These studies furnish additional evidence to support the accepted view that vegetables should be cooked in as little water as possible in order to conserve the maximum amounts of vitamins and minerals, without sacrifice of palatability.

SULFAGUANIDINE, SUCCINYLSULFATHIAZOLE, AND DEFICIENCY DISEASE

Investigations which emphasize the importance of intestinal bacteria in the nutrition of vertebrate animals have been reviewed several times in this journal (*Nutrition Reviews* **1**, *4*, *35*, *175*, *199*, *220* (*1943*)). These reviews described experiments which have shown that intestinal bacteria can synthesize vitamin K and many of the B-vitamins both *in vitro* and *in vivo*. They reviewed evidence which indicates that these synthesized vitamins apparently may furnish a substantial amount of some of the vitamin requirements both of ruminants and of those animals which have large cecums. The rumen and the cecum in these species seem to serve as "fermentation vats." In man, intestinal synthesis may supply all the biotin and vitamin K required. Furthermore, when one of the sulfonamide drugs, particularly one that is poorly absorbed like sulfaguanidine or succinylsulfathiazole, is given orally to certain experimental animals reduction in growth rate or other manifestations of deficiency disease may be produced. It seems probable that the effect of the sulfonamide drugs is to alter the intestinal flora and to interfere, consequently, with the synthesis of vitamins within the intestinal tract. Two of the questions which arise about these relationships are: (1) What type of deficiency is produced when sulfaguanidine or succinylsulfathiazole is given? and (2) What is the specific effect of the poorly absorbed sulfonamide preparation on intestinal bacteria? At least partial answers to both these questions are now available.

Most of the investigators who have studied the deficiencies produced by oral administration of sulfonamides have used white rats fed a synthetic diet. Black, Overman, Elvehjem, and Link (*J. Biol. Chem.* **145,** *137* (*1942*)) demonstrated that inadequacies of vitamin K and of para-aminobenzoic acid were produced. If the synthetic diets containing sulfaguanidine or succinylsulfathiazole were supplemented with thiamine, riboflavin, pyridoxine, niacin, calcium pantothenate, choline, inositol, para-aminobenzoic acid, ascorbic acid, and the fat soluble vitamins, the rats still showed retarded growth. This was corrected by the addition of biotin (Daft, Ashburn, and Sebrell, *Science* **96,** *321* (*1942*); Nielson and Elvehjem, *J. Biol. Chem.* **145,** *713* (1942)). Martin (*Proc. Soc. Exp. Biol. Med.* **51,** *353* (*1942*)) observed that rats fed as outlined above developed silvery-gray coats. This change was reversed by folic acid concentrates. Nielson and Elvehjem found that folic acid also had a powerful growth stimulating action on animals fed in this manner. It is apparent, therefore, that the inclusion of poorly absorbed sulfonamide drugs in synthetic diets of rats has produced deficiencies of vitamin K, para-aminobenzoic acid, biotin, and folic acid.

Welch and Wright (*J. Nutrition* **25,** *555* (*1943*)) in studies which are complimentary, observed that succinylsulfathiazole, when incorporated to the extent of 5 or 10 per cent in a complete diet of natural materials (Wayne or Purina brand), caused no demonstrable effect on the growth rate of rats. However, only 1 or 2 per cent of the same drug was effective in producing marked inhibition of growth when added to purified rations which were supplemented with a vitamin A, D, and E concentrate and with the same crystalline B-vitamins used by Black and associates. Moreover, the animals frequently, but irregularly, developed alopecia, spectacled eyes, panophthalmitis, porphyrin-caked whiskers, and achromotrichia. A yeast concentrate and a liver extract, both fed at a level of 1 per cent, partially antagonized the effect of the sulfonamide, while an extract of rice polishings was possibly more effective. Dried grass also seemed to supply the missing factors. Addition of 20 micrograms of biotin per 100 g. of the sulfonamide ration had a slight stimulating effect on growth. In other experiments, 2 micrograms of biotin and 10 mg. of "folic acid concentrate" (200,000 Snell-Peterson units per gram) were administered daily to rats by stomach tube. Each of these substances, when given singly in the amounts indicated, had some activity in overcoming the growth depression caused by succinylsulfathiazole; but when they were administered together a striking stimulation of growth resulted. The authors state that a favorable effect on the clinical signs of the deficiency state "was exerted by a combination of the folic acid concentrate and biotin, and occasionally by one or the other factor alone." One gathers from this that the response to each factor, therefore, was apparently not consistent; even so, it is regrettable that a more complete description of the therapy-induced changes was not made. One of the most interesting observations made by Welch and Wright is related to the bleeding manifestations which occurred in some of their animals fed the purified diet containing succinylsulfathiazole. As was to be expected, the prothrombin values of the rats who bled were low. However, the prothrombin returned to normal and the hemorrhages stopped after biotin and the folic acid concentrate were fed. Vitamin K administration was not necessary to bring about this result. It is suggested that the biotin and folic acid stimulated the synthesis of vitamin K by intestinal bacteria. These findings indicate that these two vitamin substances, in addition to being probable essential components of metabolic systems in the rat, function also in promoting a certain type of bacterial synthetic activity.

