Monograph on **Biotin** #117 6/18/74

BIOTIN #117

MONOGRAPH

ON

BIOTIN

M O N O G R A P H

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B I O T I N

TR-72-1552-47

Submitted Under:
Contract No. FDA 72-104

June 18, 1974

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Rockville, Maryland 20852

BIOTIN

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BIOTIN

Summary

Since the demonstration early in 1940 of the vitamin role of biotin in animal nutrition, biotin has been the subject of extensive research. Its function as a vitamin has been repeatedly expounded and its specific metabolic activities determined by innumerable studies. In short, biotin has been found to be essential to the metabolism of carbohydrates, lipids, proteins, and nucleic acids. The more recent use of biotin as a vitamin supplement in foods has prompted some research into its possible pharmacological effects, and the present work.

The metabolism of biotin has been studied primarily through i.p. injections of ^{14}C-labelled biotin into rats. Almost all of the biotin (85-99%) is excreted in the urine within 24 hours, about one half as free biotin (797, 796, 444, 303). Fractionation of the urine has revealed four metabolites - d- and l-sulfoxides of biotin, bisnorbiotin and a neutral ketone (797). No significant amounts of the injected biotin have been recovered from the feces or expired CO_2 of rats (797, 796, 444).

However, it has been reported that, due to microbial synthesis of biotin in the intestines, fecal excretion of biotin by man often exceeds intake, and is relatively unaffected by the level of intake. Nevertheless, urinary biotin excretion by man is reported to be significantly influenced by the level of intake, with biotin supplements leading to increased urinary excretion (331, 330, 465, 463, 1022).

Tissue analysis performed on rats and chicks after i.p. injections of biotin have shown that biotin is temporarily stored, to only a small extent, in the liver, kidneys, and spleen (444, 303). Tuszynska (1403), however, found no storage of biotin in the body of rats when the biotin was administered per os.

In studying the pharmacology of biotin, Crittenden (285) found that: relatively high concentrations of biotin do not alter the contractions of the isolated uterus or intestine of the normal rat; in the anesthetized normal rat large i.v. doses of biotin produce no effect on blood pressure, respiration, or intestinal circulation; the secretion of free HCl by the gastric glands appears to be depressed by d-biotin; a large single oral dose of d-biotin to normal rats produced no deviation from the normal renal function or metabolic rate; repeated large doses of biotin per os have no effect on hepatic function, renal function, blood fibrinogen, rectal temperature, sensitivity of nerve endings, skin regeneration or healing of scalded skin in the normal rat.

Schmidt and Landy (1209) reported that biotin failed to produce any changes in the following physiological functions: blood pressure, heart rate and respiration of anesthetized cats followed rapid i.v.

injection of 0.25 mg/kg; excised guinea pig and rabbit uterus and rabbit intestine suspended in concentrations up to 1:40,000; and the frog heart perfused in situ with 0.2 mg of biotin.

Biotin (0.5-2 mg) was observed by Petrelli and Farabollini (1067) to have a hyperglycemic effect when injected into rats endoperitoneally. On the basis of a study in which 55 patients were given 10 mg biotin in injection form or 100 mg in infusion form, Steiger-Wald and Boehle (1299) concluded that biotin stimulates the production of oxalacetic acid from pyruvic or lactic acid, and that it increases the oxidation of fat. Tissue respiration in rats was observed by Ivanov (627) to be significantly increased (2-7 times) by s.c. administration of biotin (0.3 mcg/100 g/day for 20 days). Wilson and Leduc (1492) observed a slight, and temporary, stimulation of mitotic activity in the livers of normal mice following s.c. injection of 2 mcg biotin/day for 3 days. Rajalakshmi (1127) found that in rats biotin (0.1 mg/day for 8 weeks, i.p.) stimulates the synthesis of cholesterol from acetate, resulting in an increase in serum, carcass, and liver cholesterol. However, the oral administration of 1 mg biotin/kg body weight/day for 9 days to normal rats has been reported to cause no significant changes in serum free fatty acids, cholesterol or triglycerides (954).

