# Perspectives in Nutrition

## An Evaluation of Mineral Mixtures Commonly Used in Diets for Experimental Animals

MARY ANN WILLIAMS, PH.D. AND GEORGE M. BRIGGS, PH.D.

IT IS hoped that Perspectives in Nutrition will review the literature selectively, interpret it moderately and present a spectrum of ideas that will serve as a continual stimulation to nutritional research applied to medical problems.

EDITORIAL NOTE: *The science of nutrition is based on experiment, using a variety of animals. Since diet is the key to most nutrition studies, every effort must be made to insure dietary adequacy under controlled conditions. This review, evaluating mineral mixtures, will materially aid nutritional investigators in planning their studies.* *W. A. K.*

THE READY availability of a number of commercially prepared mineral mixtures for use in semisynthetic diets in experimental nutrition has supported the widespread but often erroneous belief that the requirements for individual minerals have been satisfied when a given percentage of a commonly used mineral mixture has been added to the diet. It is often assumed, unjustifiably, that the different mineral mixtures are nutritionally equivalent.

We wish to point out (1) important differences in the composition of several mixtures commonly used in experimental diets, (2) essential minerals which some of the mixtures lack, (3) the failure of many published reports to include a description of or reference for the mineral mixture used, and (4) the difficulty in determining whether commonly used mineral mixtures, as described in the catalogues of typical supply houses, are prepared according to the original reference. These remarks, although directed primarily at mineral mixtures used in rat diets, apply also to mixtures used in diets for other species of experimental animals.[1]

In Table I, percentages of individual minerals in a total diet are compared with the requirements of the rat[2] when the minerals are supplied by various commonly used mixtures fed at the indicated level (the level recommended in the original reference or the level used most frequently). All values are calculated from the original reference. The wide variation in the levels of individual minerals supplied by the mixtures and the general lack of agreement with the rat's requirements are obvious.

It should be noted that the Wesson[3] mixture was originally proposed to be fed at a level of 3.5 per cent of the diet, and the Hubbell-Mendel-Wakeman[4] (HMW) mixture as 2 to 3 per cent of the diet. The HMW mixture, with a Ca:P ratio of 4.25:1, was originally prepared for use with casein diets in which the casein would provide additional phosphorus. A diet containing 20 per cent casein or 20 per cent isolated soybean protein would add about

From the Department of Nutritional Sciences, University of California, Berkeley, California.

This study was supported in part by funds appropriated under the Research and Marketing Act of 1946 as part of the Western Regional Experiment Station Project W-57, USDA.

TABLE

Essential Minerals,* Per Cent of Diet, When Each Mineral
Percentages are Taken or Calcu-

| Mineral | Wesson† (3.5%) | Hubbell, Mendel, Wakeman (HMW)‡ | | U.S.P. 14§ (4.0%) | Hawk-Oser‖ (4.0%) |
|---|---|---|---|---|---|
| | | (2.0%) | (4.0%) | | |
| | | | | | *Per Cent* |
| Calcium | 0.496 | 0.434 | 0.868 | 0.441 | 0.441 |
| Phosphorus | 0.359 | 0.102 | 0.204 | 0.267 | 0.267 |
| Potassium | 0.532 | 0.239 | 0.478 | 0.655 | 0.655 |
| Sodium | 0.146 | 0.055 | 0.110 | 0.121 | 0.121 |
| Chlorine | 0.423 | 0.191 | 0.380 | 0.424 | 0.424 |
| Magnesium | 0.0636 | 0.0195 | 0.0390 | 0.0683 | 0.0683 |
| Iron | 0.0129 | 0.0103 | 0.0206 | 0.0107*** | 0.0107*** |
| Manganese | 0.00025 | 0.00026 | 0.00052 | 0.00024 | 0.00021 |
| Iodine | 0.00014 | 0.00012 | 0.00024 | 0.00012 | 0.00012 |
| Zinc | ... | ... | ... | ... | ... |
| Copper | 0.00034 | 0.00072 | 0.00144 | 0.00008 | 0.00100 |
| Ca:P | 1.38:1 | 4.25:1 | 4.25:1 | 1.65:1 | 1.65:1 |

