FOLIC ACID, BIOTIN AND PANTOTHENIC ACID DEFICIENCY AND THE LIVER STORAGE OF VARIOUS VITAMINS IN RATS FED SUCCINYLSULFATHIAZOLE IN HIGHLY PURIFIED RATIONS

LEMUEL D. WRIGHT AND ARNOLD D. WELCH

Nutritional Laboratories, Department of Pharmacology, Medical-Research Division, Sharp and Dohme, Inc., Glenolden, Pennsylvania

(Received for publication July 9, 1943)

The concentration in the tissues of various vitamins of the B complex has been employed by many investigators as an index of the adequacy of an animal's supply of these dietary essentials. Of the various tissues, changes in the vitamin content of the liver have, in general, shown the best correlation with variations in the vitamin intake and the nutritional status of the animal. In this communication data are presented on the storage of folic acid, biotin, and pantothenic acid in the liver of the rat, as influenced by the incorporation of succinylsulfathiazole in highly purified diets adequate in the well-recognized members of the vitamin B complex.

It has been shown (Welch, Mattis and Latven, '42; Welch and Wright, '43) that the inclusion of succinylsulfathiazole in amounts up to 10% of the ration has no demonstrable deleterious effect on rats when the remainder of the diet is composed of relatively crude ingredients (stock ration [1]). The inclusion of 1 or 2% of succinylsulfathiazole in rations composed of purified ingredients and containing thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, inositol, p-aminobenzoic acid, and choline in amounts believed to be

[1] The Purina brand was employed as a stock ration in these studies.

adequate, brings about a retardation in the rate of growth of rats. On such rations growth finally ceases and death occurs after a few weeks. Accompanying the effect on growth manifestations of dietary inadequacy such as alopecia, spectacled eyes, ophthalmitis, porphyrin-caked whiskers and achromotrichia may occur. A combination of crystalline biotin and folic acid concentrate [2] will effectively prevent or cure the condition. Reasons have been presented elsewhere (Black, Overman, Elvehjem and Link, '42; Martin, '42; Nielsen and Elvehjem, '42; Welch and Wright, '43; Wright and Welch, '43a) for relating the effect of succinylsulfathiazole to its effect on the synthetic activity of intestinal bacteria.

PROCEDURE

Young male black or piebald rats of approximately 50 gm. weight were caged individually over wide mesh screening and were fed ad libitum on the various rations outlined in table 1. After the animals had subsisted on these rations for varying lengths of time, representative animals were killed by decapitation and their livers assayed for several members of the vitamin B complex by microbiological methods. With some of the animals liver autolysates prepared by the method described by Wright et al. ('41) were used. In the remainder of the series the livers were subjected to enzymatic digestion with takadiastase at their natural pH [3] (Cheldelin et al., '42). In our experience autolysis yields approximately 90% (range 89–93%) of the pantothenic acid present in normal liver, based on the amount liberated after enzyme digestion with takadiastase. However, autolysis yields only 41% (range

[2] The substance termed "folic acid" is probably not a single entity. The evidence for the probable existence of several substances which can be utilized for growth by L. casei ϵ or by Streptococcus lactis R, but which appear to be of significantly different potency, has been discussed by Stokstad ('43).

[3] Two gm. of liver fragmented with a spatula, suspended in 20 ml. water, takadiastase (equivalent to 2% of the liver weight) added, and digestion carried out for 18–24 hrs. at 37°C. under benzene. This, of several procedures, has given the highest values for folic acid in liver without the use of xanthopterin (Wright and Welch, '43b, 43c).

38–43%) of the folic acid obtainable from normal liver by takadiastase digestion. The biotin data were obtained from assays performed on samples of liver which had been digested by autoclaving at 15 pounds pressure for 1 hour with 6*N* H_2SO_4 and then neutralized with NaOH.

