SPECIAL ARTICLES

BIOTIN DEFICIENCY AND OTHER CHANGES IN RATS GIVEN SULFANILYLGUANIDINE OR SUCCINYL SULFATHIAZOLE IN PURIFIED DIETS

THERE is general agreement[1, 2, 3, 4] that sulfaguanidine (sulfanilylguanidine), added to a purified diet, reduces the growth rate of young rats. Similar results have been reported[4, 5] for sulfasuxidine (succinyl sulfathiazole). It has been suggested[1, 5] that the reduced rate of growth might be explained on the basis of suppression by the drug of intestinal synthesis of essential growth factors. It has been further suggested[4] that these sulfonamide drugs might exert a part of their effect by interference with the action, in the animal body, of one or more essential enzyme systems. If sulfaguanidine and sulfasuxidine act through either or both of these mechanisms, then it should be possible to produce characteristic deficiency syndromes, the development of which might be prevented, individually, by the use of small amounts of pure substances or concentrates.

In this laboratory, we have observed the following changes in rats given sulfaguanidine or sulfasuxidine in purified diets. First, hyaline sclerosis and calcification of blood vessels[6]; second, agranulocytosis, leukopenia, bone marrow aplasia and, occasionally, anemia[4]; third, hyalinization, necrosis and calcification of voluntary muscle[7]; and fourth, a dermatitis which can be prevented or successfully treated with biotin. We have also observed necrosis of heart muscle, hemorrhage into various organs and subcutaneous tissues, and liver damage.

In our work with the sulfonamide drugs we have to date employed variations of a single basic diet. This basal diet consists of glucose ("Cerelose") 73 per cent., leached and alcohol extracted casein 18 per cent., cod liver oil 2 per cent., cottonseed oil (Wesson oil) 3 per cent. and salt mixture 4 per cent. The drug at a level of 1 per cent. has been included at the expense of an equivalent amount of glucose. Each rat has been given a daily supplement of 100 micrograms of thiamine, 200 micrograms of riboflavin, 100 micrograms of pyridoxine, 200 micrograms of calcium pantothenate, 1 mg of niacin and 10 mg of choline chloride.

Thirty rats receiving sulfaguanidine and 10 receiving sulfasuxidine have been kept on this régime until their deaths. Twenty additional rats receiving one or the other of these drugs have been given a daily supplementary feeding of 0.05 cc of an impure biotin concentrate,[8] beginning after growth of these animals had ceased; 35 others have been given, similarly, 40 to 100 mg of a liver extract[9] daily.

Of the 40 rats given neither biotin concentrate nor liver extract, only 2 have survived longer than two months. Examination of the blood of 22 of these rats shortly before death revealed an agranulocytosis and a leukopenia and, less frequently, an anemia.[4] Histological examination revealed some degree of bone-marrow aplasia in 16 of 18 marrows examined,[4] a hyalinization, necrosis or calcification of voluntary muscle in 12 of 15 rats examined for this condition,[7] but blood vessel changes in only 5 of the 29 of these rats examined to date.[6] Any degree of biotin deficiency present in these animals was difficult to diagnose.

The use of the biotin concentrate has proved to be of value in the routine development of the blood vessel changes. Of the 20 rats given 0.05 cc portions of this concentrate, 11 have survived for 2 months or longer and 3 have lived for more than 3 months; 13 of these 20 animals have been examined histologically to date; 11 of these 13 have shown hypocellularity of bone marrow, 12 have shown muscle hyalinization and necrosis, and 10 have shown calcification of blood vessels.

The use of the liver extracts has been of particular value in the development, in a mild form, of the characteristic dermatitis of biotin deficiency. No attempt has been made to determine the span of life of these animals, but of the 35 rats used in this phase of the work, 33 have been kept on experiment for 3 months or longer. The dermatitis has developed very slowly but has been noted in 24 of the 35 animals. It is similar in appearance to that produced by raw egg white but does not attain the same severity.[10] Remission of symptoms has occurred in all 10 animals which were given crystalline biotin[11] orally or parenterally in daily doses of 0.5 to 10 micrograms for 14 days.

[1] S. Black, J. M. McKibbin and C. A. Elvehjem, *Proc. Soc. Exp. Biol. and Med.*, 47: 308, 1941.

[2] J. B. Mackenzie, C. G. Mackenzie and E. V. McCollum, SCIENCE, 94: 518, 1941.

[3] W. J. Dann, *Jour. Biol. Chem.*, 141: 803, 1941.

[4] S. S. Spicer, F. S. Daft, W. H. Sebrell and L. L. Ashburn, Prevention and Treatment of Agranulocytosis and Leukopenia in Rats Given Sulfaguanidine or Succinyl Sulfathiazole in Purified Diets, *Pub. Health Rep.* (in press).

[5] A. D. Welch, *Federation Proceedings*, Part II, Fed. of Amer. Soc. for Exper. Biol., Vol. 1, p. 171, March 16, 1942.

[6] F. S. Daft, L. L. Ashburn, S. S. Spicer and W. H. Sebrell, *Pub. Health Rep.*, 57: 217, 1942.