* None of the mixtures contains molybdenum or selenium.
† WESSON, L. G. *Science*, 75: 339, 1932;[3] contains aluminum and fluorine, also.
‡ HUBBELL, R., MENDEL, L. B. and WAKEMAN, A. J. *J. Nutrition*, 14: 273, 1937;[4] contains aluminum and fluorine, also.
§ U.S. Pharmacopoeia, 14th ed., p. 789, 1950;[6] contains aluminum and fluorine, also.
‖ HAWK, P. B., OSER, B. L. and SUMMERSON, W. H. Practical Physiological Chemistry, 13th ed., p. 1375. New York, 1954. McGraw-Hill Book Co.; contains aluminum and fluorine, also.
¶ JONES, J. H. and FOSTER, C. *J. Nutrition*, 24: 245, 1942;[7] contains cobalt also; this is equivalent to the mineral mixture of the U.S. Pharmacopoeia, 16th ed., p. 886, 1960.
\# HEGSTED, D. M., MILLS, R. C., ELVEHJEM, C. A. and HART, E. B. *J. Biol. Chem.*, 138: 459, 1941;[8] fed as 5.0 per cent in chick diets and as 4.0 per cent or 5.0 per cent in rat diets.
** PHILLIPS, P. H. and HART, E. B. *J. Biol. Chem.*, 109: 657, 1935.[24]

0.14 gm. additional phosphorus to change the Ca:P ratio to 1.8:1. Feeding the HMW mixture as 4 or 5 per cent of the diet greatly increases the calcium intake. Under these conditions, even with a 20 per cent casein diet, the Ca:P ratio is still greater than 2.5:1. Van Reen et al.[5] have shown that young rats fed diets containing 15 to 20 per cent casein and 4 per cent of the HMW mixture are very susceptible to the development of calcium citrate stones in the kidneys and bladder. Unfortunately, few investigators show concern for this finding since the HMW mixture is often fed as 4 per cent of the diet or more. This observation can be made by reading a current issue of any journal of experimental animal biology or nutrition.

The practice of feeding the HMW mixture as 4 per cent of the diet may have developed because mineral mixtures such as U.S.P. 14[6] and Jones-Foster[7] were formulated to be fed as 4 per cent of the diet. From this, it may have been assumed that all mineral mixtures are equivalent, and that 4 per cent is the correct level for any mineral mixture in a rat diet. The mineral mixture of Hegsted et al.[8] ("salts 4") was initially prepared as a mineral mixture for chick diets, to be fed at a level of 5 per cent, but it is commonly fed in rat diets at either 4 or 5 per cent.

Although zinc was found to be an essential mineral for rats as long ago as 1934,[9] a source of zinc is lacking in the following commonly used mineral mixtures: U.S.P. 14, Hawk-Oser, HMW, Wesson and Dunn.[10] Copper, another essential trace mineral,[11] is also lacking in the Dunn mixture, and zinc, copper, manganese[12] and iodine[13] are not included in the U.S.P. 13

I
Mixture is Fed at the Level Specified in Parentheses
lated from the Reference Listed