TABLE 1

Composition of diets employed in feeding experiments.

	s-5	s-6	s-6A	s-7	s-8
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Casein (Labco)	18.0	18.0	18.0	18.0	18.0
Fat (Primex)	...	10.0	10.0	10.0	10.0
Corn oil	2.0	2.0	2.0	2.0	2.0
Sucrose	72.4	59.9	59.9	59.9	59.9
Salts [1]	4.0	4.0	4.0	4.0	4.0
Cellu flour	1.5	4.0	4.0	4.0	4.0
A, D, and E concentrate [2]	.. [4]	.. [4]	.. [4]	0.08	0.08
Choline chloride	0.1	0.1	0.1	0.1	0.1
Succinylsulfathiazole [3]	2.0	2.0	2.0	2.0	2.0
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Thiamine hydrochloride	3.0	.. [5]	.. [6]	0.2	0.2
Riboflavin	3.0	.. [5]	.. [6]	0.4	0.4
Pyridoxine hydrochloride	3.0	.. [5]	.. [6]	0.2	0.2
Nicotinic acid	2.5	.. [5]	.. [6]	4.0	4.0
Calcium pantothenate	2.0	.. [5]	..	4.4	...
p-Aminobenzoic acid	...	...	.. [6]	4.0	4.0
Inositol	...	...	.. [6]	8.0	8.0
2-methyl-1, 4-napthohydroquinone diacetate	...	...	...	1.0	1.0

[1] Osborne and Mendel ('13) or Hubbell, Mendel and Wakeman ('37).

[2] Compounded as follows: fish liver concentrate containing 450,000 U.S.P. units of vitamin A and 90,000 U.S.P. units of vitamin D per gram, 7 gm.; α-tocopherol, 2 gm.; corn oil, 41 gm.

[3] In rations containing no succinylsulfathiazole this compound was replaced by an equivalent amount of sucrose.

[4] Animals in these groups were given 2 drops per week of percomorph oil fortified with α-tocopherol (1 mg. per 25 mg. oil).

[5] Animals in this group were given a daily subcutaneous injection of 1 ml. of a solution containing (per ml.) thiamine hydrochloride, 10 μg.; pyridoxine hydrochloride, 10 μg.; riboflavin, 20 μg.; nicotinic acid, 200 μg.; calcium pantothenate, 220 μg.

[6] Animals in this group were given a daily subcutaneous injection of 1 ml. of a solution containing (per ml.) thiamine hydrochloride, 10 μg.; pyridoxine hydrochloride, 10 μg.; riboflavin, 20 μg.; nicotinic acid, 200 μg.; p-aminobenzoic acid, 200 μg.; inositol, 400 μg.

In general one microbiological assay for each factor was performed on all the tissues of a given experiment. While there may be slight differences between the results of consecutive assays, all values within a single experiment are strictly comparable.

Riboflavin was determined by the method of Snell and Strong ('39). The determinations of pantothenic acid were carried out by either the method of Pennington, Snell and Williams ('40) or that of Landy and Dicken ('42). The methods of the latter workers were employed in the determinations of folic acid, biotin, and nicotinic acid. Titration of the lactic acid produced after a growth period of approximately 72 hours was employed as a measure of the response of Lactobacillus casei to the factor in question. Samples of crystalline biotin from two sources served as reference standards for biotin determinations. A folic acid concentrate [4] served as the folic acid standard. This was assayed against a sample of liver extract (Wilson's fraction B) and the results have been calculated in terms of micrograms of "potency 40,000 units," as described by Cheldelin et al. ('42).

RESULTS

Wright et al. ('41) and Mitchell and Isbell ('42) have presented data on the B vitamin content of normal rat tissues. We have found that the inclusion of succinylsulfathiazole in highly purified rations does not cause a significant deviation from the normal values for the concentrations of either riboflavin or nicotinic acid in the liver. The level of riboflavin in the livers of several series of sulfonamide-fed rats averaged 25 μg. per gram (range 18–27 μg. per gram). Nicotinic acid levels in several groups of animals, likewise receiving succinylsulfathiazole in highly purified rations, averaged 155 μg. per gram (range 119–189 μg. per gram). Since these observations indicate that no gross change in the composition of the liver occurred during the period of sulfonamide administration, they support the hypothesis that the storage of specific

[4] Kindly furnished by Dr. E. L. R. Stokstad.

