um-channel inactivation (Thompson), repetitive firing (Connor), and burst production (T. Smith, Gorman *et al.*). Neurons in cerebellar and brainstem slices are shown to have action potentials that differ substantively from those of axons (Llinás). A densely packed report on acetylcholine-activated channels in snail neurons (Marty) includes an Eyring model that can explain the effects of blocking ions. A slow inhibitory input can abolish the negative resistance characteristic of an *Aplysia* neuron (Wilson), as can the application of dopamine. One report (Shapiro) shows great persistence in trying to apply voltage-clamp analysis to nerve terminals that are clearly not under potential control.

The two papers on behavior are convincing if not earthshaking. The secretion of ink by a specialized gland in *Aplysia* occurs only when the appropriate motor neurons are excited. Thus "inking" may be taken as the end point of a reflex arc that includes sensory neurons. That motor neurons do not fire action potentials until 1 to 2 seconds after the start of a stimulus and that inking is similarly retarded are explainable by a strong early outward potassium current. Hence, "biophysics explains behavior" (Byrne and Koester). Another behavior in *Aplysia*, the gill-withdrawal reflex, exhibits habituation, or a decrease in amplitude with repetition, and sensitization, or a restoration of amplitude with a certain novel type of stimulus. The first may be explained by an inactivation of inward calcium current in the presynaptic terminals, and the second by a decreased potassium current (Klein). (A persistent quibble: At a meeting intended to bring biophysicists and behaviorists together, where were Davis, who has so carefully dissected the neural basis of classical conditioning in *Pleurobranchaea*, Willows, who has extensively studied the motor control of swimming and feeding in *Tritonia*, or Kater, who has similarly analyzed feeding in *Helisoma*?)

I did not find any typographical errors, but Llinás's given name is misspelled throughout. Much credit should go to the editors and their graduate-student "scribes" for a carefully produced book with an even level of discussion. The book should be welcomed by all who work in the field, who may happily look forward to further Cold Spring Harbor Reports in the Neurosciences, of which this is the first.

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Nutrition During Pregnancy

Diet in Pregnancy. A Randomized Controlled Trial of Nutritional Supplements. DAVID RUSH, ZENA STEIN, and MERVYN SUSSER. Liss, New York, 1980. xxviii, 200 pp. $26. Birth Defects: Original Article Series, Vol. 16, No. 3.

In the early 1970's research on dietary factors that influence pregnancy outcome took a new direction. The large body of evidence from previous epidemiological studies was mixed concerning the likelihood that nutritional intervention during pregnancy would substantially improve fetal growth and reduce perinatal mortality. Studies conducted in the United States and England suggested that nutritional supplementation during pregnancy would have no effect. Studies from developing countries, although inconclusive, suggested that further research was warranted. The questions that had to be addressed dealt with the efficacy of specific types of nutritional intervention. At that time protein deficiency was considered by most authorities to be the major nutritional problem of the developing nations. Therefore, most of that generation of nutrition intervention studies focused on the efficacy of providing a protein supplement to the normal diets of pregnant women. In most cases an increase in birthweight was to be the measure of the success of the supplementation. Though the focus of these intervention trials was the developing countries, Rush, Stein, Susser, and colleagues conducted such a study in what they considered to be a high-risk population of poor black women from New York City. This book is a description of that study, bringing together in one place the study rationale, design, and results, a summary version of which has been published in *Pediatrics* (vol. 65, April 1980). The authors' justification for publishing the work in book form stems from the controversial nature of their results, interpretation, and conclusions, the most important being the failure of the intervention to raise birthweight.

The objective of the study was to improve the prenatal and early-postnatal development of offspring by supplementing maternal diets with protein during pregnancy. The experiment called for a randomized controlled trial of two liquid nutritional supplements, administered as either a high-protein (40 grams per day), high-energy (470 kilocalories per day) "supplement" beverage or a similar "complement" beverage with very little protein (6 grams per day) and slightly less energy (322 kilocalories per day). Controls received multivitamin-mineral tablets that were similar in composition to the vitamin-mineral component of the liquid supplements. The study did not confirm the hypothesis that provision of a nutritional supplement during pregnancy will increase birthweight.

The authors report no significant benefit of either protein or caloric supplements in elevating birthweights above those for the control group. The only significant effects on birthweight were observed in the offspring of a subsample of smoking mothers who received the supplement or the complement. In addition, mothers who received the supplement delivered infants who at one year of age scored better on three of ten behavioral tests.

Given that past analyses of the efficacy of nutritional supplements have generally led to the conclusion that birthweight can be increased in the offspring of women who truly are malnourished, the authors' conclusion that the women they studied were generally not sufficiently malnourished to benefit from a protein supplement is probably correct. It is unfortunate that this possibility was not recognized earlier. The authors' evaluation of the protein intake of the population prior to the study was based on data obtained from 24-hour dietary recalls. Since underreporting of intakes of all nutrients by this method is common, the data on protein intake should have been evaluated relative to data on total caloric intake. A diet in which protein accounts for less than 12 percent of the total caloric intake is widely accepted as indicative of protein deficiency, and by this criterion the population in question would not be considered deficient. When the diets of the study subjects at recruitment were analyzed from 24-hour recalls, the average protein intake of each of the three groups was higher than previously reported values for the population under study, with the 61 to 69 grams of protein ingested per day accounting for about 14.5 percent of the total calories ingested (1700 to 1900 kilocalories). (The 24-hour recalls were also used to screen individual prospective subjects for a history of low protein intake and therefore at risk of bearing children with low birthweight, although a single 24-hour recall should not be used to evaluate diet histories for individuals.)

