese consider their newborns to be 1 year of age at birth.

## Nutritional and Socioeconomic Factors During Pregnancy

Dr. Behrman talked to us about the placenta in malnutrition, and reviewed several aspects of placental transport. He made the point that physiologic adaptation of a mother to environmental stress is often adjusted to the disadvantage of the fetus. The release of maternal catecholamines, for example, could cause vasoconstriction and compromise the fetal circulation. This is important in terms of current concepts of the normal structure and function of the placenta. The placenta is not only an organ that contains arteries and veins

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with smooth muscles that are sensitive to various sympathomimetic substances, but it also contains a lot of free and randomly distributed myosin and aggregates of actin-myosin in blood vessels and throughout the so-called mesenchymal stroma.<sup>1</sup> Thus, an increase in maternal sympathomimetic substances in the blood could cause vasoconstriction not only of the placental vessels but of the entire organ. This could result in compromised fetal circulation and possible fetal wastage.

Next, Dr. Ousa reported that substances that are necessary for the differentiation and proliferation of tissues, and that cross the placenta (for example, iron) should be given in large amounts during pregnancy. Other substances, such as vitamin A, do not seem to be transported very well across the placenta, but one can make up for this deficiency by giving vitamin A to either the lactating mother or the child.

Similar observations were reported by Dr. Arroyave. He told us about iron and vitamin A deficiencies during pregnancy, and showed that vitamin A is probably not transported across the placenta. He extended his observations to riboflavin and various amino acids. In Guatemala, iron and calories are two of the principal deficiencies in pregnancy.

During the course of these comments, I could not help but think of previous work on vitamin A regarding its effects on tissue differentiation,<sup>2</sup> adjuvanticity,<sup>3</sup> labilization of lysosomes,<sup>4</sup> virus susceptibility,<sup>5</sup> and the immune response.<sup>6</sup> Perhaps the elegant studies of Drs. Ousa and Arroyave will stimulate new efforts in this important area of human biology.

The next area for consideration was that of the socioeconomic and cultural determinants as factors in pregnancy and its outcome. Dr. Rush plunged us into a quandary by showing that it is very difficult, if at all

possible, to accept many of the existing definitions of malnutrition. I think his skepticism, which he maintained with some vigor throughout the conference, was useful. We often talk rather glibly about malnutrition, and when pinned down about what it really means, we tend to climb back onto our favorite hobbyhorses. He showed us that many of these, although commonly used, are subject to serious doubt, and he put forward the use of energy expenditure and energy balance coefficients as more acceptable definitions.

We heard next from Dr. Lechtig on the socioeconomic variables in a developing country. He maintained that maternal height and socioeconomic status can be related to birth weight. When he and his co-workers provided caloric supplementation to shorter mothers of lower socioeconomic class, this resulted in a decrease in the number of low-birth-weight infants. Conversely, he showed that if they gave caloric supplementation to taller mothers of higher socioeconomic status, they were not able to alter the birth weight significantly. These data allowed him to lead us to the conclusion that socioeconomic status could be considered as a form of biological variable. Although this type of reasoning is often useful, I am compelled to point out one exception to its use. That is the example of Queen Anne, the last Stuart monarch of England. She was a tall and heavy woman, and she was generally considered to be of high socioeconomic status. It was also rumored that she was well supplemented calorically. Queen Anne sustained 17 pregnancies and only one of these produced a child that survived past the perinatal period.<sup>7</sup> This was an unhealthy lad who died at the age of 12 years. I do not tell that story to detract from the Lechtig hypothesis, because as you may know, Queen Anne had rather severe gout. As Dr. Sinclair and several other people have tried to point out, metabolic disease

is an important correlate to birth weight and intrauterine growth retardation. However, if Queen Anne had not been queen, and if she had been short, poorly supplemented, but still had gout, she would have fit nicely into the Lechtig hypothesis and would have further confused an already complicated phenomenon.

#### The Problem of Infection

We next began to talk about infection during pregnancy. Dr. Kass told us that the role of nutrition in the production of low-birth-weight infants must be juxtaposed against at least two other factors. These are bacteriuria and genital T *Mycoplasma* infection, both reversible by therapy. This presented us with a clear example of a nonnutritional effect on the fetus that was reversible and that seemed to be closely related to the production of low-birth-weight infants.

He then made several comments on the propensity of placentas to Schwartzman reaction. The Schwartzman reaction can be generated by products from Gram-negative bacteria, many of which reside in the gastrointestinal tract. So it seems that the ammunition for generating a Schwartzman reaction in placentas at some time during pregnancy is available, and it is possible that such events might occur subclinically during so-called normal pregnancy.

If one studies sections of human placentas with fluorescein-labeled serum reacting with fibrinogen, one finds massive depositions of fibrinogen.\* This is seen not only around the vessels and in basement membranes, but in the areas of so-called fibrinoid necrosis. Placentas from children who have sustained certain intrauterine insults are filled with areas of fibrinoid necrosis, as, for example, in erythroblastosis fetalis and congenital nephrosis.

We then heard from Dr. Stiehm on the mysteries of fetal host defense mechanisms. He treated us to a nice review of what is known about T- and B-cell function, phagocytosis, opsonization, and complement metabolism in the neonate. He came to the conclusion that the neonatal host defense

system may not be as effective as that in adults, but it is intact and can function when necessary.

