HEMOGLOBIN MAINTENANCE UPON SYNTHETIC DIETS

THE point of view, developed mainly by Whipple and Robscheit-Robbins,[1] that certain foodstuffs possess special virtues as curative agents in experimental secondary anemia has been generally accepted. Indeed, anemias of various types have come to be regarded (perhaps uncritically) as problems in nutrition. It seemed pertinent to inquire whether a synthetic diet, which has been shown to maintain experimental animals in apparent health, is also a maintenance diet in respect to hemoglobin. Such a diet, composed in large part of purified food materials, is the Karr-Cowgill[2] ration for dogs. Will dogs, fed exclusively upon this diet over long periods of time, maintain hemoglobin at a "normal" level?

Five dogs, fed upon the Karr-Cowgill diet for periods of from six to eighteen months, maintained their hemoglobin at fairly constant normal levels from week to week. During the course of this work there were published the very interesting papers of Hart and his associates[3] upon the importance of salts of copper in the cure of severe anemia in rats due to an exclusive milk diet. Although it is not wise to assume that experiments upon one species apply to another, it seemed very desirable to determine the copper content of our synthetic ration. Copper was determined by the potassium ethyl xanthate method,[4] which was checked as to accuracy by analysis for small amounts of added copper. The results of these analyses served to modify the course and, indeed, the original purpose of our investigation.

Sixteen grams (the quantity fed per kilogram body weight) of the Karr-Cowgill diet was found to contain 0.052 mgm of copper. In the experiments of the Wisconsin investigators, 35 cc of milk consumed by a rat per day contained 0.01 mgm of copper. For a 50-gram rat this represented 0.2 mgm copper per kilogram body weight. Therefore, in respect to the ingestion per kilogram of body weight, the amount of copper in the diet fed to our dogs was approximately one fourth that supplied to Hart's rats on the whole milk (anemia-producing) ration. The hemoglobin maintenance levels of our dogs and the speed of recovery after single large bleedings were not influenced by the addition of $CuSO_4$ to the diet, raising the level of copper fed daily to 1.3 mgm per kilogram of body weight. The method of feeding the ration to which copper had been added was slightly modified to avoid the possibility of incompatibility between $CuSO_4$ and KI in our salt ration (*i.e.*, formation of insoluble CuI). Furthermore, analysis of the excreta for copper indicated that most of the copper administered was retained by the animal.

It was of interest to determine the source of the copper in our original diet. Surprisingly, analysis of the individual salts in the "salt mixture" proved negative, while the copper content of the commercial casein (the protein component of the diet) and Vitavose (used as the source of vitamin B) accounted for the total copper. Modifying the Karr-Cowgill diet by using coagulated egg albumin as the protein and Harris yeast concentrate and dry brewers' yeast as the sources of water-soluble vitamins and with other slight changes, we constructed a "copper-free" synthetic diet suitable for rat-feeding experiments. By the addition of $CuSO_4$ to this diet, we had two similar synthetic rations complete in all known dietary essentials and differing only in the presence of copper. When fed *ad libitum* approximately 10 g of synthetic diet were consumed per rat per day. In several analyses of 10 to 20 g samples of diet by the xanthate method, which is sensitive to 0.01 mgm, no copper was found in the ration designated as copper-free. The small quantity of copper added to the copper-free ration in making up the copper-containing diet was quantitatively checked by the xanthate method.

Most of the rats used were of a "skin-parasite free" strain. They were kept in a special room in individual cages. The possibility of copper contamination of the rats upon the copper-free ration was eliminated. The animals were placed upon the special diets at the age of twenty to twenty-four days. The growth, general appearance and hemoglobin maintenance were normal in the rats on the copper-containing and also in those on the copper-free diet. Five rats upon a whole milk diet developed a severe anemia. This anemia was promptly cured by placing these rats upon the copper-free synthetic ration. It should be mentioned that diarrhea invariably accompanied milk feeding and in two of the milk rats growth, which had been retarded, was promptly resumed on the copper-free diet.

Further experiments are in progress.

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