B-vitamins in the liver may be used as a criterion for the estimation of the status of an animal with reference to the nutritive essentials measured.

Liver from animals fed purified rations adequate for normal growth contained only a fraction of the folic acid found in the hepatic tissue of animals subsisting on the stock ration. The inclusion of succinylsulfathiazole in the same purified rations (which rendered them inadequate for normal growth) resulted in only a slight further diminution in the amount of folic acid found in the liver. Animals which were fed rations adequate except that pantothenic acid was omitted, had a slightly higher concentration of folic acid in the liver than did those which consumed entirely adequate synthetic rations (in which neither succinylsulfathiazole nor folic acid were present).

The biotin content of the liver from animals maintained on the stock ration was found to be about 1.5 μg. of biotin per gram of fresh tissue. When the animals were reared on synthetic rations without added biotin, but adequate in the other well-recognized constituents of the B complex, the biotin content of the liver was considerably depressed, although evidence of a biotin deficiency was either absent or appeared only in a mild form after the animals had subsisted on such rations for several months. The inclusion of 2% succinylsulfathiazole in the ration caused a further depression in the amount of biotin found in the liver and signs of biotin deficiency were frequently evident (Nielsen and Elvehjem, '41). Neumann, Krider and Day ('43) have also studied the biotin deficiency which develops in rats fed a purified diet containing succinylsulfathiazole and p-aminobenzoic acid. Our data suggest that for signs of biotin deficiency to make their appearance in the rat, the biotin content must be reduced to approximately 0.35 μg. per gram of fresh liver. Two micrograms of biotin per day, administered subcutaneously, were found sufficient to maintain a concentration of biotin, in the liver of rats fed 2% succinylsulfathiazole, equal to that of animals on the stock ration.

The level of pantothenic acid in the liver was uninfluenced by the addition of succinylsulfathiazole to the extent of 10% in the stock ration. However, when succinylsulfathiazole (2%) was added to highly purified rations containing hitherto adequate amounts of pantothenic acid, low concentrations of pantothenic acid in the liver were invariably found. In such cases the content of pantothenic acid in the liver was consistently found to be comparable to the low levels encountered in induced pantothenic acid deficiency, produced by a ration (S–8) containing neither pantothenic acid nor succinylsulfathiazole. Changes seen in pantothenic acid deficiency (Unna, '40; Unna and Richards, '42) such as marked achromotrichia and porphyrin-caked whiskers, were noted when rats were maintained on purified rations to which succinylsulfathiazole was added (2%). Similar observations with regard to achromotrichia have also been described by Martin ('42), who employed sulfaguanidine. Evidence for pantothenic acid deficiency in rats receiving purified rations, low in protein and containing sulfapyridine, has recently been presented by West, Jefferson and Rivera ('43). These workers found that the oral administration of 1 mg. of calcium pantothenate daily caused more or less complete abolition of the signs of pantothenic acid deficiency within a period of 2 to 4 weeks. In our experiments in which the B-vitamins were given subcutaneously (diets S-6, table 1), neither the subcutaneous administration of calcium pantothenate (0.22 mg. daily), nor a marked increase in the amount of the vitamin in the diet (11 mg. per 100 gm.) raised the depressed pantothenic acid content of the hepatic tissue of rats fed succinylsulfathiazole in purified diets (table 2).

Our analyses of the pantothenic acid content of the livers of rats maintained on rations adequate for normal growth, except with respect to pantothenic acid, indicate that evidence of pantothenic acid deficiency in the rat may be anticipated when the concentration in the liver falls below about 50 μg. per gram.

TABLE 2

Pantothenic acid, biotin and folic acid in the liver of rats on various diets.

