the chemical determination of carotene. The basal ration was composed of white corn, wheat bran, solvent-extracted soybean meal, dry skim milk, dry brewer's yeast, bone meal, limestone, salt, manganese sulfate, and a vitamin premix containing adequate levels of all vitamins except vitamin A. The feeds were mixed at frequent intervals, and were never over 14 days old when consumed by the chicks. None of the chicks receiving the basal diet alone lived more than 26 days after hatching.

The data in Table IV show that chicks receiving the meals treated with *N,N′*-diphenyl-*p*-phenylenediamine grew less than chicks fed the untreated alfalfa meal. In general, death losses and condition of the eyes also indicated that the vitamin A potency of the diamine-treated meal was less than was indicated by carotene determinations. Thus, the chick assay data support the observation that noncarotene pigment is eluted from the chromatographic columns during carotene analysis of meal treated with *N,N′*-diphenyl-*p*-phenylenediamine, resulting in high carotene values.

The results of the growth tests (gains in weight, deaths, and eye condition) with meals treated with 2,5-di-*tert*-butylhydroquinone were less favorable than those with meals containing no antioxidant. The differences, although small, were consistent, and some interference with carotene determination may have occurred with this antioxidant.

Acknowledgment

The authors are indebted to B. F. Goodrich Chemical Co., Monsanto Chemical Co., and Universal Oil Products Co. for certain of the chemicals used in this study.

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Received for review March 26, 1953. Accepted May 21, 1953. Presented before the Division of Agricultural and Food Chemistry at the 123rd Meeting of the American Chemical Society, *Los Angeles, Calif. Contribution 490, Department of Chemistry, Kansas State College.*

TRACE ELEMENT DEFICIENCIES

Water-Culture Crops Designed to Study Deficiencies in Animals

J. F. McCLENDON and JACOB GERSHON-COHEN
Northern Division, Albert Einstein Medical Center, Philadelphia, Pa.

Experiments were carried out in order to obtain diets lacking halogens and sodium, particularly for the study of iodine and fluorine deficiency in rats. A water-cultured crop was obtained with successful elimination of halogens or sodium. With natural foodstuffs devoid of iodine, fluorine, or sodium, the effect on experimental animals can be definitively determined. Conversely, the optimum amount of these halogens or sodium for fertilization can be ascertained.

Water culture might be one solution to the problem of obtaining foodstuffs deficient in essential elements. Small-scale water culture has been used to show the necessity of certain trace elements in plants, but the use of large-scale water culture of the same chemical purity to grow foodstuffs for animals has never been described (*1*). Recently reported were successful studies of diets which were composed of foods obtained by water culture free of fluorine. The effect of these fluorine-free diets on dental caries and bone calcification was strikingly demonstrated by this procedure (*4*). This report deals with the technique evolved during the past 9 years for water culture of plants devoid of halogens or sodium.

Analytical Methods

Owing to the large quantity of pure water required, rain water was analyzed. Even though halogens pass into the atmosphere from sea spray, the burning of coal, and fluorine from open-hearth furnaces, there was only 0.002 to 0.004 p.p.m. of fluorine in rain water at the experimental farm 25 miles from Philadelphia. This value might be increased by contamination. For instance, when maple leaves were dried and extracted with double-distilled water, 2 p. p. m. of fluorine were extracted from them. Therefore, leaves should not be allowed in the water-collecting system. Rain water collected from an aluminum roof was freed from halogens and sodium by passage through Amberlite ion exchange resins.

Wooden tanks 2 feet wide and 12 feet long, lined with aluminum foil and coated with asphalt varnish, were filled with 200 liters of nutrient solutions each. A porous carbon tube filled with air at 15 pounds per square inch pressure, which was down the middle of each tank, supplied oxygen for the roots. Wooden frames, 2 × 2 feet with bottoms of 1-inch wire mesh, were used to support the root crowns of the plants. Aluminum foil was laid over the wire and on it a litter of last year's crop. A wick made of the previous year's stalks extended into the solution through the middle of the foil and wire mesh, and on top of the wick the seeds were planted.