Spicer, Daft, Sebrell, and Ashburn (*Pub. Health Rep.* **57,** *1559* (*1942*)) also observed that the use of sulfaguanidine or succinylsulfathiazole in purified diets fed to rats led to the development of granulocytopenia and anemia which could be cured with whole dried liver or certain liver fractions. Their studies have recently been extended to include the use of three of the more soluble sulfonamides: sulfathiazole, sulfadiazine, and sulfanilamide (Kornberg, Daft, Sebrell, *Science* **98,** *20* (*1943*)). The basic ration allowed was composed of glucose (Cerelose), purified casein, cottonseed (Wesson) oil, a salt mixture, and 1 per cent sulfathiazole, sulfadiazine, or sulfanilamide. Supplements of thiamine, riboflavin, pyridoxine, calcium pantothenate, niacin, and choline chloride were fed daily. Fifty-two of 105 rats developed either a severe granulocytopenia (polymorphonuclear granulocyte count of less than 150 cells per cmm.), an anemia (less than 7.5 g. hemoglobin per 100 ml. blood and a hematocrit value under 25 per cent), or both. The changes in peripheral blood were found as early as the tenth experimental day and as late as the eighty-sixth. Animals which remained free of these hematologic abnormalities were observed as long as one hundred and sixty-nine days. The blood dyscrasias appeared in 28 out of 34 animals given sulfathiazole, 22 out of 36 given sulfadiazine, and in only 2 out of 35 given sulfanilamide. Granulocytopenia was twice as common as severe anemia in animals fed sulfathiazole. These frequencies were reversed for sulfadiazine. Therapy with liver extract or whole dried brewers' yeast brought about prompt improvement in the peripheral blood. Unfortunately, the bone marrow changes which accompanied the blood dyscrasias were not described. It would also be of interest to know whether biotin or folic acid concentrate possess therapeutic value for the granulocytopenia and anemia. The mechanism by which these soluble sulfonamide compounds bring about the abnormalities described is not clear. They may, possibly, alter the synthesis of essential factors by intestinal bacteria as seems to be the case with sulfaguanidine and succinylsulfathiazole. However, as the authors point out, the possibility that the drugs may produce these effects by toxic action, either direct or indirect, is not eliminated. They may interfere with tissue enzyme systems, the integrity of which is restored by some unidentified factors in liver and yeast.

The most consistent change produced in the intestinal flora of animals and men fed sulfaguanidine or succinylsulfathiazole is a reduction in the number of coliform organisms (Corwin, *Bull. Johns Hopkins Hosp.* **69,** *39* (*1941*); Poth, *et al. Arch. Surg.* **44,** *187* (*1942*); Kirby and Rantz, *J. Am. Med. Assn.* **119,** *615* (*1942*); and Gant, Ransone, McCoy, and Elvehjem, *Proc. Soc. Exp. Biol. Med.* **52,** *276* (*1943*)). Enterococci are often increased in number so that the total bacterial count per unit of fecal material may not be significantly altered. Gant and coworkers made the interesting observation that the decrease in number of colon bacilli in rats (Sprague-Dawley colony, chosen because of freedom from protozoan and metazoan parasites) began as early as the third or fourth day after feeding with the sulfonamide-containing diet was begun. However, after eleven to twenty-five days the colon bacilli gradually increased and ultimately became as numerous as they had originally been. In spite of this fact, the animals showed no growth. The colon bacilli, therefore, had apparently lost their ability to synthesize the growth promoting factors, presumably biotin and folic acid. Animals fed a succinylsulfathiazole-containing diet supplemented with liver extract or crude folic acid maintained their normal growth in spite of a marked reduction in *E. coli.* These results indicate that normally the colon bacillus is mainly responsible for the synthesis of biotin and folic acid or related growth promoting factors. When sulfonamide drugs, poorly absorbed from the intestinal tract, cause deficiencies of biotin and folic acid to develop, they do so by decreasing the number of *E. coli* in the intestinal tract. The observation by Gant and colleagues that *E. coli* may reappear in great numbers while the sulfonamide is still being administered but then are not able to synthesize biotin and folic acid is important. It needs, however, to be verified.

SUPPLEMENTARY FOOD AND THE NUTRITION OF SCHOOLCHILDREN

The incorporation of all the essential vitamins and minerals in a single or minimum number of food articles is a useful goal if directed toward mass feeding, and particularly if considerable transportation difficulties must be faced. The object of nutrition education in this country, however, has been to advocate a diversified palatable diet so as to include all the essential nutrients. There has, of course, been emphasis on certain foods for special purposes, such as the consumption of citrus fruits for their vitamin C content, dark green leafy vegetables for provitamin A, milk for calcium, riboflavin, protein, etc. There are indeed justifiable instances of food supplementation such as the use of fish liver oils and enriched flour; but indiscriminate supplementation is definitely opposed by the majority of nutritionists.

Nevertheless there are many reports to indicate that a rather large proportion of our people are malnourished and that the most direct solution to the problem is by means of food supplementation. Harris, Weeks, and Kinde (*J. Am. Dietet. Assn.* **19,** *182* (*1943*)) are of the belief that a high proportion of schoolchildren that they examined showed evidence of malnutrition and that the situation was improved by feeding a special soup designed to meet, in itself, the daily requirement of vitamins and minerals.

The dehydrated soup mixture developed by Harris *et al.* contains in every 100 g., 10