

Second Report of the ad hoc Committee on Standards for Nutritional Studies

The Committee has communicated with a number of investigators who have been using the AIN-76 diet since its first report in 1977 (*J. Nutr.* 107: 1340, 1977). In studies with both rats and mice, these investigators generally have had very good results in terms of animal growth, appearance and survival. A few reports have come to the Committee's attention, however, of problems encountered when the diet was modified or possibly stored improperly. The primary problem appeared to be excessive destruction of menadione or interference with its utilization, with a resultant vitamin K deficiency, and has been noted previously in the *Journal of Nutrition*, vol. 109, p. 924, 1979. A case of marked rancidity when the diet was not kept refrigerated has also been noted.

Two laboratories which fed the AIN-76 diet to mice for a year or longer reported a significant number of trichobezoars (hair balls) upon autopsy. Without controls fed other purified diets, the relevance of these observations to this diet is unknown.

There have been comments from AIN members questioning the use of sucrose as the predominant carbohydrate. The possible adverse effects of sucrose, particularly in long-term studies, were mentioned in the original report. Alternatives which have been suggested are (1) substituting glucose for sucrose, (2) replacing at least half of the sucrose with glucose, (3) using all cornstarch and no sugars, (4) variable proportions of glucose, sucrose and starch depending on the nature of the study in question. It should be pointed out that commercial glucose (dextrose) is hydrated; anhydrous glucose is more expensive.

In view of the above observations, the Committee makes the following recommendations regarding the AIN-76 diet:

(1) The amount of vitamin K source be increased 10-fold to 500 $\mu\text{g}/\text{kg}$ diet, and that a stabilized form of menadione be used, such as menadione sodium bisulfite complex (MSBC) or menadione dimethylpyrimidinol bisulfite (MPB). When thus modified, the diet should be designated AIN-76A.

(2) That an antioxidant be added to the corn oil (or other oil if substituted), as noted in table 2, footnote 4 of the original report (*J. Nutr.* 107: 1340, 1977). Satisfactory are BHT or Santoquin at 0.01–0.02% of oil. Investigators should be aware that the most popular brand of corn oil now has no added antioxidant. The endogenous tocopherols are not very effective in stabilizing the oil when it is mixed into purified diets.

(3) That investigators modify the sucrose content, as noted above, if they believe the metabolic effects of sucrose may complicate their experimental plan.

ad hoc Committee on Standards
for Nutritional Studies
John G. Bieri, Chairman