Editorial

Man, the Laboratory Animal

In a recent important article on the influence of dietary fats on serum lipid levels in man, Ahrens and his colleagues from the Rockefeller Institute[1] made a particularly pertinent statement: "The unique design of these studies was based on the belief that nutritional experiments can be carried out as precisely in man as in animals."

Their detailed findings suggest that unsaturated fatty acids produce lowering of lipid levels and that this may be related to the number of double bonds (iodine value) in the oils. However, the significance of their findings should be considered in the total view of the experimental design. It is this particular point which motivates this editorial.

In their reported studies, all recognized extra-dietary factors influencing serum-lipid levels were kept to a minimum. The patients were carefully selected and observed in the closely supervised environment of metabolic wards for periods of from four to six months in most cases, and uninterruptedly for as long as 36 months in one patient. Dietary intakes were simplified and rigidly standardized. Even the variable of cooking was eliminated by the feeding of what is known as a formula diet. All the known requirements for essential foodstuffs, minerals, and vitamins were supplied. In addition, medical and hospital care were provided to the patients without charge and constant attention was paid to social-economic factors which "might disrupt their adjustment to the study situation."

Although these subjects were hypercholesteremic or hyperlipemic, all were free from complicating medical conditions and were ambulatory. This is important because so often experiments are carried out on hospitalized subjects who do have conditions complicating long-term metabolic studies.

In a typical feeding experiment, the patient ate an *ad libitum* diet for eight weeks. (This was a truly prolonged control period.) After each change of formula, there was a transition period during which the lipid levels became adjusted. This interval was followed by a new steady stage in which the plateau of serum levels was marked only by minor fluctuations. (The body weight remained unchanged during the study.) The length of the transitional phase was determined on an individual basis by inspection of the data and varied from three days to eight weeks but usually was completed in two or three weeks. Measurements during the transitions were omitted in the calculations. Consequently, with each change in formula, at least the first two weeks' determinations were discarded when selecting data for comparisons.

As noted in an editorial in the same issue of *Lancet*,[2] "Advancement of our understanding of several important pathologic processes would be much accelerated by the study of patients under similarly controlled conditions for periods to be measured in months." Of course, a study of this type involves a tremendous outlay in personnel. To care for and investigate thoroughly five patients, a staff of twenty-five to fifty persons may be necessary. This does not include the problems of floor space for the laboratory and the expensive problem of the accurate collection and analysis of data. The editorial concluded: "Properly safeguarded, *Homo sapiens* can be a very useful laboratory animal."

Along these lines, attention should be called to a recent critique of the conduct of clinical trials in infant nutrition prepared by the

American Academy of Pediatrics.[3] In discussing some of the principles useful in conducting nutritional research the report points out that the type of population for which the conclusion is intended should determine the choice of subjects. Thus the value of foodstuffs for *all* infants should be studied in subjects from a heterogenous population. On the other hand, a dietary supplement for treating a deficiency must be assayed in the deficient subjects for such a supplement would probably produce no significant changes in normal subjects. And even if such changes were effected in normals, these results would not necessarily apply to the treatment of the deficiency.

The problem that nutritional research faces today can be clearly seen in the criteria of measurements in a clinical trial *The aim of good nutrition is the achievement and maintenance of optimal health*;[3] however, there are neither ideal standards of, nor simple methods for, measuring health. Indirect measurements, such as that of total hemoglobin and serum protein, may be useful but certainly are not at all conclusive. Furthermore, maturation rates, as determined by epiphyseal growth, the length of long bones, and dentition, although often used to evaluate pediatric nutrition, correlate rather poorly with height and weight indices. An example of this is the patient with iron-deficiency anemia in whom height and weight may be reasonably normal although epiphyseal maturation is retarded.

Unfortunately, *ideal* patterns for growth of healthy children are not known although there are many studies which provide data of growth averages for selected populations. It may be a debatable point, moreover, whether growth at a rate greater than the average of established groups is beneficial. Thus, an increased consumption of a special diet may accelerate weight gain of normal infants, but this increase may be temporary because the subjects at some later time may stop growing while chronologic age catches up to morphologic age. Therefore, since there are no universally accepted standards for ideal growth in children, each study should provide appropriate growth standards from control subjects and avoid comparing growth of an experimental group with arbitrary growth standards.