| Jones-Foster¶ (4.0%) | Hegsted# (5.0%) | Phillips-Hart** (5.0%) | Dunn†† (5.0%) | U.S.P. 132‡‡ (4.0%) | McCollum-§§ Davis 185 (3.7%) | NRC‖‖ Requirements |
|---|---|---|---|---|---|---|
| *of Diet* | | | | | | |
| 0.610 | 0.687 | 0.637 | 0.842 | 0.256 | 0.254 | 0.600 |
| 0.355 | 0.354 | 0.334 | 0.547 | 0.383 | 0.380 | 0.500 |
| 0.447 | 0.726 | 0.650 | 0.712 | 0.432 | 0.429 | 0.180 |
| 0.219 | 0.329 | 0.295 | 0.191 | 0.127 | 0.126 | 0.050 |
| 0.339 | 0.508 | 0.456 | 0.811 | 0.106 | 0.105 | 0.050 |
| 0.0463 | 0.0504 | 0.0452 | 0.0430 | 0.0542¶¶ | 0.0537 | 0.040 |
| | | | | (0.1107)## | | |
| 0.0217 | 0.0142††† | 0.0127††† | 0.0317 | 0.0198 | 0.0197 | 0.0025 |
| | (0.0230)‡‡‡ | (0.0206)‡‡‡ | | | | |
| 0.00524 | 0.00615 | 0.00039 | 0.00027 | ... | ... | 0.005 |
| 0.00242 | 0.00306 | 0.00274 | 0.00412 | ... | ... | 0.000015 |
| 0.00050 | 0.00060 | 0.00054 | ... | ... | ... | 0.0012 |
| 0.00048 | 0.00038 | 0.00034 | ... | ... | ... | 0.0005 |
| 1.72:1 | 1.94:1 | 1.91:1 | 1.54:1 | 0.67:1 | 0.67:1 | 1.20:1 |

†† BOULLIN, D. J. *Brit. J. Nutrition*, 15: 577, 1961;[10] contains fluorine also.
‡‡ U.S. Pharmacopoeia, 13th ed., p. 721, 1947.
§§ MCCOLLUM, E. V. and SIMMONDS, N. *J. Biol. Chem.*, 33: 55, 1918.
‖‖ Mineral requirements of the rat, per cent of diet, National Research Council, Committee on Animal Nutrition, Publication 990, 1962.[2]
¶¶ Based on $MgSO_4 . 7H_2O$.
\## Based on $MgSO_4$ anhydrous.
*** Based on 17.5 per cent iron in ferric ammonium citrate, Merck Index, 7th ed., p. 444, 1960.
††† Based on the formula $Fe(C_6H_5O_7)_2 . 6H_2O$ given in the original reference.
‡‡‡ The figure in parentheses is based on 16.7 per cent iron in ferric citrate, $FeC_6H_5O_7 . 5H_2O$, given in the Merck Index, p. 445, 1960.

salt mixture No. 2 or the McCollum-Davis mineral mixture. Despite their deficiencies these mixtures are very often used in highly purified diets, e.g., diets containing amino acids in place of intact proteins. The Hegsted and the Jones-Foster mineral mixtures both contain copper and zinc, but the levels which they provide are relatively low in view of the more recent studies on the requirements of the rat for these minerals.[14,15]

Fortunately, even if a zinc supplement is not added to a ration, it is generally believed that a deficiency does not readily occur in short-term experiments with rats fed purified diets containing intact proteins, especially if the rats are housed in galvanized cages and given tap water. To prove this point, we have recently compared the growth of male weanling rats fed diets containing zinc-deficient mineral mixtures with and without the addition of zinc (78 p.p.m.). The dietary protein was supplied by 20 per cent "vitamin-free" casein or 20 per cent isolated soy bean protein (C-1 Assay Protein, Archer-Daniels-Midland Co.). Soybean protein was tested since it is often used in rat diets and has been found to reduce the availability of dietary zinc for the rat.[15] Both the U.S.P. 14 and the HMW mineral mixtures were used. No attempt was made to eliminate the zinc present in the drinking water or available from the galvanized wire cages. Over the short four-week test period, the addition of zinc caused no improvement in growth. Under more rigorous conditions, however, i.e., with more highly purified diets, longer experimental periods, exclusion of zinc contamination, and the like, it is to be expected that the total intake of zinc might be inadequate unless a source of zinc, as a zinc salt, were added to the diet. In recent years, zinc deficiency has been induced quite readily in poultry and swine fed experimental diets, even when no special effort was made to reduce zinc contamination.[16,17] (One wonders how many rat experiments reported in the literature have been unknowingly affected by a deficiency of zinc, such as "unknown growth factor" responses or enzyme changes.)