[7] F. S. Daft, L. L. Ashburn and W. H. Sebrell. Unpublished results.

[8] S.M.A. No. 1000.

[9] Eli Lilly's No. 343 or Lederle's 80 per cent. alcohol insoluble.

[10] The results of microbiological assays indicate the following approximate figures for the biotin content of these liver extracts: Lilly's No. 343 1.5 micrograms per gram; Lederle's 80 per cent. alcohol insoluble 0.06 micrograms per gram. We are indebted to Dr. Roy Hertz for these assays.

[11] Supplied through the courtesy of Dr. Hans Molitor, Merck Therapeutic Institute.

SUMMARY

The following pathological changes have been observed in rats given sulfaguanidine or sulfasuxidine in purified diets.

1. A granulocytopenia, leukopenia, hypocellularity of bone marrow and, occasionally, an anemia.

2. Hyalinization, necrosis and calcification of voluntary muscle.

3. Hyaline sclerosis and calcification of blood vessels.

4. A dermatitis which can be prevented or successfully treated with crystalline biotin.

We have also observed necrosis of heart muscle, hemorrhage into various organs and subcutaneous tissues, and liver damage.

FLOYD S. DAFT
L. E. ASHBURN
W. H. SEBRELL

NATIONAL INSTITUTE OF HEALTH,
U. S. PUBLIC HEALTH SERVICE,
BETHESDA, MD.

ATYPICAL RESPONSE OF THE RABBIT TO DESOXYCORTICOSTERONE ACETATE

WE have found[1,2] that administration of desoxycorticosterone acetate, progesterone, alpha-estradiol benzoate, testosterone proprionate and diethylstilbestrol to dogs results in a marked increase in the rate of entrance of Na and Cl into 5.5 per cent. dextrose solution introduced into the peritoneal cavity. No such effect was noted in rabbits following administration of desoxycorticosterone acetate, progesterone and diethylstilbestrol.[2] Certain additional findings are of interest in this connection.

Injection of 2.5 mg of DOCA daily for three days in two rabbits had no significant influence upon the urinary excretion of water or Cl during the experimental period. Two rabbits (2.0 and 1.8 kg) received 2.5 mg of DOCA daily for three months, the animals being weighed and the blood-pressure determined[3] bi-weekly. There was no significant change in weight for six weeks in one animal and eight weeks in the other, with a subsequent increase to a maximum of 47 and 40 per cent., respectively, over the pre-treatment levels at the end of the experimental period. There was no significant alteration in blood-pressure and no edema or other evidence of toxic effect of DOCA, as has been reported in the dog.[4] Two pregnant rabbits treated in the same manner showed no significant gain in weight, no edema and no increase in blood-pressure. Both aborted at about four weeks' gestation, one developed marked weakness of the hind limbs after five weeks of treatment and both died after six weeks. The weakness of the hind limbs may have been due to decrease in the serum K concentration; the serum Cl concentration was unaltered in the non-pregnant animals, but no chemical studies were performed in the pregnant rabbits. Dexter and Weiss[5] have reported a similar absence of effect of large doses of DOCA in pregnant and non-pregnant rabbits in experiments of shorter duration.

These observations suggest the existence of a marked species difference in the influence of DOCA and perhaps also progesterone, estradiol, testosterone and other steroid hormones upon water and electrolyte metabolism. This has an important bearing, perhaps, on the use of the rabbit in studies of the relation of these hormones to hypertensive "toxemia" of pregnancy.

A. E. RAKOFF
K. E. PASCHKIS
A. CANTAROW

JEFFERSON MEDICAL COLLEGE
AND HOSPITAL,
PHILADELPHIA

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UNIFORMITIES IN THE CONTENT OF B VITAMINS IN MALIGNANT NEOPLASMS

FOR two years[1] this laboratory has been interested in the possibility that cancer tissues might show peculiarities in vitamin distribution, characteristic of this type of growth. We have now completed an extensive series of determinations of eight B vitamins not only on various types of human, rat and mouse cancer material but also upon normal tissues from these same animals. This material is now in press.[2]

By analysis of the values obtained, highly interesting and important uniformities are observed which we wish to set forth briefly here. We shall use the term "vitamin uniformity" to designate the similarity of vitamin content in a group of tissues. For example, if in a number of samples of muscle tissue pantothenic acid had a mean level of 5 γ per gram with a standard deviation of 1, then $1/5 \times 100$, or 20, would equal the average deviation from the mean in per cent., or, to express the relationship in the opposite manner, there would be an average degree of uniformity in pantothenic acid content of 80 per cent. The average of the "vitamin uniformities" in a series of tissues calculated for the individual vitamins is designated the "Total B Vitamin Uniformity."

[5] L. Dexter and S. Weiss, "Preeclamptic and Eclamptic Toxemia of Pregnancy." Boston: Little, Brown and Company, 1941.

[1] R. J. Williams, SCIENCE, 92: 579, 1940.

[2] University of Texas Publication 4237, 1942.