It was found best to surround the tanks with a glass windbreak and to have the top open to the weather. In order to prevent rain water from entering the tanks, each frame had a roof of aluminum foil, except for the hole through which the plant grew.

For 3 years the nutrient solutions were analyzed frequently and records taken of salts added as they were used up; after that, iron and manganese were added every day and the other salts once a month.

Nutrient salts of reagent purity were used, but the calcium salts were analyzed as though they had been prepared from calcium oxide containing 4 to 6 p. p. m.

of fluorine. Therefore, a special procedure was used to free them of halogens. The method of McClendon and Bratton (*2*) (1938) was used in iodine determinations and of McClendon and Foster (*3*) (1941) in fluorine determinations. Recrystallization was depended on for removal of sodium.

Reagent grade sulfuric acid was freed from traces of halogens by boiling. The reagent grade salts were dissolved separately and freed of halogens by "adsorption" to freshly prepared hydroxyapatite as follows:

> The month's supply for a tank containing 200 liters of halogen-free water was prepared from reagent chemicals, each dissolved separately in 1 gallon of halogen-free water, except that the potash salts were mixed before dissolving: 60 grams of tripotassium phosphate, 100 grams of calcium nitrate, 150 grams of potassium nitrate, 50 grams of magnesium sulfate, and 25 grams of ammonium sulfate. Five per cent of the potassium phosphate was transferred to a beaker and 5% of the calcium nitrate to another beaker; 95% of the potassium phosphate was mixed with the potassium nitrate and then 5% of the calcium nitrate was added, causing a precipitate. The remaining 5% of the potassium phosphate was added to the remaining 95% of the calcium nitrate, causing a similar precipitate.
>
> When the first precipitate had settled, the clear supernatant fluid was siphoned into a flask containing 12.5 ml. of concentrated sulfuric acid and more was added until a pH of 4.5 (green with bromocresol green) resulted, and this solution was added to the tank. The supernatant fluid from 95% of the calcium nitrate was siphoned into a flask, adjusted to pH 4.5, and added to the opposite end of the tank. Both precipitates were washed once with water and the supernatant fluid was siphoned into the tank. The magnesium sulfate solution was mixed with one precipitate and the ammonium sulfate with the other and, after settling, the supernatant solutions were siphoned into the tank and mixed with the 200 liters of water by aeration. The pH was adjusted periodically with the aid of bromocresol green indicator to about 4.5 (green) with phosphoric acid or potassium phosphate as necessary.

Minor Elements. It was found that sufficient zinc was supplied as impurities in the salts and water and that sufficient copper was supplied in the salts and in condensed water in the copper tube leading air from the pump to the porous carbon tube (rubber tubing being used only for convenient connections). Adding traces of zinc, copper, or molybdenum made no difference in growth or color of the plants. Boron was supplied by adding 0.6 gram of boric acid to the tank, making the concentration of boron about 0.5 p. p. m.

Iron and manganese are constantly being precipitated as phosphate and it is better to add them daily. In a 100-ml. bottle of water were dissolved 30 grams of ferrous sulfate and in another 10 grams of manganese sulfate, and 1 ml. of each solution was added to each tank daily. This is a ratio of 2 parts of iron to 1 of manganese, as recommended by Somers, Gilbert, and Shive (*5*) (1942).