An even more important omission, we believe, is the failure of many papers in the literature to list or give a reference for the composition of the mineral mixture used in the experimental diet, although other aspects of the diet may be stated in great detail. Under these conditions, the reader can only speculate to what extent the experimental results might reflect the inadequacy of the mineral mixture.

In contrast to the omission of essential minerals, several of the mixtures specifically provide minerals for which no dietary need has been established for the rat. For example, aluminum and fluoride are present in the U.S.P. 14, Hawk-Oser, HMW and Wesson mineral mixtures. The Dunn mixture also provides fluoride, and the Jones-Foster mixture contains cobalt. (The reasons for the additions are not readily apparent.)

The investigator using experimental diets assumes, or hopes, that individual dietary components are reasonably stable when mixed into the complete diet, under the usual conditions of diet preparation and refrigerated storage. As a precaution, however, high levels of vitamins are often added to "insure" adequate intakes if losses do occur during storage. The instability of thiamine in purified diets has been known for some time.[18–20] Waibel et al.[21] found that the destruction of thiamine resulted chiefly from the presence of $K_2HPO_4$ in the mineral mixture. The following mineral mixtures contain large amounts of $K_2HPO_4$ (more than 20 per cent by weight): Hegsted, Phillips-Hart, U.S.P. 14 and Hawk-Oser.

Fox and Mickelsen[22,23] have studied in detail the effect of minerals on the development of oxidative rancidity and the Maillard browning reaction in purified diets. They have found that oxidative rancidity in diets containing 4 per cent corn oil was accelerated by $CuSO_4 \cdot 5H_2O$, $FePO_4 \cdot 4H_2O$ or $MnSO_4 \cdot H_2O$. The Maillard browning reaction was greatly accelerated by $K_2HPO_4$. Mineral mixtures such as the HMW mixture and the Wesson mixture, which do not contain $K_2HPO_4$, did not accelerate browning under the ordinary conditions of diet preparation. Obviously, both of these destructive effects should be avoided as much as possible in nutritional experiments.

The increasing use of commercially prepared mineral mixtures is based, in part, on the assumption and implied claim that these prepared mixtures have been made according to the specifications listed in the original reference. In some cases, this assumption is justified. In other cases, however, the mixtures described in the supplier's catalogue do not give the specific chemical formulas of the compounds used. Consequently, it cannot be determined from the catalogue description whether the salt form used is the one originally stated, especially when several hydrated forms of the salt may be available. This situation could be remedied in these catalogues by giving the specific chemical formulas of the individual salts used in each mineral mixture.

Table II shows differences which exist between the composition of the mineral mixtures as taken or calculated from the literature reference and as stated in the most recent catalogues* of two of the principal suppliers in the United States. When necessary for comparison, the percentage composition of the mixtures was calculated from the weights of the individual salts given in the literature reference and from the weights given in the current catalogues. In the Phillips-Hart mixture, the percentage of manganese sulfate in the original reference[24] is less than one-tenth of the amount listed in the two catalogues, and the percentages of all other major components differ by at least 1 per cent for each salt (from 1 to 5 per cent). These commercial mixtures are called "Phillips-Hart salt mixture IV" in both catalogues although there is no mention of a "mixture IV" in the reference cited in the catalogues. The composition of the two

* Names furnished upon request.

