New Concepts in Biological Evaluation of Novel Protein Foods

SANFORD A. MILLER, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA USA

ABSTRACT

Technology has advanced to where it is now possible to fabricate new foods having high acceptability and providing better nutrient balance than the traditional foods they replace. If such products ultimately represent a major portion of the diet, then a new approach to toxicology and nutritional evaluation has to evolve to allow proper estimation of the safety and adequacy of the products. Traditional approaches to these problems are based upon the elucidation of major changes in the test organism, i.e., evaluation of substances of high biological potency. For food analogs, any deleterious effect will probably be at levels just above the natural noise level of the system. To compensate for this, most traditional evaluations of analogs have been made by feeding the test substances at extremely high, nonphysiological levels with the hope that this will reveal any toxic response. With this approach, however, many traditional foods would appear to be inadequate. A better technique may be designed to explore the functional consequences of feeding the test analog over several generations, using stress as a modifier to increase sensitivity of the system. Other sensitive measures such as behavior and resistance to disease should be included. The conceptual basis of this approach was used to test a vegetable protein analog (VPA). The feeding of VPA to animals as a significant part of the diet resulted in no change in the capacity of the animal to grow and reproduce and to function under stress conditions. Preliminary results of feeding this material through three generations also produced no changes in the parameters studies.

Application of modern technology to problems of food production have enormously expanded the base of our food supply. Nutritional sources of exotic and nontraditional origin are being made available to provide nourishment to ever increasing needs of expanding world population. Technology not only has been able to provide new direction in production of new food resources, but perhaps, even more importantly, it has provided the foundation for organoleptic equivalence and exchange of such new foods for more traditional products. Since these properties are by far the most important stimuli for making "food" food, these developments in technology now permit high acceptability for a variety of nutrient sources no matter what their origin. Furthermore, this concept of organoleptic equivalence of new nutrient sources also permits for the first time, the construction of foods to the specification of human need rather than dependence upon the vagaries of natural products.

As with all such advances, however, there are potential problems of equal magnitude. For the first time, a significant and profound change can be made in the diet of a significant part of the human population within a very short period of time. Thus, no time is available for adaptation and selection to provide optimal utilization of such new products. The result is a need for assurances of safety and wholesomeness far beyond any required in the past. The level of biological function required to be investigated is orders of magnitude more specific than has been considered in the past.

The extent to which these problems need to be solved is a function of the nutritional uses to which the product is to be put. In the case of a product designed to replace a significant nutrient resource, the problem becomes important only when the intake of the analog becomes a significant part of the daily total intake. Thus, if a meat substitute represents a snack food consumed in addition to the major diet, it has different standards of nutritional equivalence to meat and safety than if it represents 50% of the total animal protein intake.

The possible absence of nutrients for which requirements have not been determined is also significant. These include both known and unknown materials. Finally, the problem of meeting needs of a variety of age groups and under a variety of health conditions becomes important when the product is a major source of nutrients. At present this is not normally considered in estimating quality or safety.

Problems associated with the development of products designed for nutritional superiority in relation to natural products can mostly be associated with two questions. First, what indeed represents a more optimal pattern of nutrient? Second is best described as the "Australian Rabbit" problem. That is, by modifying a pattern of long standing are we developing other problems of unknown dimension?

For all sorts of analogs the problem of possible nutritional imbalance must also be considered, not only in terms of interactions within the food but even more importantly as result of a changing dietary pattern overall, and thus the interaction among foods.

The problems of toxicology and safety are equally difficult to resolve and made more complicated by legitimate demands of consumers resulting in increasing resistance to additive inclusion in the food supply. This results in new products that must not only perform as claimed, but must do so with a minimal, highly rationalized additive mixture. Traditional techniques for the evalution of safety (which involve studies having as their end point only survival) are not sufficiently sensitive to provide information appropriate for estimating the safety of substances such as new formulated food products. For most such products, the potential hazard involved is not that of acute or even chronic death, but rather concern over the effect of such substances on the long term capacity of the animals to perform. Thus, it seems clear that any study which involves the determination of wholesomeness and safety of such formulated products must take into account the effect of feeding such substances on several functional parameters such as resistance to infection, behavior, and so on.