If it was not evident before the study

started it certainly should have been evident at this point that the sample was not protein-deficient. At about this stage in the project new reports from nutritional supplementation trials in Guatemala pointed toward the theretofore unforeseen role of energy supplementation during pregnancy. A shift in focus from protein to energy effects could have been justified, even with the same research design. It is unfortunate that the authors did not use this opportunity to validate the Guatemala study results. They continued with their original objectives for four more years of data collection and several more of data analysis.

The important question of whether the subjects actually consumed sufficient amounts of the supplement and complement and did not use them as a substitute for the normal diet is addressed by the authors. Though the evidence is not conclusive, it is sufficiently strong that one is led to the conclusion that an experiment did indeed take place.

In studies that focus on one major outcome variable, in this case birthweight, consideration should be given to the sensitivity of the variable. No mention is made of the contribution of measurement error in reducing the statistical power of the design.

At this point it is appropriate to explore confounding effects that might militate against a significant difference in birthweight among treatment and control groups. The authors were reasonably successful in their random assignment of subjects; major confounding was avoided and covariation in such factors as maternal smoking, gestational age, and time on the supplement or complement were controlled statistically. However, some of these factors should have been explored further. Given that some mothers may have been either protein- or energy-deficient, an analysis of the treatment effects on women with the lowest prepregnancy weights or smallest mid-upper-arm muscle and fat dimensions or even on those who were consuming the least calories and protein at recruitment would have been useful. The only place where any of these variables is considered is in a series of multiple regression analyses (appendix 3.5) that includes weight at conception as one of at least ten independent variables; it is impossible to quantify treatment effects in subgroups from this type of analysis. When the authors do analyze treatment effects for certain subgroups, the results are interesting. For example, the significant effect of the supplement in heavy smokers is an intriguing finding. It receives very little discussion, however, and further analysis of the relationship would have been welcome.

In studies of this kind in which a considerable quantity of data have been collected, analysis of ancillary data often reveals interesting statistical relationships. It is from this type of secondary analysis that the most controversial conclusions of the study emerge.

The authors present evidence for what they consider to be important negative effects of the supplement, which are manifested by shorter gestation as well as by fetal growth retardation in prematurely delivered infants and higher neonatal mortality than is found in controls. The complement group falls midway between the supplement and control groups in these outcome measures. Considerable space is devoted to explaining the implications of these results, concluding in a strong recommendation to suspend high-protein supplementary food programs, at least for women who are not protein-deficient. This conclusion is not supported by the data.

It is important to recognize that the criteria for statistical inference in such secondary analyses are different from those for rigorous hypothesis testing and that it was not originally intended to use differential mortality as a measure of the effect of dietary supplementation. The sampling procedure clearly lacks the statistical power to demonstrate an effect on mortality. The supplement group suffered more neonatal deaths than the complement group (3.9 percent versus 2.3 percent), but the difference is not statistically significant. To determine whether high-protein supplementation is the cause of the deaths, the contrast between supplement and complement groups should be the test, not the contrast between supplement and control groups (which is significant at $p < .05$). The number of neonatal deaths (19 of 823 births including twins, 14 of 805 excluding twins) certainly is insufficient evidence on which to base such strong conclusions.

Even if the supplement had a statistically significant effect on mortality, no plausible biological mechanism has been presented by the authors to indict protein. From the data presented on all births including twins, it is possible to make nearly as strong a case against caloric supplementation as against protein supplementation, but no one has suggested that an average of 2300 calories is too high during pregnancy. One could also use the authors' own argument to implicate calcium, zinc, or copper as the cause of the higher mortality, since these minerals are found in considerable excess in the protein supplement. Other more plausible alternative hypotheses were overlooked. For example, one could argue that the excess protein in the supplement group prevented some early fetal deaths, perhaps with the survivors manifesting a greater degree of fetal growth failure, premature delivery, and neonatal deaths. The data on total perinatal losses are much less convincing with respect to a supplement effect than the data on neonatal deaths by themselves. This is especially evident with singleton deliveries for which the supplement group had 18 perinatal deaths (6.9 percent), the complement group had 16 deaths (5.9 percent), and the control group had 16 deaths (5.4 percent).

One of the most insightful parts of the book is the excellent evaluation of the various experimental and quasi-experimental studies of diet and pregnancy conducted during the past ten years. Though the authors are very critical of other studies, they fail to apply the same measure of criticism to their own work. What is disturbing about this book is that the authors have fallen short of their obligation to completely diagnose the shortcomings of the study and have overstepped the interpretation of ancillary data. The criteria for inferences that could be made from the principal experimental design are shifted in favor of an exposition of weak post hoc inferences that are unsupported by the secondary analysis. Moreover, the book falls short of meeting the authors' stated expectation that presentation of sufficient data will convince critics of the validity of their results and interpretation.

An uncritical reader would be left with the impression that protein supplements are potentially hazardous during pregnancy, at least to women who are ingesting sufficient protein before supplementation, and that protein supplementation studies like this one should never be repeated. The important questions that remain to be addressed after this fruitful decade of research deal with the effects of maternal malnutrition on infant survivorship, lactation performance, and future fertility. Carefully designed experimental trials on human subjects are becoming increasingly more difficult and expensive to conduct. However, they remain our best method for evaluating the basic nutritional mechanisms functioning in human populations. This study should not be used as support for abandoning such research.

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