EXPERIMENT NO.	SUCCINYLSULFATHIAZOLE	TYPE OF RATION	DURATION OF EXPERIMENT	PANTOTHENIC ACID	BIOTIN	FOLIC ACID
	%		*weeks*	*μg./gm.*	*μg./gm.*	*μg. "potency 40,000"/gm.*
1	..	S-5 Purified ration	4	145(110–201) (4)[1]	2.0(1.5–2.5) (4)[1]	1.0(0.6–1.7) (4)[1]
1	2	S-5 Purified ration	4	58(46–66) (4)	1.3(1.2–1.5) (4)	0.84(0.56–0.96) (4)
2	2	S-6 Purified ration; B-vitamins subcutaneously	6	41(39–53) (5)	0.89(0.72–1.1) (5)	.....
2	2	S-6 + 2 μg. biotin per day subcutaneously	6	.....	1.2(1.2–1.3) (3)	.....
2	2	S-6 + 16 μg. biotin per day subcutaneously	6	.....	1.6(1.4–1.8) (3)	.....
2	2	S-6A + 220 μg. Ca. pantothenate per day subcutaneously	6	45(42–50) (3)	.....	.....
2	2	S-6A + 11 mg. % Ca. pantothenate in ration	6	46(44–48) (3)	.....	.....
3	..	S-7 Purified ration	6	81(61–120) (5)	0.81(0.70–1.1) (5)	1.1(0.76–1.6) (5)
3	2	S-7 Purified ration	6	39(32–48) (5)	0.57(0.41–0.73) (5)	0.48(0.17–0.56) (5)
3	..	Stock ration	..	78(62–104) (5)	1.5(1.3–1.7) (5)	13(10–16) (5)
4	..	Stock ration	6	73(67–79) (4)	1.4(1.2–1.7) (5)	.....
4	10	Stock ration	6	78(73–88) (4)	1.3(1.0–1.7) (5)	.....
5	..	Stock ration	..	89(88–91) (4)	.....	20(14–26) (8)
6	..	S-7 Purified ration	8	96(93–99) (2)	0.41(0.40–0.42) (2)	2.6(2.6–2.6) (2)
6	2	S-7 Purified ration	8	44(35–49) (3)	0.29(0.20–0.40) (3)	1.6(1.5–1.6) (3)
6	..	S-8 Purified ration, no pantothenic acid	8	38(33–47) (3)	0.62(0.58–0.65) (3)	3.6(3.5–3.7) (3)
7	..	S-7 Purified ration	12	83(82–85) (2)	0.41(0.38–0.44) (2)	2.5(2.2–2.7) (2)
7	2	S-7 Purified ration	12	40(33–46) (3)	0.25(0.18–0.32) (3)	1.6(1.0–1.9) (3)
7	..	S-8 Purified ration, no pantothenic acid	12	46(44–49) (3)	0.79(0.75–0.84) (3)	4.6(2.4–7.0) (3)

[1] Accompanying the average values presented are (1) the range in values obtained, and (2) the number of animals studied.

Biotin extraction was done by acid hydrolysis; pantothenic acid and folic acid by enzymatic digestion, except in experiments 1, 2 and 4 where autolysis was employed. All data are based on the weight of fresh liver employed.

TABLE 3

Liver analyses and changes in weight of rats[1] on a purified diet containing succinylsulfathiazole (2%) given various supplements by stomach tube.