Another major problem in making hazard assessments for such products is associated with the question of the

effects of age and physiological condition mentioned earlier. Data on the effects of additives in the young and old and under stress conditions are not generally available. To date most such studies begin with a healthy weanling animal and in no way, *directly* involve the neonatal or aged animal nor do they examine the effects of stress or of ill health on response to the test product.

In the case of vegetable protein mixtures, there are a large number of nutritional studies available. They are, however, entirely concerned with the problems of protein quality and utilization rather than those of product safety, or for that matter, *total* product utilization. This is not surprising in view of the fact that the principle reason for the development of such products was in fact to expand protein resources. In general these studies demonstrate that a mixture of high protein quality can be devised either as single source supplemented with the most limiting amino acids, or a mixture of such proteins. For example, there are several papers describing studies in animals and man that clearly demonstrate the high nutritional quality of textured soy protein supplemented with methionine. More recently, a number of papers have also indicated that the use of egg albumin as binder in such products also helps improve the biological value of the product. Thus, it seems clear that the protein quality of such mixtures is assured, or is at least *capable* of being assured. In fact, the data suggests that, even in long term exposures, these products appear capable of maintaining maximal growth of weanling animals and maintenance of adults when included in a normal diet otherwise complete. Thus, in my opinion, any study of wholesomeness of an analog does not have to concern itself primarily with protein quality.

There are, however, several other questions of nutritional importance to which existing studies have not really addressed themselves. There are, for example, only limited studies of the capacity of young children (or equivalent animal models) to utilize these products. The data on metabolism in general and protein metabolism in particular appear clear that this capacity may be different at different ages. More importantly, the effect of such early exposure may be relatively subtle and not easily observable under the usual experimental approaches. If, in fact, textured vegetable protein products are to become a major source of protein, the capacity of the young child and infant to handle this product needs to be explored.

It is clear that none of these studies has examined the ability of these products to act as a major food source. In contrast to its current use as a simple source of protein, the major role of TVP in the future will be in the development of food products which, as described earlier, must be a source of several nutrients not only protein, since these products may replace significant amounts of traditional nutrient sources. Even here, the subtle effects of a marginal deficiency may not be revealed in standard protocols. For example, in a number of papers, Newburn has shown that marginal intakes of methionine, lipotrops and vitamin B_{12} during early life result in a significant decrease in the ability of the animal to resist infection later in life even though no other significant effect was observed at the time of infection. Thus, products expected to have a broad impact on the food habits and patterns of a society require special, more sensitive and precise determinants of overall nutritional quality.

Such tests must include exposure of infants, measurements of response to stress, and determination of the status of the organism for several micro elements.

Determination of the potential toxicity of the other components of such formulated products requires equally innovative approaches. Although each additive component has been cleared individually and is, in fact, generally GRAS, the effect of their combination, and their consumption as a component of a major food product has not been investigated. Moreover, even more importantly, most, if not all, have not been studies in the infant or young or in the stressed animal.

It is apparent that a new approach to the study of nutritional quality and toxicity is needed for the engineered food product. Considering the potentially broad exposure of society to these products and the possibility that they can become a significant component of our food supply makes it more important than ever that we better understand their utilization and have a more extensive understanding of their quality under the variety of conditions in which human society lives. Such investigations have never been done before, neither in terms of the scope of conditions required nor in the context of *product* safety and quality rather than *ingredient* safety alone. However, neither has there been before such a product with the potential of modifying and expanding human food resources.

The investigation reported here was designed to be the initial phase of a detailed examination of the nutritional quality and safety of one such analog. More importantly it was designed to provide for an evaluation of the way in which a study of this kind can be organized.

Two principle goals were developed for these investigations, nutritive value and wholesomeness. The nutritive value of such foods can be defined using three areas. First, it must have the ability to satisfy metabolic needs. Second, it must be assured of being wholesome in the context of its use. And third, it must provide palatability, because it has been said before, food is not food until somebody eats it.

