the gravimetric section correspond to the ion groups of the ordinary scheme of qualitative analysis. The volumetric section is divided according to type of reaction, such as oxidation-reduction, acid-base neutralization, and others. This new arrangement makes for a much more attractive and useful volume.

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SPECIAL ARTICLES

THE ROLE OF "FOLIC ACID" AND BIOTIN IN THE UTILIZATION OF PANTOTHENIC ACID BY THE RAT

THE inclusion, in *highly purified diets* fed to rats, of sulfaguanidine or of succinylsulfathiazole brings about a retardation of growth; after growth ceases the animals lose weight and usually die within a few weeks. The effect of these drugs has been attributed to their interference with the synthesis of essential factors by intestinal bacteria.[1] This explanation is supported by the fact that sulfaguanidine is partially retained in the intestine,[2, 3] while about 95 per cent. of ingested succinylsulfathiazole fails to be absorbed from the alimentary tract.[4, 5] Recently Nielsen and Elvehjem,[6] using succinylsulfathiazole, reported that supplementation with biotin and concentrates of "folic acid" caused a striking resumption in growth which was maintained during several weeks therapy. Martin,[7] who used sulfaguanidine, obtained similar results. We[8] have presented detailed evidence in confirmation of the findings of Nielsen and Elvehjem.

In addition to the cessation of growth, rats fed the sulfonamide in purified diets[9] frequently develop signs of marked pantothenic acid (PA) deficiency, such as achromotrichia and porphyrin-caked whiskers.[8] The fact that "folic acid" (biotin was also present) will cure the achromotrichia produced by sulfaguanidine feeding was attributed by Martin[7] to a chromotrichial action of this factor.

We have found that the signs of PA deficiency in rats are borne out by microbiological assays of the PA content of the liver.[10] Liver obtained from rats on a complete diet[11] or on the highly purified diet without sulfonamide contained 80–90 μgm of PA per gram. Rats fed the purified diet, without either PA or succinylsulfathiazole, developed signs of severe PA deficiency, and the liver tissue contained only 40–50 μgm of PA per gram. Rats fed the purified diet with succinylsulfathiazole (2 per cent.) showed marked evidence of PA deficiency, despite the presence in the diet of adequate amounts of PA (4 mgm per 100 gm), and the PA content of the liver was reduced to only 40–50 μgm per gram. Further increase in the PA content of the diet (10 mgm per 100 gm) caused no favorable effect. Administration of PA subcutaneously, 200 μgm daily, also was without influence on the diminished content of PA in the liver or on the severely depressed rate of growth.

When the purified diet was supplemented with succinylsulfathiazole (2 per cent.) and dried grass[12] (5 per cent.) the PA content of the liver was raised to a value (50–70 μgm per gram) intermediate between normal levels and those produced by the basal diet with added sulfonamide. However, this moderate increase in liver PA content was accompanied by a growth rate comparable to that of rats on the basal diet.

The oral administration of crystalline biotin[13] (5 μgm daily) for a period of 3 weeks caused a slight increase in the PA content of the liver and some improvement in the rate of growth of the succinylsulfathiazole-fed rats. When, however, in addition to biotin (5 μgm), a "folic acid" concentrate[14] (20 mgm

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

[2] E. K. Marshall, Jr., A. C. Bratton, L. B. Edwards and E. Walker, *Bull. Johns Hopkins Hosp.*, 68: 94, 1941.

[3] W. M. Firor and E. J. Poth, *Annals of Surg.*, 114: 663, 1941.

[4] E. J. Poth and F. L. Knotts, *Proc. Soc. Exper. Biol. Med.*, 48: 129, 1941.

[5] A. D. Welch, P. A. Mattis and A. R. Latven, *Jour. Pharmacol. and Exper. Therap.*, 75: 231, 1942.

[6] E. Nielsen and C. A. Elvehjem, *Jour. Biol. Chem.*, 145: 713, 1942.

[7] G. J. Martin, *Proc. Soc. Exper. Biol. Med.*, 51: 353, 1942.

[8] A. D. Welch and L. D. Wright, *Jour. Nutrition*, in press.

[9] Composition of diet: casein (Labco) 18 gm, fat (Primex) 10 gm, corn oil 2 gm, sucrose 61.9 gm, salts 4 gm, cellu flour 4 gm, vitamins A, D and E concentrate 0.08 gm, choline chloride 0.1 gm, thiamine hydrochloride 0.2 mgm, riboflavin 0.4 mgm, pyridoxine hydrochloride 0.2 mgm, nicotinic acid 4 mgm, calcium pantothenate 4.4 mgm, p-aminobenzoic acid 4 mgm, inositol 8 mgm, 2-methyl-1,4-naphthohydroquinone diacetate 1 mg.

[10] Some of the liver samples were prepared for assay after autolysis as described by L. D. Wright, *et al.*, University of Texas Publication, 4137: 38, 1941. We now employ enzyme digestion of the liver samples with taka-diastase, as recommended by V. H. Cheldelin, *et al.*, University of Texas Publication 4237: 15, 1942. With liver samples autolysis liberates approximately 90 per cent. of the pantothenic acid found after enzyme treatment. Pantothenic acid was determined by either the method of D. Pennington, *et al.*, *Jour. Biol. Chem.*, 135: 213, 1940, or the method of M. Landy and D. M. Dicken, *Jour. Lab. and Clin. Med.*, 27: 1086, 1942.