TABLE II

Examples of Discrepancies Between Compositions of Mineral Mixtures as Described in Original References and in Catalogues of Two Commercial Suppliers

| Reference | Mineral Mixture Ingredients | Per Cent Composition in | | | |
|---|---|---|---|---|---|
| | | Original Reference | Catalogue of Supplier A | Catalogue of Supplier B | Hegsted Mix[8] (shown for comparison) |
| Phillips, Hart[24] | NaCl | 15.0 | 16.7 | 16.7 | 16.7 |
| | $K_2HPO_4 \cdot 3H_2O$ | 37.8 | 32.2 | 32.2 | 32.2 |
| | $Ca_2H_2(PO_4)_2 \cdot 4H_2O$ | 8.51 | 7.5 | 7.5 | 7.49 |
| | $MgSO_4 \cdot 7H_2O$ | 9.14 | 10.2 | 10.2 | 10.2 |
| | $CaCO_3$ | 26.9 | 30.0 | 30.0 | 30.0 |
| | $Fe(C_6H_5O_7)_2 \cdot 6H_2O$ | 2.46 | 2.75 | 2.75 | 2.75 |
| | KI | 0.0717 | 0.08 | 0.080 | 0.0799 |
| | $MnSO_4 \cdot 4H_2O$ | 0.0314 | 0.51 | 0.510 | 0.500 |
| | $ZnCl_2$ | 0.0224 | 0.025 | 0.025 | 0.0250 |
| | $CuSO_4 \cdot 5H_2O$ | 0.0269 | 0.03 | 0.030 | 0.0300 |
| | Cobalt chloride | 0.0000 | 0.005 | 0.005 | 0.0000 |
| Hegsted, Mills, Elvehjem, Hart[8] | $MnSO_4 \cdot 4H_2O$ | 0.500 | 0.365 | 2.687 | ... |
| | | gm. in one lot of complete mineral mixture | | | |
| Jones, Foster[7] | $CoCl_2 \cdot 6H_2O$ | 0.0476 | 0.476 | * | ... |

* The weight of cobalt chloride was not given in the catalogue of supplier B but the percentage stated agreed with the percentage of cobalt chloride calculated from the original reference, if it is assumed that $CoCl_2.6H_2O$ was the salt used in the catalogue of supplier B.

"Phillips-Hart mixtures," however, is very similar to the "salts 4" of Hegsted et al. (Table II). (The Hegsted mixture, "salts 4," is a modification of the original Phillips-Hart mixture.) In addition, both of these so-called "Phillips-Hart mixtures" contain cobalt chloride, which is not present in either the original Phillips-Hart mixture or in the Hegsted mixture. Supplier A does state specifically that cobalt has been added, but supplier B merely lists cobalt chloride with the components of the mixture, without calling attention to the addition.

The Jones-Foster mixture, as formulated originally, contains cobalt chloride, 0.0476 gm. of $CoCl_2.6H_2O$ in 2,099 gm. of complete mixture. The Jones-Foster mixture described by supplier A contains 0.476 gm. of $CoCl_2.6H_2O$ although the weights of the other salts are the same as in the original reference. This apparent difference in the amount of cobalt chloride may be a typographical error in this catalogue, but the same error has appeared in at least one previous catalogue from this supplier. In the Hegsted mixture, however, the difference in the levels of manganese sulfate cannot be explained so easily. The original mixture contains 0.500 per cent $MnSO_4.4H_2O$ by weight. The mixture from supplier A contains 0.365 per cent "manganous sulfate" by weight (water of hydration not stated), while the mixture from supplier B contains 2.687 per cent "manganese sulfate" by weight (water of hydration not given). Fortunately, these increases in the concentrations of cobalt and manganese do not introduce a toxic level of either mineral into the diet if the mineral mixtures are fed at the recommended levels.[25,26] Also, the composition of the Hawk-Oser mixture No. 3 given in the catalogue of supplier A is identical with that of the U.S.P. 14 mineral mixture. The original Hawk-Oser mixture, however, contains over ten times more copper (Table I) than the U.S.P. 14 mixture. Inaccurate statements such as these in the catalogues of commercial suppliers lead one to wonder if the mineral mixtures may also have been prepared inaccurately.