The evaluation of each of these areas can be presented in the form of several questions. The ability to satisfy metabolic needs would be assured if the following questions were answered. First, does the product support growth of the young and maintainence in the adult? In addition, does it allow conception, normal gestation, and support lactation and development of the young? Will it allow appropriate responses to stress? And last, will it maintain the function of cells and cell components? Similar wholesomeness might be evaluated by providing responses to the following questions. First, does the feeding of vegetable protein analog affect energy metabolism or protein utilization? Second, does the feeding of VPA interfere with micro nutrient utilization? And third, does the feeding of VPA produce pathological changes in cellular morphology?

In the study reported here these questions were investigated by the use of several parameters. For example, the questions concerning growth of the young and maintainance in the adult were explored with the use of measurements of weight gain, food intake, whole life survival, body composition and changes in tissue pathology. The capacity of the material to provide for needs of conception and gestation were studied using the traditional reproduction studies, measurement of growth through several generations and perhaps most important of all, determination of functional parameters in each succeeding generation. The capacity of the organism to respond to stress included measurements of the capacity of the animal to resist infection as well as examinations of mortality patterns during life of the animal. And finally, the ability of the test product to maintain the function of cells was evaluated using measurements of cellular protein, DNA and so on. Function at the cellular level particularly was studied at the membrane level using techniques of measuring permeability of basement membrane of isolated epithelium as well as using traditional approaches to the problem such as the measurement of organ weights and the growth of various organ systems.

Wholesomeness of the product, at least in nutritional terms, was evaluted using measurements of a variety of metabolic parameters such as a serum glucose, uric acid, organ fat and protein levels, as well as serum protein

measurements. The utilization of protein, of course, can be estimated using traditional techniques such as PER or other such methods.

It has been argued that the feeding of VPA causes a reduction in the utilization of several micronutrients, therefore, any measurement of nutritive value of such products must take this into account and must include measurement of serum levels of several micronutrients such as calcium, magnesium, and iron, as a reflection of their utilization. The functional utilization of these materials were estimated using the measurement in bone of calcium and other components of bone structure as well as measurements of serum components such as hemoglobin and transfern. Finally, changes in cellular pathology of course need to be included using the techniques of histopathological examination as well as measurement of both relative and absolute organ weight changes during the course of the study. For the past two years, such a study has been in progress at MIT.

Using these parameters is an experiment which has been in progress for more than 2 years. It determines the capacity of a new vegetable protein analog, to substitute for meat in the diet of the rat and support good growth and proper development in young animals and maintenance of function in the adult when fed as a significant part of the diet for a significant portion of the life span. This study also explored the capacity of substituting VPA in the diet in maintaining resistance to disease and reproductive potential. The possibility of trace nutrient deficiency resulting from VPA substitution was also explored by evaluating metabolic changes associated with the nutrients in question.

The data presented here represent only the first portion of the study and include information obtained during the first year of investigation.

Five experimental cells were used. These were: 1) a standard control group, fed an adequate semisynthetic diet; 2) a minimal control group similar to the standard control group but fed a diet in which all vitamins and minerals were added at a level just equal to the published requirement of the albino rat; 3) the third group was a replacement in the minimal control diet of an amount of hamburger equal to the proportion of calories provided by meat in the average American diet; 4) a group in which VPA was added at the same level as was hamburger; and 5) a group fed a similar product as the vegetable protein analog, but one in which no color or flavor additives were used. The diets were constructed assuming that meat provides ca. 15% of the total dietary calories in the American diet. All diets were isocaloric and isonitrogenous. In addition to the studies already described, although not completed yet, behaviorial tests and measurements of brain catacholamines are also being performed. The studies were designed to run through three generations with the first two generations being allowed to continued through a life time.

No significant differences were found in most of the parameters studies. As a result of feeding VPA growth patterns were statistically indistinguishable among the various groups and show no differences that can be attributed to diet. Since this included the animals on the minimal control diet, it demonstrated that we had indeed selected a dietary level of vitamins and minerals that just satisfied the needs of these animals *under nonstress conditions*. In addition, no differences were found in organ weights among the various groups and no changes were found in relative organ weights at all ages investigated. Similarly, no differences were found in the measurements of uric acid, glucose or serum protein. It is interesting to note that there was a tendency (not statistically significant) for male animals to show high uric acid levels when fed hamburger diets. This may be a reflection in part of the high purine and pyrimidine base levels of these diets.