This point was rather well amplified by Dr. Chandra, who told us about immunologic indices in neonates who had been exposed to intrauterine growth retardation. Practically every one of the indices, except perhaps B-cell function, was significantly depressed. He thought it possible that a fetus whose mother is subjected to severe malnutrition, or whose placenta is compromised because of inadequacies of flow may have an impaired development.

This area strikes at the heart of our workshop, because if Dr. Chandra's point is indeed true, it would suggest a plausible mechanism for the seemingly higher incidence of neonatal and infantile infections in low-birth-weight infants. This idea does not fit into any unitarian type of hypothesis, but it is possible that the malnourished mother compromises nutrition of the fetus, and that the malnourished child is compromised in the development of his immune system.

We were then told by Dr. Plotkin about the routes of fetal infection and the mechanism of fetal damage. Damage can be manifest in several ways. First, a virus-infected cell could undergo destruction. Second, cells infected by viruses can manifest certain measurable metabolic defects. Third, virus-affected cells may sustain an inhibition in cell division. In the case of fibroblasts, virus infection could possibly compromise the growth and differentiation of the connective tissues. Fourth, viruses may cause immunosuppression. He then came to the conclusion, which we all share, that verified intrauterine virus infections can cause low birth weight. I could not help but wonder if his comments were relevant to the children who had intrauterine infections, but who have normal IgM level. We know that IgM is produced early in utero. If the B cells are infected by a virus, is it not possible that the virus may interfere with the manufacture of IgM?

This talk was logically followed by the excellent papers of Drs. Alford and Sever. Their information left the clear message that intrauterine virus

infections occur, but when these are compared to the number of low-birth-weight or intrauterine-growth-retarded infants, they constitute a very small group. Dr. Urrutia presented a convincing study showing that low-birth-weight infants had an excess of infections. The conference gave some consideration to this apparent paradox. These differences may merely reflect differences between industrialized and developing societies. They could be a manifestation of the number of infections to which different groups of children are subjected, or a phenomenon of the intactness of the immune response. These observations point out the sort of geopathologic factors that are currently engaging the attention of scientists and public health authorities around the world.

#### Nutritional Interrelationships

We have then at least three variables that have been discussed as determinants in the cause of low-birth-weight infants. One of these is nutritional status, another is host defense, and the third is the phenomenon of intrauterine infections. These three phenomena may or may not be interrelated, but it will be difficult to talk about interrelationships until more information is accumulated.

Along these lines, we got very nice information from Dr. Sinclair, who told us that nutrition was *pathogenetically* associated with low birth weight in a small percentage of his cases. However, he found similar associations when he looked at either high blood pressure in the mother or maternal smoking. Thus, nutrition does not seem to be the only variable, and I doubt that anyone went away from this symposium thinking that it is. Some of the major contributions in this field have been made by the IN-CAP group, and several of these were reported at this symposium. For instance, Dr. Mata showed us that the small-birth-weight infant often does not catch up in growth, and his colleagues also showed that small-birth-weight infants are at very high risk for infectious diseases and suffer greater morbidity and mortality than infants of normal birth weight. Dr. Lechtig showed us that the number of

low-birth-weight infants can be decreased by caloric supplementation, suggesting that nutrition, although it is not the only variable, is strongly associated with low birth weight. Finally, Dr. Lasky showed us that the psychomotor development of these children could be associated with measurements in the mother, which

could perhaps be related to infection as measured by IgM levels.

In this symposium, we have done two things. First, there has been a serious and a concerted effort to define the problems. Second, attention was called to these problems as they exist around the world.

Let me close by rephrasing the Or-

tega y Gasset quotation cited by Dr. Béhar, that man has no nature, he only has history. Surely it would seem that man does not just have history, but that man makes history, and that if some progress has been made through meetings such as this, one might eventually expect a better history of man.

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#### CORRECTION

**Paragraph and Table Omitted.**—In the article, "Survival and Physical Growth in Infancy and Early Childhood: Study of Birth Weight and Gestational Age in a Guatemalan Indian Village," published in the May JOURNAL (129:561-566, 1975), two omissions occurred.

On page 563, the following should have appeared as the first paragraph after the centerhead "Survival": "A direct correlation was noted between birth weight and survival in the first year of life. Whereas only one half of the infants of less than 2,000 gm birth weight survived the first year of life, all those of 3,000 gm or more lived through that period. Survival during the neonatal period seemed to be associated with large weights (< 2,750 gm, Table 3). There were two deaths in infants of more than 2,750 gm, precipitated by infectious diseases."

In the seventh line of the copy in the next paragraph, the parenthetical reference to Table 3, should have been to Table 4. Table 4, omitted from the published article, is printed below.

Table 4.—Infant Deaths in Relation to Gestational Age, Santa Maria Cauque, Guatemala, 1964-1973

Gestational Age, Weeks	No. of Infants	Period of Life			First Year
		29 Days	<29 Days-5 mo	6-11 mo	
31-32	3	2 (667)*	0	0	2 (667)
33-34	8	3 (375)	3 (375)	0	6 (750)
35-36	20	5 (250)	1 (50)	2 (100)	8 (400)
37-38	47	0	2 (43)	0	2 (43)
39-40	261	6 (23)	7 (27)	8 (31)	21 (80)
41-42	77	0	1 (13)	0	1 (13)
<b>Total</b>	<b>416</b>	<b>16 (38)</b>	<b>14 (34)</b>	<b>10 (24)</b>	<b>40 (96)</b>

\* Deaths, and in parentheses, rate per 1,000 live births by gestational age category.