DAILY SUPPLEMENT	NO. OF ANIMALS	DURATION OF EXPERIMENT	CHANGES IN WEIGHT	PANTOTHENIC ACID CONTENT OF LIVER	BIOTIN CONTENT OF LIVER	FOLIC ACID CONTENT OF LIVER
		weeks	*gm.*	*μg./gm.*	*μg./gm.*	*μg. "potency 40,000 units"/gm.*
None	3	0	0	40(33–46)	0.25(0.10–0.32)	1.5(1.0–1.9)
None	3	3	− 8	48(47–49)	0.26(0.19–0.31)	1.2(0.72–1.6)
Crystalline biotin, 5 μg.	3	3	+ 16	57(55–61)	0.76(0.49–0.97)	1.2(0.96–1.5)
Crystalline biotin, 5 μg. and folic acid concentrate,[2] 20 mg.	3	3	+ 46	81(80–82)	0.89(0.81–0.94)	4.2(2.6–6.1)

[1] These animals had received for a period of 12 weeks the same purified diet (S-7) containing succinylsulfathiazole (2%).

[2] The concentrate of grass employed contained approximately 800,000 Snell-Peterson units of folic acid per gram. Biotin extraction was done by acid hydrolysis; pantothenic acid and folic acid were determined after takadiastase digestion of tissue samples.

Curative experiments demonstrate that concentrates of folic acid, administered with crystalline biotin, are effective in reestablishing excellent growth in rats which had previously reached a plateau in their growth curve, following the ingestion for several weeks of purified rations containing succinylsulfathiazole. The results obtained from one such experiment are presented in table 3. The folic acid concentrate employed was prepared from a grass juice powder [5] by a procedure similar to that described by Hutchings, Bohonos and Peterson ('41). Accompanying the resumption of growth and marked improvement in the general condition of the animals, which followed daily oral administration for 3 weeks of a concentrate of folic acid and crystalline biotin, there was a marked deposition in the liver of pantothenic acid, as well as of folic acid and biotin. The amount of folic acid and biotin found in the liver was intermediate between those amounts encountered in rats on synthetic rations adequate for growth, and those found in rats on stock rations. In addition, the simultaneous oral administration of folic acid concentrates and crystalline biotin to the deficient rats, increased the pantothenic acid content of the liver to the same level as that found in rats on the stock ration.

DISCUSSION

Data obtained from the assay of the hepatic tissue of rats receiving synthetic rations containing succinylsulfathiazole parallel the results of growth experiments, and the appearance of signs of deficiency, in that they show such animals to be deficient in both folic acid and biotin. In addition, a failure in the utilization of pantothenic acid has been demonstrated. Supplements of folic acid and biotin permit rats receiving succinylsulfathiazole to grow essentially normally for a period of several weeks (Nielsen and Elvehjem, '42; Martin, '42; Welch and Wright, '43) and folic acid concentrates, in the presence of crystalline biotin, not only cure the achromotrichia encountered in rats receiving sulfanilylguanidine (Martin,

[5] Supplied through the kindness of Dr. W. R. Graham of the Cerophyl Laboratories.

'42), but also cause a restoration of the utilization of pantothenic acid.

An explanation of this effect of folic acid concentrates and biotin is not yet possible. It is conceivable, however, that folic acid and biotin are involved in enzyme systems to which pantothenic acid is related. Pilgrim, Axelrod and Elvehjem ('42) found that liver slices from rats deficient in either pantothenic acid or biotin showed a decreased rate of pyruvate oxidation. It was concluded that the two factors are associated in metabolism.

Data presented in table 2 indicate that no impairment of pantothenic acid metabolism occurs when high levels of succinylsulfathiazole are included in a ration composed of natural crude materials. When a synthetic diet is fed, which contains succinylsulfathiazole, and an amount of pantothenic acid similar to that found in the stock ration, adequate hepatic storage of pantothenic acid fails, unless folic acid and biotin are given.

The question may be asked whether a certain type of bacterial activity in the intestine is essential for the proper utilization of "uncombined" pantothenic acid. Much information is available to indicate that pantothenic acid, as it occurs in animal and plant tissue (Cheldelin et al. '42), blood (Wright, '42), and yeast cells (Teague and Williams, '42), is largely in a combined or complex state. It is conceivable that folic acid and biotin are required, when succinylsulfathiazole is included in a highly purified ration, to maintain an intestinal flora which in some manner conjugates free pantothenic acid to an active complex or in some other fashion facilitates the utilization of this factor by the rat. It might be pointed out that the inclusion of zinc chloride in synthetic rations has been reported (Gross, Harvalik and Runne, '41) to produce signs in experimental animals closely similar to those encountered in pantothenic acid deficiency. Although the mechanism by which the effect is produced has not been explained, it is possible that the action of zinc chloride on the animal results from an influence on the intestinal flora. Such an effect might

be either on pantothenic acid or on the production of folic acid or biotin.