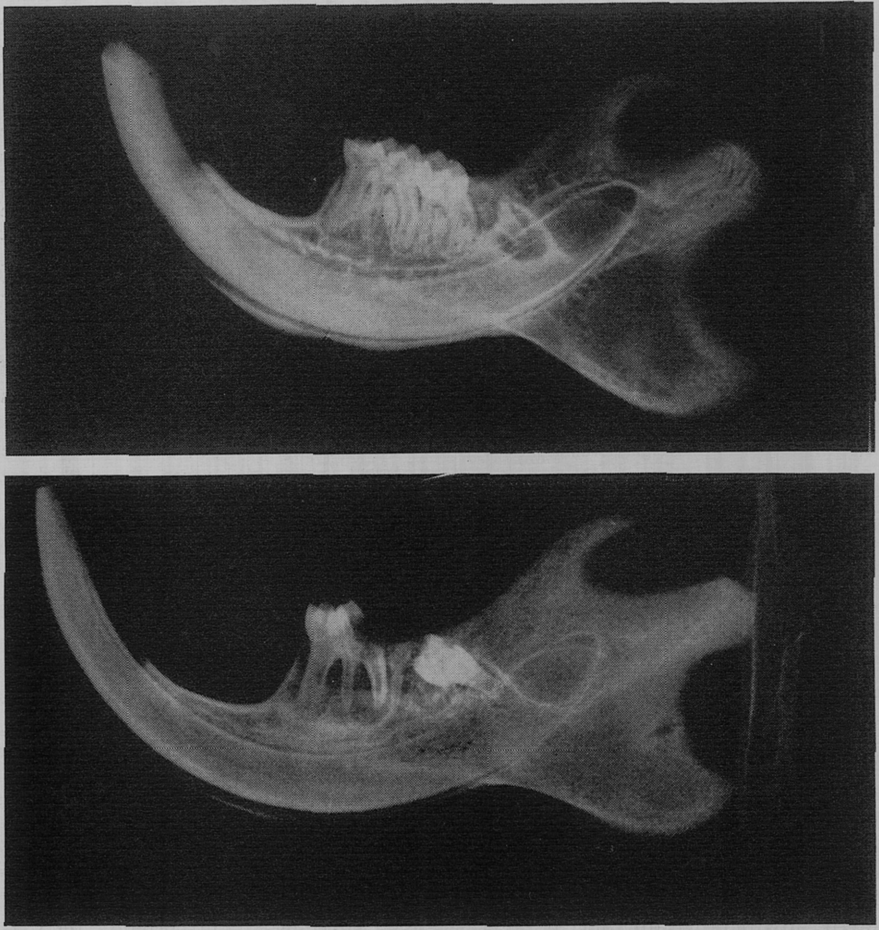

Figure 1. Roentgenograms of lower jaw of rat

A. Healthy molars in control rat on diet containing 20 p.p.m. of fluorine in drinking water
B. Extensive caries of molars in litter mate rat in fluorine-free water-grown diet

The best crop yields were obtained from sunflowers. Corn was not always well pollinated. Legumes produced lower yields and rice did not head in the short season.

Yeast. Although growing yeast for food is well enough known, the authors were unable to buy halogen-free yeast. Halogen-free corn and sunflower stalks were passed through a rubber roller clothes wringer and the juice and stalks were boiled in halogen-free water. To 10 liters of this extract was added the following: 200 grams of glucose or sucrose (only half added at the start), 30 grams of ammonium nitrate, 20 grams of potassium dihydrogen phosphate, 1 gram of *l*-aspartic acid, and 0.005 gram of inositol.

The mixture while boiling was poured into a 6-gallon crock fitted with a triangle of the porous tubing in the bottom, connected by rubber and glass tubing to a source of compressed air at about 15 pounds' pressure per square inch. When it had cooled to 30° C., it was seeded with *Saccharomyces cerevisae* or *Torulopsis utilis*. When the foam reached the top of the crock it was broken by spraying with ethyl alcohol from an ordinary throat atomizer. After all the sugar had been used up, the porous carbon tubes were removed and the crock was placed in a refrigerator until the yeast had settled. The supernatant fluid was siphoned off and the yeast spread on trays lined with paraffined paper to dry.

The initial pH of 4.5 tended to inhibit the growth of bacteria. Further inhibition was obtained by small additions of potassium bisulfite, but this must be tested on a sample in order not to kill the whole yeast culture. Bacteria are probably good food for rats, but they do not precipitate in the refrigerator and they use up the nutrients in the culture.

In some yeast experiments a complete mineral mixture was supplied, but the yield did not seem to be any larger and subsequently the authors relied on the juice from the stalks to supply calcium, magnesium, boron, iron, manganese, zinc, and copper as well as the vitamins.