The interaction of raw egg white and biotin is a well known and extensively studied phenomenon. When raw egg white is fed to animals one of its protein components, avidin, complexes with, and thereby inactivates, biotin. When large quantities are administered this can result in characteristic biotin deficiency (576, 1226, 1487).

Sulfa drugs and antibiotics have been shown to produce effects in rats similar to those of egg white injury. Since much of the biotin utilized by the rat is obtained through microbial synthesis in the intestine, the effects of these drugs have been attributed to their bactericidal action and resultant inhibition of biotin synthesis (292, 389, 550, 963, 972, 1461).

Orlandi and Rabaglia (1024) reported that the simultaneous oral or i.v. administration of sodium salicylate (1 g) and biotin (0.2 g) to children enhanced the action of the salicylate and slowed its elimination.

A number of studies on the toxicology of biotin have been reported. The effect of biotin on reproduction of the rat and of the turkey was studied by Paul et al. (1058) and Waibel et al. (1434), respectively. Adult female Holtzman rats were treated, s.c., by Paul et al. (1058) with 100 mg biotin/kg body weight at the diestrus stage of the cycle. These rats showed complete irregularity of the estrus cycle, with a progressive increase of the vaginal leucocyte numbers up to the 14th day and a sharp decline thereafter. The biotin treatment enhanced the process of the formation of corpora leutea, but a large number of the corpora lutea and ovarian stroma showed atrophic changes. The concentration and total liver glycogen of the rats was reduced significantly 14 days after biotin treatment, but returned to the control level after 21 days. The concentration and total uterine glycogen showed a slight reduction only 21 days after biotin treatment.

Waibel et al. (1434) found a dietary supplement of 300 mcg biotin/

kg given to 144 breeder hens for 16 weeks caused no effects on egg production, fertility, hatchability of fertile eggs, body weight, or feed efficiency. However, when progeny of these hens were given a dietary supplement of 100 mg biotin/kg they exhibited reduced weight gain and an increased incidence of hock disorders starting at 1 week of age and continuing for about 10 weeks before recovery.

Crittenden (285) reported that biotin produced no signs of local irritation upon application to the cornea of rabbits (0.1% d-biotin, pH 7.3), intradermal injection into the abdominal skin of guinea pigs (0.1 ml), or i.m. injection into rabbits (0.5 or 1.0 ml of 0.1% d-biotin).

Keller (683) reported the case of a female lab worker who, after occupational exposure, developed hypersensitivity to topically applied biotin, manifested in red, itching patches on her arms and hands.

Biotin has been employed in the treatment of a number of diseases, including atherosclerosis, hypertensive illness, Leiners disease and various forms of dermatitis. Several cases of such treatment are included in this review to show the dosage of biotin administered and its pharmacological effects or lack of effect. The only contraindication reported in these studies was the development of temporary (4-5 days) diarrhea observed by Gompertz (488) in a 5-month-old infant receiving 10 mg of biotin/day. The dosage and duration of treatment reported in the other studies, in which no adverse effects were reported, are presented in the following table. The highest dose reported was 120 mg of biotin/day, which was tolerated for 40 days without the production of any harmful side effects (1459).

No. of Subjects	Dose	Duration	Route	Ref.
116	1-5 mg/day	7-14 days	Internal	166
152	1-5 mg/day	7-14 days	Internal	164
1	10 mg/day	5 days	Oral	83
11	up to 40 mg	2 months	Injected	986
28	0.1 or 20 mg/day	3-6 weeks	i.m. or p.o.	1333
8	250 mcg/day	4-8 weeks	Oral	1023
-	0.50-0.75 mg/day	3 months	Oral	234
403	5-20 mg/day	1-3 weeks	i.m. or p.o.	954
28	10 mg/day	28 days	-	1060
5	120 mg/day	40 days	Oral	1459
30	5 mg/day	6-10 days	i.m.	467