Of the criteria used to evaluate nutritional progress, the simplest and least liable to error is rate of weight gain. Unfortunately, this procedure lacks popular appeal, perhaps because there is a "lack of sophistication" in this method.[3] The disadvantages here are readily apparent. These include the absence of a standard for ideal weight gain and the uncertainty as to whether the gain is due to an accumulation of fat or water rather than protoplasmic synthesis.

In addition, because use of published tables on food composition does not always furnish sufficiently accurate data, the diet itself as well as the stool and urine must be analyzed chemically. Finally, publication of basic data, such as variations in replicate determinations and recovery experiments will show the degree of precision of laboratory procedures and will help in the evaluation of the experiment.

Before the details of the experiment are planned, the investigator should determine either from the literature or from a pilot study, the estimates of variation in the control and test groups, and an estimate of the expected differences between these groups at the end of the experiment. Then, after deciding on the level of significance, "it is possible to estimate the minimum number of observations necessary to give the expected differences the desired precision." The interested reader is referred to this article[3] for an example of the calculation of the minimum number of subjects and experimental (balance) periods theoretically necessary in an example in which certain statistical requirements have been established.

It is heartening to see in these interesting papers the development of the concept that nutritional experiments in man can be done with the same precision as they are done in the better animal studies, although it is certainly recognized that at present this is a relatively impractical and expensive procedure for all but a few organizations. Nevertheless, it is significant that a scientific academy felt the need for a "statement of the essential principles governing scientific appraisal of nutritional substances through clinical trials with hu-

mans."[3] It is clearly essential that this new point of view be extended to all investigators in clinical nutrition for it seems obvious that only in such a frame of reference lies the future of research on that most unique of laboratory animals—man.

—S. O. WAIFE, M.D.

REFERENCES

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2. Editorial: Long-term studies in man. *Lancet* 1:977, 1957.
3. LOWE, C. U., and PESSIN, V.: The conduct of clinical trials of substances proposed for the nutrition of infants and children. *Pediatrics* 19:694, 1957.

Letter to the Editor

DIABETIC RETINOPATHY: CORRELATION WITH VITAMIN B_{12} EXCRETION

Dear Sir:

In 1953, Becker, Lang, and Chow demonstrated marked elevation in the urinary excretion of injected vitamin B_{12} in diabetes with retinopathy as compared to non-diabetics and diabetics without retinopathy (J. CLIN. NUTRITION 1:417, 1953).

In view of the difficulty of the microbiologic assay of vitamin B_{12} in urine, a considerable amount of attention was given to validating the experimental and assay procedures with recovery experiments. In addition to the microbiologic assay, using *Lactobacillus leichmannii*, radioactive cobalt-labeled vitamin B_{12} was used as a tracer, and this method gave results which were virtually identical with those of the microbiologic assay.

In a recent paper (AM. J. CLIN. NUTRITION 5:26, 1957), Bookman and his associates report an attempt to reproduce our findings. A number of samples were assayed with three different micro-organisms, but no radioactive tracer studies are reported. Although the data are not presented in sufficient detail to allow fully adequate statistical analysis, the results with one of the micro-organisms *L. leichmannii* seem to yield a significant difference, at the 0.05 level, between diabetics with and without retinopathy, although in the opposite direction from that found in the earlier work. The difference indicated by the ochromonas assay was almost significant at the 0.05 level, but, in contrast with the *L. leichmannii* results, the ochromonas difference was in the same direction as that reported by Becker, Lang, and Chow. (The *L. leichmannii* and ochromonas results of Bookman *et al.* are, of course, significantly different from one another.) We are at a loss to explain the statement by Bookman *et al.*: "No P values of less than 0.1 were found between any groups, indicating a total lack of significance for the average difference found between groups in all three methods."

In view of the considerable variability and inconsistent results of the assays using the different organisms, and the lack of any evidence to validate the procedures, the negative finding reported by Bookman *et al.* cannot be considered to contradict the results of the earlier work.

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