In contrast to several other reports in the literature, no significant differences were found in the serum level of several minerals such as, calcium, magnesium, copper, zinc and iron when measured at the various periods in this experiment. When measured in long bone, the calcium content also did not appear to be affected by the feeding of the vegetable protein mixtures. Bone growth in general appeared to be perfectly normal. Liver composition was also unchanged as a result of the feeding of VPA, as was brain. No differences were found in the protein, DNA and RNA content of these tissues, not in the ratios among these measurements, indicating that both cell number and cell size in these organs as well as in bone were unaffected by the feeding of VPA.

The capacity of cell membrane to act as a barrier to the passage of large molecules was also measured as part of the functional evaluation of this experiment. Using the rat eyelid as a source of nonkeratinized epithelium, the basement membrane was separated and the penetration of triturated dextran of known molecular weight through this cell component was measured using a technique previously described by our group. The feeding of VPA had no effect on the penetrability of the basement membrane of this epithelium. However, feeding of the diet containing minimal levels of vitamins and minerals did result in increased permeability of this structure.

In previous studies with this penetrability technique, we had suggested that the capacity of the basement membrane to act as a barrier to passage of large molecules was a process of considerably greater sensitivity to marginal micronutrient deficiency than any other parameter measured thus far. Thus, the requirements for vitamin C appear to be much larger to maintain a epithelial cell barrier function than that associated with the maintainance of saturated levels of the vitamin in white blood cells, the classical standard of ascorbate status. Results of this study seem to confirm this observation, since it represents the only nonstress parameter measured in the study in which any difference was found among the various groups. It is interesting to note that, in spite of the fact that the VPA product was added to the diet containing minimal amounts of vitamins and minerals, no reduction in epithelial barrier capacity similar to that for the minimal group was noted. This suggests that the micronutrients contained in the VPA were sufficient to provide the additional levels of these micronutrients required to maintain this function, when added to the minimal diet.

Under the stress of pregnancy, differences were also found when the group fed the minimal vitamin and mineral mixture was compared to the other groups. Again, no differences were found when animals fed these diets were supplemented with VPA or hamburger. However, when the minimal diet fed animals are considered, there was a significant reduction both in birth weight and in conception among these animals. This again confirms the observation that, under conditions of stress of pregnancy and reproduction, there is an increased requirement for vitamins and minerals not capable of being met by the levels which have been established as adequate for the nonpregnant rat. The addition of VPA to the mixture again appears to provide sufficient additional micronutrients to compensate for this lack.

Finally, capacity of the animals to resist stress of infection was also unaffected by feeding VPA. In a series of experiments in which animals were tested with standard doses of a murine strain of salmonella typhimurium, animals fed the minimal control diet demonstrated decreased capacity to respond to the stress of this infection. Greater numbers of them became ill and a significant number died. In contrast, when these diets were supplemented with VPA, there was no difference in the response rate when compared to animals fed the control diet.

In summary then, the feeding of VPA to animals as a

significant part of the diet resulted in no change in the capacity of the animal to grow and reproduce and, much more importantly, to function under conditions of stress. Preliminary results obtained from animals of second and third generation also suggest that the feeding of this material produces no changes in the parameters studied.

The increasing needs of society for food make mandatory the developments by which the food supply may be expanded. The capacity of food technology to utilize and upgrade low and marginal quality materials in the formulation of food products of high acceptability is an important component of this armory. However, the safety of such products are also an important and equal concern in the determination of their use. It is no longer sufficient to depend entirely on the use of classical toxicological measures which involve death as their only end point. The lack of sensitivity of such measures and the difficulty of applying them to food reduces their effectiveness. Man in the modern world requires considerably greater assurance of the safety of the substances that he consumes. He is not only concerned with the questions of survival, but he is also concerned with the questions of the quality of his life. The results presented thus far in this investigation are to our knowledge a first attempt to include in the evaluation of hazard, parameters which may be associated with the components of life style. Such parameters are difficult to measure, are expensive and are time consuming to perform. However, I cannot see any alternative if we are going to assure the public consuming our product that what we are providing for them is of the highest quality, nutritionally, toxicologically, as well as, organoleptically. Without these assurances, it will become increasingly difficult to convince regulatory agencies and their congress of their usefulness and value. The choice is ours and the time for action is now.