Short-term feeding studies on biotin have been conducted on mice, gerbils, rats and dogs. Crittenden (285) reported no signs of toxicity in groups of young, growing mice given daily oral doses of 1 mg of d- or dl-biotin for 60 days. Mongolian gerbils given, s.c. 1 mg biotin/kg/day for 3-6 weeks exhibited a significant body weight loss, but no other untoward effects (954). Five male rats which received 50 mg of d-biotin daily for 10 days were reported by Crittenden (285) to remain normal in all respects. However, in another study by Crittenden (285), groups of 5 male rats given, for 120 days, daily doses of 5 mg d- or dl-biotin per os showed reduced weight gains of 35 and 48 g, respectively, compared to untreated controls. Finally, no signs of toxicity were manifested by 4 dogs treated i.v. with 10 mg of d-biotin/day for 10 days (285).

BIOTIN

Chemical Information

I. Nomenclature

A. Common Names

1. Biotin
2. Vitamin H
3. Coenzyme R

B. Chemical Names

1. cis-Tetrahydro-2-oxothieno(3,4-d)imidazoline-4-valeric acid

2. cis-Hexahydro-2-oxo-1H-thieno(3,4)imidazole-4-valeric acid

C. Trade Names

1. Bios II
2. Bioepiderm

D. Chemical Abstracts Registry Number

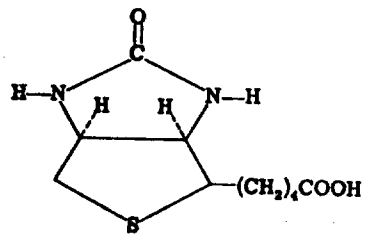
000058-85-5

II. Empirical Formula

$C_{10}H_{16}N_2O_3S$

III. Structural Formula

The biotin molecule contains three asymmetric carbon atoms and thus there are four diastereoisomers - dl-biotin, dl-epibiotin, dl-allobiotin, and dl-epiallobiotin. Natural biotin, however, exists only in the form of d-biotin as shown below.



IV. Molecular Weight

244.31

V. Specifications

Food Chemicals Codex

Assay	Not less than 97.5% of $C_{10}H_{16}N_2O_3S$
Melting range	Between 229 degrees and 232 degrees
Specific rotation	$[\alpha]_D^{25°}$. Between +89 degrees and +93 degrees
Limits of Impurities	
Arsenic (as As)	Not more than 3 ppm
Heavy metals (as Pb)	Not more than 10 ppm

VI. Description

A. General Characteristics

Purified biotin appears as a practically white, crystalline powder, in the form of fine, long needles.

B. Physical Properties

The following properties are those of biotin isolated from liver or milk (Kogl's beta-biotin): Specific optical rotation at 25 degrees C for D line is +91 degrees (C-1 in 0.1 N NaOH). Isoelectric point at pH 3.5. pH of 0.01% aqueous solution 4.5. Solubility in water about 22 mg/100 ml at 25 degrees, more soluble in hot water and in dilute alkali. Solubility in 95% alcohol about 80 mg/100 ml at 25 degrees. Insoluble in other common organic solvents.

C. Stability

Pure biotin is stable to air and heat. Moderately acid and neutral solutions are stable several months; alkaline solutions are less stable, but appear reasonably stable up to a pH of about 9. Aqueous solutions are very susceptible to mold growth. Acidic solutions can be heat sterilized. Biotin is incompatible with nitrous acid, oxidizing agents, formaldehyde, chloramine T, strong acid or alkali.