Molitor ('42) states that in the acute stage of induced pantothenic acid deficiency in the dog, the intravenous injection of pantothenic acid results in prompt recovery from the previously critical condition. Accordingly, on the basis of the evidence available at present, it is quite possible that folic acid and biotin produce their effects on pantothenic acid and other systems within the tissues of the animal and not through reactions occurring within the intestinal tract. A comparison of the minimal effective doses administered orally with those given parenterally may yield information bearing on the sites of action of these compounds.

However the effects of these factors are produced, it is clear that when folic acid concentrates and biotin are not administered to rats fed succinylsulfathiazole in highly purified rations, there develops decisive evidence of a failure properly to use pantothenic acid. In addition to a growth defect, black rats turn gray and present other characteristics of pantothenic acid deficiency not primarily because folic acid is a chromotrichial factor per se but because pantothenic acid metabolism is impaired when inadequate amounts of folic acid and biotin are available (Wright and Welch, '43a).

SUMMARY

A study of the storage of riboflavin, pantothenic acid, nicotinic acid, folic acid, and biotin in the liver was made in rats receiving various types of rations. A highly purified diet, adequate in those members of the vitamin B complex required for the production of excellent growth in rats, caused a marked reduction in the hepatic stores of folic acid and biotin compared with the amounts of these factors found in the liver of animals maintained in stock rations. The hepatic storage of these factors was further reduced by the incorporation of succinylsulfathiazole in such synthetic rations. The storage of riboflavin and nicotinic acid was not demonstrably influenced, a finding which indicates that no gross change in the liver

occurred during sulfonamide administration. Despite the presence in the diet of a previously adequate amount of pantothenic acid, the inclusion of succinylsulfathiazole caused a reduction in the pantothenic acid content of the liver to a level as low as that produced by a diet devoid of pantothenic acid. Increasing the dietary intake of pantothenic acid or giving the vitamin parenterally did not cause a renewal of growth, failed to modify the signs of pantothenic acid deficiency, and left unaffected the severely reduced amount of pantothenic acid in the liver. Administration of crystalline biotin and a concentrate of folic acid caused a prompt restoration of growth, recovery from the signs of pantothenic acid deficiency, and a restoration of the pantothenic acid content of the liver to normal. It is suggested that folic acid (or a constitutent of the folic acid concentrate employed) and biotin are essential for the maintenance of growth and of the general health of rats given purified rations containing succinylsulfathiazole. At least a portion of the effect of these factors is attributed to their playing an essential role in the utilization of pantothenic acid.

LITERATURE CITED

BLACK, S., R. S. OVERMAN, C. A. ELVEHJEM AND K. P. LINK 1942 The effect of sulfaguanidine on rat growth and plasma prothrombin. J. Biol. Chem., vol. 145, p. 137.

CHELDELIN, V. H., M. A. EPPRIGHT, E. E. SNELL AND B. M. GUIRARD 1942 Enzymatic liberation of B vitamins from plant and animal tissues. The University of Texas Publication, no. 4237, p. 15.

GROSS, P., Z. HARVALIK AND E. RUNNE 1941 Vitamin-deficiency syndrome in the albino rat precipitated by chronic zinc chloride poisoning. J. Invest. Dermat., vol. 4, p. 385.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

HUTCHINGS, B. L., N. BOHONOS AND W. H. PETERSON 1941 Growth factors for bacteria. XIII. Purification and properties of an eluate factor required by certain lactic acid bacteria. J. Biol. Chem., vol. 141, p. 521.