These suppliers also offer a large variety of purified diets to be used as "complete" diets or adapted to produce deficiencies of vitamins or minerals. An inspection of the literature shows that these diets are used by many investigators. Because of the increasing use of these commercially prepared diets, we wish to emphasize the inadequacy of many of them with regard to their mineral content. The catalogue of supplier A does not clearly define the mineral mixture of the "Vitamin B Complex Test Diet." It is simply described as "U.S.P. Salt Mixture No. 2." From the mineral mixtures listed in the catalogue, this mixture appears to be the mineral mixture No. 2 of the 13th edition of the U. S. Pharmacopoeia. The composition given in the catalogue for this mineral mixture shows that it lacks the following minerals essential for the rat: manganese,[12] copper,[11] iodine[13] and zinc.[9] Supplier A uses the mineral mixture "McCollum-Davis 185" in a "Chick Basal Ration," and supplier B uses this mixture also in a "Riboflavin Deficient Rat Test Diet." This mineral mixture also lacks manganese, copper, iodine and zinc. We have previously mentioned the development of calcium citrate stones in the kidneys of rats fed diets containing 20 per cent casein and 4 per cent of the HMW mineral mixture. Supplier B uses the HMW mixture as 4 per cent of the diet in a "Vitamin B-Complex Deficient Test Diet," and both suppliers provide the HMW mixture as 4 per cent of the diet in the "Tocopherol Deficient Test Diet." All these salt mixtures and diets are used by investigators often with little or no mention of these inadequacies or excesses.

The inadequacies of several of the mineral mixtures and the unnecessary complexity of many of them point to the need for the development of an adequate and less complex mixture for use in rat diets. Such a mineral mixture has been developed for chick diets.[27] A modification of this mixture for rat diets is being studied by us and will be published in the near future.[28]

In conclusion, we wish to emphasize that much more attention should be given to the composition of the mineral mixtures used in purified diets. Several of the mineral mixtures commonly used in rat diets are lacking in one or more of the minerals essential for the rat and other animals. If these mineral mixtures are used in experimental diets, the diets should be supplemented with sources of the minerals in which that particular mineral mixture is deficient, in order to insure the nutritional adequacy of the diet. For example, a source of zinc should be added to diets containing the HMW, Wesson or U.S.P. 14 mixtures. Moreover, if a commercially prepared mineral mixture is used in experimental diets, it is essential, for proper evaluation of the experimental report, that the mixture correspond to the specifications of the original reference. If the commercial mixture is a modification of the original mixture, the changes should be clearly stated in the experimental report, and the modified mixture should not be described in the literature as the mixture of the original reference. We wish also to recommend that an investigator should prepare his own experimental diets until he can be absolutely sure that the commercially prepared diets are as carefully prepared as the diets which the investigator himself would make.

## REFERENCES


1. Briggs, G. M. Inadequacy of certain salt mixtures used in studies of unidentified growth factors for chicks. *Poultry Sc.*, 35: 740, 1956.
2. Committee on Animal Nutrition, National Academy of Sciences, National Research Council, 1962. Nutrient Requirements of Laboratory Animals. Publication 990.
3. Wesson, L. G. A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. *Science*, 75: 339, 1932.
4. Hubbell, R., Mendel, L. B. and Wakeman, A. J. A new salt mixture for use in experimental diets. *J. Nutrition*, 14: 273, 1937.
5. van Reen, R., Lyon, H. W. and Losee, F. L. Urolithiasis in the rat. I. The influence of diet on the formation and prevention of calcium citrate calculi. *J. Nutrition*, 69: 392, 1959.
6. United States Pharmacopoeia, 14th ed., p. 789, 1950.
7. Jones, J. H. and Foster, C. A salt mixture for use with basal diets either low or high in phosphorus. *J. Nutrition*, 24: 245, 1942.
8. Hegsted, D. M., Mills, R. C., Elvehjem, C. A. and Hart, E. B. Choline in the nutrition of chicks. *J. Biol. Chem.*, 138: 459, 1941.