VII. Analytical Methods

Although biotin is present in all living cells, it occurs in such trace amounts as to exclude the use of direct chemical and physical methods for assay purposes. Consequently, the most commonly used assay methods are based on microbiological techniques. These methods have the advantage of being technically simple and short-term procedures; however, they have certain limitations. While biotin is present in biological material chiefly in conjugated or bound form, most of the microorganisms used for the assay of biotin will respond only to free biotin. Thus concentrations must be prepared by enzymatic or acid

hydrolysis in order to have all of the biotin in free form and in aqueous solution. Another complication is the possible presence of biotin analogs, such as desthiobiotin, oxybiotin, etc., or decomposition products that may have in varying degree stimulating and in some cases inhibitory activity for the test microorganisms. A major problem for microbiological assays of biotin is presented by the interference of unspecific stimulating compounds, such as oleic and related fatty acids, pimelic acid, and aspartic acid. There are also inhibitory unspecific compounds such as lysolecithin (1226).

The most widely used method of microbiological assay of biotin is that of Wright and Skeggs, which utilizes Lactobacillus plantarum as the test organism. The growth of L. plantarum increases over the range 0-0.2 ng/ml of medium. Standards and unknown are added to tubes containing 5 ml double-strength medium; each tube is diluted to 10 ml, capped, autoclaved, cooled, and inoculated. The response measured is either titration of the acid produced after 72-hour incubation at 30-37 degrees or turbidity after 24 hours(1515).

The concentration of biotin may also be determined through bioassay using higher animals. Rats or chicks which have been made biotin-deficient by the use of special diets are generally used. These tests have the advantage of measuring both bound and free biotin without pre-treatment; however, they require more equipment and time than micro-biological assays (1226).

VIII. Occurrence

Biotin is present in very minute amounts in every living cell, where it occurs mainly bound to proteins or polypeptides. The richest sources are liver (less than 0.0001%), kidney, pancreas, yeast, and milk. (For more information see Biochemical Aspects VI.).

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage mg/kg Body Weight	Measurement	Ref.
Biotin	Mice	-	p.o.	>10,000	LD_{50}	954

Crittenden reported that mice weighing an average of 20 g tolerated single i.v. injections of 1 g of dl-biotin/kg body weight without signs of toxic effects (285).

II. Short-Term Studies

Mice

In a study by Crittenden, two groups of mice of approximately 7 and 14 weeks of age were treated for 60 days as follows: 15 of the younger group and 10 of the older group were given daily oral doses of 1 mg of dl-biotin in 0.2 ml saline, an equal number of 1 mg of d-biotin and 10 of each group 0.2 ml of physiological saline only. No toxic signs attributable to biotin were observed and there were no significant differences in the death rates of the groups (285).

Gerbils

Doses of 1 mg biotin/kg/day were given orally or s.c. to Mongolian gerbils fed a normal or a hypercholesterolemic diet. After 3 or 6 weeks treatment the animals were sacrificed and body weight, organ weight, serum lipids, adrenal and liver cholesterol and fecal lipid excretion were determined. No consistently significant changes were found in serum FFA, triglycerides or cholesterol in normal or hypercholesterolemic animals and little change was observed in adrenal or liver cholesterol concentration. As shown in Tables 1 and 2, biotin treatment resulted in little change in body, adrenal or liver weight in the hypercholesterolemic groups. However, significant body weight loss was observed in the normal diet groups. No significant changes were observed in fecal lipid excretion (954).

Rats

The toxicity of biotin was tested by Crittenden through the administration, by stomach tube, of 50 mg of d-biotin daily for 10 days to 5 male rats weighing an average of 141 g. No toxic signs were observed and there were no changes in the number of erythrocytes, leucocytes, or amount of hemoglobin. Autopsy of the rats revealed no gross changes (285).

Table 1. Effect of Biotin[1] on Body and Organ Weight in Mongoligan Gerbils

Treatment	NORMAL DIET			HYPERCHOLESTEROLEMIC DIET[2]		
	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.
Control	65.0 ± 2.3	59.5 ± 2.9	2.95 ± 0.07	71.6 ± 3.7	64.3 ± 3.4	5.58 ± 0.17
Biotin 1 mg/kg p.o.	58.4 ± 2.3	58.8 ± 2.5	3.1 ± 0.09	69.6 ± 3.1	66.9 ± 3.4	5.59 ± 0.06
Biotin 1 mg/kg s.c.	55.0 ± 4.2	70.7 ± 6.5	3.2 ± 0.06*	66.0 ± 3.5	68.9 ± 4.9	5.11 ± 0.21

[1] Daily treatment for 3 weeks.