LANDY, M., AND D. M. DICKEN 1942 A microbiological assay method for six B vitamins using Lactobacillus casei and a medium of essentially known composition. J. Lab. and Clin. Med., vol. 27, p. 1086.

MARTIN, G. J. 1942 "Folic acid" in nutritional achromotrichia. Proc. Soc. Exp. Biol. and Med., vol. 51, p. 353.

MITCHELL, H. K., AND E. R. ISBELL 1942 B vitamin content of normal rat tissues. The University of Texas Publication, no. 4237, p. 37.

MOLITOR, H. 1942 Vitamins as pharmacological agents. Federation Proceedings, vol. 1, p. 309.

NEUMANN, F. W., M. M. KRIDER AND H. G. DAY 1943 Biotin deficiency in rats fed purified diets containing succinylsulfathiazole and p-aminobenzoic acid. Proc. Soc. Exp. Biol. and Med., vol. 52, p. 257.

NIELSEN, E., AND C. A. ELVEHJEM 1941 Cure of spectacle eye condition in rats with biotin concentrates. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 349.

———— 1942 The growth-promoting effect of folic acid and biotin in rats fed succinylsulfathiazole. J. Biol. Chem., vol. 145, p. 713.

OSBORNE, T. B., AND L. B. MENDEL 1913 The relation of growth to the chemical constituents of the diet. J. Biol. Chem., vol. 15, p. 311.

PENNINGTON, D., E. E. SNELL AND R. J. WILLIAMS 1940 An assay method for pantothenic acid. J. Biol. Chem., vol. 135, p. 213.

PILGRIM, F. J., A. E. AXELROD AND C. A. ELVEHJEM 1942 Metabolism of pyruvate by liver from pantothenic acid- and biotin-deficient rats. J. Biol. Chem., vol. 145, p. 237.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed., vol. 11, p. 346.

STOKSTAD, E. L. R. 1943 Some properties of a growth factor for lactobacillus casei. J. Biol. Chem., vol. 149, p. 573.

TEAGUE, P. C., AND R. J. WILLIAMS 1942 Pantothenic acid and the utilization of glucose by living and cell-free systems. J. Gen. Physiol., vol. 25, p. 777.

UNNA, K. 1940 Pantothenic requirement of the rat. J. Nutrition, vol. 20, p. 565.

UNNA, K., AND G. V. RICHARDS 1942 Relationship between pantothenic acid requirement and age in the rat. J. Nutrition, vol. 23, p. 545.

WELCH, A. D., P. A. MATTIS AND A. R. LATVEN 1942 A toxicological study of succinylsulfathiazole. J. Pharmacol. and Exper. Therap., vol. 75, p. 231.

WELCH, A. D., AND L. D. WRIGHT 1943 The role of "folic acid" and biotin in the nutrition of the rat. J. Nutrition, vol. 25, p. 555.

WEST, H. D., N. C. JEFFERSON AND R. E. RIVERA 1943 The effect of aromatic hydrocarbons on growth. The reversible inhibition of pantothenic acid by sulfapyridine. J. Nutrition, vol. 25, p. 471.

WRIGHT, L. D. 1943 The state of pantothenic acid in blood. J. Biol. Chem., vol. 147, p. 261.

WRIGHT, L. D., J. R. MCMAHAN, V. H. CHELDELIN, A. TAYLOR, E. E. SNELL AND R. J. WILLIAMS 1941 The "B" vitamins in normal tissues (autolysates). The University of Texas Publication, no. 4137, p. 38.

WRIGHT, L. D., AND A. D. WELCH 1943a The role of "folic acid" and biotin in the utilization of pantothenic acid by the rat. Science, vol. 97, p. 426.

———— 1943b The metabolism of folic acid. Am. J. Med. Sci., vol. 206, p. 128.

———— 1943c The production of folic acid by rat liver in vitro. Science, vol. 98, p. 179.