9. Todd, W. R., Elvehjem, C. A. and Hart, E. B. Zinc in the nutrition of the rat. *Am. J. Physiol.*, 107: 146, 1934.
10. Boullin, D. J. Thiamine requirement of rats given a high protein, carbohydrate-free diet. *Brit. J. Nutrition*, 15: 577, 1961.
11. Hart, E. B., Steenbock, H., Waddell, J. and Elvehjem, C. A. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. *J. Biol. Chem.*, 77: 797, 1928.
12. Waddell, J., Steenbock, H. and Hart, E. B. Growth and reproduction on milk diets. *J. Nutrition*, 4: 53, 1931.
13. Parker, H. E., Andrews, F. N., Hauge, S. M. and Quackenbush, F. W. Studies on the iodine requirements of white rats during growth, pregnancy, and lactation. *J. Nutrition*, 44: 501, 1951.
14. Hundley, J. M. Achromotrichia due to copper deficiency. *Proc. Soc. Exper. Biol. & Med.*, 74: 531, 1950.
15. Forbes, R. M. and Yohe, M. Zinc requirement and balance studies with the rat. *J. Nutrition*, 70: 53, 1960.
16. Supplee, W. C., Blamberg, D. L., Keene, O. D., Combs, G. F. and Romoser, G. L. Observations on zinc supplementation of poultry rations. *Poultry Sc.*, 37: 1245, 1958.
17. Tucker, H. F. and Salmon, W. D. Parakeratosis or zinc deficiency disease in the pig. *Proc. Soc. Exper. Biol. & Med.*, 88: 613, 1955.
18. Hankes, L. V. and Elvehjem, C. A. A nervous syndrome produced with phenylalanine and methionine. *Proc. Soc. Exper. Biol. & Med.*, 72: 349, 1949.
19. Lyman, R. L. and Elvehjem, C. A. The lability of thiamine in certain purified rations. *Proc. Soc. Exper. Biol. & Med.*, 77: 813, 1951.
20. Waibel, P. E., Cravens, W. W. and Baumann, C. A. Effect of dietary antibiotics on the growth of chicks receiving suboptimum amounts of thiamine by mouth or by injection. *J. Nutrition*, 50: 441, 1953.
21. Waibel, P. E., Bird, H. R. and Baumann, C. A. Effect of salts on the instability of thiamine in purified chick diets. *J. Nutrition*, 52: 273, 1954.
22. Fox, M. R. S. and Mickelsen, O. Salt mixtures for purified-type diets. I. Effect of salts in accelerating oxidative rancidity. *J. Nutrition*, 67: 123, 1959.
23. Fox, M. R. S. and Mickelsen, O. Salt mixtures for purified-type diets. II. Effect of salts on the Maillard browning reaction. *J. Nutrition*, 68: 289, 1959.
24. Phillips, P. B. and Hart, E. B. The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. *J. Biol. Chem.*, 109: 657, 1935.
25. Tappan, D. V., Lewis, U. J., Register, U. D. and Elvehjem, C. A. Further studies on the rat growth assay for vitamin $B_{12}$ activity. *Arch. Biochem.*, 29: 408, 1950.
26. Holtkamp, D. E. and Hill, R. M. The effect on growth of the level of manganese in the diet of rats, with some observations on the manganese-thiamine relationship. *J. Nutrition*, 41: 307, 1950.
27. Fox, M. R. S. and Briggs, G. M. Salt mixtures for purified-type diets. III. An improved salt mixture for chicks. *J. Nutrition*, 72: 243, 1960.
28. Briggs, G. M. and Williams, M. A. A new mineral mixture for experimental rat diets and evaluation of other mineral mixtures. *Fed. Proc.*, 22: 261, 1963.