[2] Diet consists of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic acid.

Significant change from control * $p < .05$.

Table 2. Effect of Biotin[1] on Body and Organ Weight in Mongolian Gerbils

Treatment	NORMAL DIET			HYPERCHOLESTEROLEMIC DIET[2]		
	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.
Control	70.0 ± 8.9	57.2 ± 2.3	3.19 ± 0.07	62.5 ± 1.7	74.5 ± 4.2	5.82 ± 0.2
Biotin 1 mg/kg p.o.	64.0 ± 1.8*	57.3 ± 3.4	3.34 ± 0.03	61.2 ± 2.9	76.0 ± 1.5	5.87 ± 0.4
Biotin 1 mg/kg s.c.	56.8 ± 3.5*	59.7 ± 2.8	3.69 ± 0.24	60.0 ± 2.7	76.6 ± 3.5	5.65 ± 0.2

[1] Daily treatment for 6 weeks.

[2] Diet consists of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic acid.

Significant change from control * $p < .05$.

Crittenden conducted a study in which two groups of 5 male rats each were given per os daily for 120 days 5 mg of dl- or d-biotin, while a group of 5 control rats were given physiological saline (1 ml). At the end of the test period the average body weight of the control group was 48 g greater than that of the group receiving dl-biotin, and 35 g greater than that of the group receiving d-biotin. In general, the animals appeared normal throughout the experiment; there were no significant changes in the number of blood cells or amount of hemaglobin and the hepatic and renal functions were normal (285).

Dogs

Four dogs weighing from 6-10.8 kg were injected intravenously by Crittenden for 10 successive days with 10 mg of d-biotin. No changes were observed in any of the following: general behaviour; body weight; rectal temperature; condition of the fur, skin, buccal membranes, tongue or teeth; corneal reflex; scratch reflex; pupillary light reflex; heart rate; and respiratory rate. The urine showed no deviation from the normal with regard to volume, pH, specific gravity, sediment (microscopic examination), and presence of albumen or blood. There were no changes in the number of erythrocytes, leucocytes, or amount of hemoglobin. No signs of thrombosis in the injected vein were observed. No gross tissue changes were noted in the one animal sacrificed (285).

III. Long Term Studies

None

IV. Special Studies

Reproduction

The effects of an acute dose of biotin on the reproductive organs of the female rat were studied by Paul et al. Eighteen adult female rats of the Holtzman strain (approximately 3 months old), exhibiting normal estrus cycles, were given biotin at a dose of 5 mg/100 g body weight dissolved in 0.2 ml of 0.1 N NaOH subcutaneously in two injections, morning and evening, at the diestrus stage of the cycle. Groups of 6 rats each were sacrificed 7, 14 and 21 days after the biotin injections. As controls, a group of 6 rats was given an equal volume of 0.1 N NaOH at the diestrus stage and was sacrificed 7 days after the injections. During the test period, the estrus cycle of all the animals was studied daily through vaginal smears. Upon sacrifice the liver and uterine glycogen was estimated and the ovaries were subjected to histological examination. The control animals appeared normal in all respects. Indications of a sterilizing effect of biotin were observed in the test rats - the biotin-treated rats showed complete irregularity of the cycle and the vaginal leucocyte numbers progressively increased up to the 14th day and sharply declined thereafter. Biotin treatment did not significantly affect the weights of the ovary, uterus, liver and normal body weight gain. The histological examination of the ovarian sections indicated that biotin

treatment enhanced the process of the formation of corpora lutea, but a large number of the corpora lutea and stroma showed atrophic changes. The concentration and total liver glycogen was reduced significantly 14 days after biotin treatment, but returned to the control level after 21 days (Table 1). The concentration and total uterine glycogen showed a slight reduction only 21 days after biotin treatment (Table 3). It was suggested that biotin may exert its effect on hepatic and uterine glycogen through an altered ratio of progesterone and estrogen release from the ovary (1058).