Rats on Halogen- or Sodium-Free Diets

In all rat-feeding experiments, littermate albino rats at weaning (21 days old) were used. The controls were either on the same diet with the missing elements added or on a diet of the same propor-

Table I. Effect of Fluorine-Free Water-Grown Diet on Weight and Dental Caries in Rats

	Number	Average Weight, Grams 22 days	88 days	No. of Carious Molars per Rat
Controls	18	41.2	128.1	0.5
Fluorine-free diet	19	40.8	51.2	10.2

Table II. Effect of Iodine-Free Water-Grown Diet on Thyroid Weight in Rats

	Number	Average Weight, Grams 22 days	88 days	Thyroid, Mg./100 G. Body Weight
Control	14	39.6	147.0	8
Iodine-free diets	17	41.3	99.2	26.4

tions made from the same varieties of plants but grown in soil. The basal diets comprised 30% yellow corn, 30% sunflower seed, 20% sunflower leaves, 10% yeast, and 10% sucrose. The test diets then were made by adding sodium salts or halogens, except the one which was to be absent for the test. At the end of the experiments after 2 months, tissues of liver, pancreas, spleen, kidney, stomach, uterus, thyroid, parathyroid, pituitary, adrenal, and thymus of representative rats were studied by histological methods, and the teeth and bones were studied by x-rays and chemical analysis.

The results of rat feeding in a sample experiment are shown in Tables I and II. The rats either failed to grow or grew at subnormal rates with diets deficient in either fluorine or iodine. Most of the molar teeth in rats on fluorine-free diets were carious (Figure 1). Normally grown crops comprised the diet of the control rats, whose drinking water contained 20 p. p. m. of fluorine. The thyroid gland of the control rats averaged 8 mg. per 100 grams of body weight, but on the iodine-free diet, the thyroid gland weighed 18 to 42 mg. per 100 grams of body weight. In other words, the rats had goiters.

In one experiment, six rats were placed on a sodium-free diet and these showed a subnormal growth rate, but six rats on a chloride-free diet showed no difference from the controls. Evidently there was enough chloride stored in the body at weaning to last for 2 months, the period of the experiment.

Discussion

Perhaps the most logical method of preparing diets devoid of any particular trace element would be to make a mixture of pure chemical substances lacking that element. Anyone who has prepared vitamin-free or calcium-free casein realizes the difficulty of extracting any particular component from a natural food substance. Furthermore, there is doubt as to whether all essential food components are known. The early name of vitamin B-12 was "animal protein factor" and was inadvertently added to diets. The use of amino acids instead of protein has not made the problem easier. The great difficulty of obtaining a diet devoid of iodine has led to the use of iodine antagonists (thiouracil, etc.). Such substances have other antioxidant effects as well as antagonizing iodine. The growth of a foodstuff devoid of any element depends on whether that element is essential to the plant. It has been claimed that iodine stimulates the growth of certain plants and it is thought that sodium may replace part of the potassium in certain other plants; but the authors have demonstrated that halogens or sodium are not essential for the crops they grew.

Acknowledgment

The authors are indebted to the Chilean Iodine Educational Bureau for a grant.

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Received for review March 14, 1953. Accepted May 16, 1953.

Pesticide Toxicity Can Be and Is Being Avoided

PESTICIDES FORMULATION

Relation to Safety in Use

WAYLAND J. HAYES, Jr., and GEORGE W. PEARCE
Communicable Disease Center, Public Health Service,
U. S. Department of Health, Education, and Welfare, Savannah, Ga.

In arriving at improved safety of formulations, substitution of less toxic active ingredients, and use of emetics, warning colors, and antidusting agents and compounds to retard absorption are important. Highly toxic compounds may be employed with markedly decreased hazard if prior consideration is given to the incorporation of safety factors into the formulation itself, in addition to precautions usually advised.

Factors in pesticidal formulation which may lead to greater safety in their use are enumerated here, and certain examples are discussed. Very little has been written on this specific subject, although it is worthy of extensive consideration.

Increasing Safety of Formulations

The safety of formulations may be increased in a number of ways, but not all of them are applicable to any single situation. Some remarkable successes have been achieved in recent years in developing relatively harmless pesticides.