[11] Purina chow.

[12] Powdered grass ("Cerophyl") was generously supplied by Dr. Richard Graham, of the Cerophyl Laboratories, Kansas City, Mo.

[13] We are indebted to Dr. Hans Molitor, Merck Institute for Therapeutic Research, and Dr. W. H. Engels, Research Laboratories, Merck and Company, for a generous gift of crystalline biotin.

[14] Prepared from "Cerophyl" by the procedure described by B. L. Hutchings, N. Bohonos and W. H. Peterson, *Jour. Biol. Chem.*, 141: 521, 1941. This concentrate contained approximately 800,000 Snell-Peterson units of "folic acid" per gram.

daily) was administered orally, not only was the growth rate of the rats restored to a rate comparable to that produced by the basal diet, but also the PA content of the liver was increased to a level entirely within normal limits.

TABLE I

CHANGE IN WEIGHT AND PANTOTHENIC ACID CONTENT OF LIVER OF RATS FED SUCCINYLSULFATHIAZOLE*

Group	Daily supplement	Number of animals	Change in weight gm	Pantothenic acid content of liver μgm/gm
1	None	3	− 8	48 (47–49)
2	Cryst. biotin 5 μgm.	3	+16	57 (55–61)
3	Cryst. biotin 5 μgm + "folic acid" concen. 20 mgm	3	+46	81 (80–82)
–	Pantothenic acid deficient rats (for reference)	3	–	46 (44–49)
–	Rats on "Purina" chow (for reference)	4	–	89 (88–91)

* The groups (1–3) were composed of animals which had received succinylsulfathiazole in the purified diet for a period of 12 weeks. At that time the livers of 3 such animals were found to contain 40 (33–46) μgm of PA per gram. The supplements were administered once daily (except Sundays) by stomach tube during a period of 3 weeks.

These data suggest that for the proper utilization of PA by the tissues of the rat "folic acid," in addition to biotin, must be available. It is probable that the effect of "folic acid" on PA utilization occurs only in the presence of biotin, since a significant growth response in the rat follows the administration of "folic acid" only when biotin is also given.[8] It is unlikely that orally administered PA is inactive because of any interference with its absorption from the alimentary tract caused directly or indirectly by the sulfonamide, since parenterally administered PA is also quite ineffective.

Conceivably, the effect of these substances might be due to counteraction of the effect of the sulfonamide. However, *in vitro* studies of the effect of the "folic acid" concentrate on the bacteriostatic action of sulfathiazole (1:8,000,000) on *E. coli* showed that in a concentration of 1:3500 it had no activity not attributable to p-aminobenzoic acid, or to other heat-acid stable constituents.[15] p-Aminobenzoic acid, it will be noted, was present in the diet to the extent of 4 mgm per 100 gm.

An attractive hypothesis suggests that in the absence of "folic acid" and biotin, PA can not be utilized by the organism. Whether the influence of these trace-factors on PA utilization results from an effect on the synthesis of PA derivatives by intestinal bacteria, from an effect on the synthesis of such derivatives by the tissues of the rat, or from other effects, has not yet been established. Experiments bearing on these points are now in progress.

[15] We are indebted to Dr. Lawrence Peters of this laboratory for the experiments with *E. coli*.

According to the views presented, the basic fault, when hair pigmentation ceases following the use of purified diets containing poorly absorbed sulfonamides, actually may be the improper utilization of PA, so that the end result may be comparable to the exclusion of PA from the diet. Rather than as chromotrichial agents, *per se,* either "folic acid" or biotin might be considered as concerned primarily with PA metabolism. Possibly a similar explanation may be applied to the growth effects of these factors.

The growth stimulation sometimes caused by p-aminobenzoic acid is now believed to result from the stimulation of intestinal microorganisms to produce unknown factors. It has been shown that p-aminobenzoic acid will stimulate the synthesis of "folic acid" by the intestinal bacteria of the chick, *in vitro*.[16] It might be reasoned that the controversial anti-gray hair action and growth effects of the former substance result from a stimulation of the bacterial synthesis in the intestine of "folic acid," and perhaps of biotin. These, in turn, improve the utilization of available PA. Such a hypothesis suggests that any chromotrichial action of p-aminobenzoic acid may be mediated through "folic acid" and biotin, and that the chromotrichial effects of the latter substances are secondary to that of pantothenic acid.[17]

SUMMARY

The addition of succinylsulfathiazole to *highly purified diets* containing all dietary factors known to be required by the rat, including pantothenic acid, results in the appearance of signs of severe pantothenic acid deficiency, including achromotrichia and porphyrin-caked whiskers. These changes are accompanied by a marked reduction in the pantothenic acid content of the liver, and are corrected by the inclusion in such diets of crystalline biotin and "folic acid" concentrates. The utilization of pantothenic acid by the rat appears to depend on the availability of biotin and "folic acid." Under normal conditions these are supplied by the diet and synthesized by intestinal bacteria.

A hypothesis is offered in explanation of the chromotrichial actions of "folic acid," biotin and p-aminobenzoic acid.

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[16] G. M. Briggs, Jr., J. D. Luckey, R. C. Mills, C. A. Elvehjem and E. B. Hart, *Proc. Soc. Exper. Biol. Med.*, 52: 7, 1943.

[17] This interpretation of certain effects of "folic acid" and biotin obviously does not exclude them from the performance of other roles in living processes.