TABLE 3

The effect of biotin on the liver and uterine glycogen

Treatment	Liver glycogen		Uterine glycogen	
	Concentration mg/100 mg tissue	Total mg/liver	Concentration mg/100 mg tissue	Total mg/uterus
NaOH-treated control for 7 days	1·334 ±0·093	109·5 ± 1·12	0·136 ±0·112	0·469 ±0·028
7 days after biotin injections	0·567 ±0·058	43·4 ± 0·81	0·124 ±0·009	0·633 ±0·029
14 "	0·369 ±0·064	23·1 ± 0·58	0·144 ±0·019	0·465 ±0·025
21 "	1·232 ±0·095	103·8 ±1·52	0·083 ±0·010	0·305 ±0·036

There were six rats in each group.

Waibel et al. conducted a study of the effects of supplementary biotin on the performance of turkeys fed corn-soybean meal type diets. Three strains of Broad White turkeys, each consisting of low and high blood pressure lines were used. A total of 288 breeder hens were divided into two treatment groups of 12 pens each. For a period of 16 weeks, one group was fed the breeder diet (144 mcg biotin/kg) shown in Table 4 and the other group was fed the same diet supplemented with 300 mcg biotin/kg (analysis indicated a total of 413 mcg/kg). Records of egg production, fertility, hatchability of fertile eggs, body weight and feed efficiency revealed no significant differences between the biotin-supplemented and unsupplemented groups (1434).

In a further study, progeny of the last three hatches, all from eggs laid after the hens were in production for 10 weeks, were divided equally according to variables and fed starting and growing diets with or without a 100 mcg/kg supplement of biotin (analysis indicated 292 and 420 mcg/kg, respectively, for the unsupplemented and supplemented starting diets). In addition to recording body weights at 1, 6, 11, and 18 weeks of age, the turkeys were scored at the first three ages for foot dermatitis, leg weakness, and feathering (see Table 5). Birds receiving the biotin supplement showed no weight difference at 1 week of age. However, at 3, 6, and 11 weeks of age a significant reduction in body weight was associated with added biotin. Poults receiving added biotin showed a greater incidence of hock disorder at 3 and 6 weeks of age; however, by 11 weeks of age a very excellent recovery had been made so that all treatments were similar. Males which received added biotin showed a greater number of toe callouses at 3 weeks of age while females fed added

biotin had a lesser incidence at all ages. The effects of added dietary biotin on condition of foot pads were inconsistent: biotin added to the diet resulted in somewhat roughened pads in males at 3 and 11 weeks of age. Regarding condition of feathers, all differences were of a small magnitude (1434).

TABLE 4—*Composition of breeder diet in experiment 1 and poult diet in experiment 2*[1]

Ingredient	Breeding Exp. 1	Starting Exp. 2
	%	%
Corn, ground yellow	76.84	60.28
Soybean meal, solvent (44% protein)	16	—
Soybean meal, dehulled (48½% protein)	—	30
Fish meal, herring (70% protein)	—	5
DL-methionine (98%)	—	0.08
L-lysine (50%)	—	0.14
Dicalcium phosphate	2.25	2.4
Calcium carbonate	4.25	1.5
Salt	0.5	0.3
Trace mineral mixture	0.1[2]	0.03[3]
Vitamin mixture	0.033[4]	.27[5]
Choline chloride (70% solution)	0.032	—

[1] Starting and growing diets in experiment 1 were reported by Krista *et al.* (1969) as diet series 3.

[2] Trace mineral mixture ("Z-2 Delamix"®): 6% manganese, 0.12% iodine, 2% iron, 0.2% copper, 2% zinc, 0.2% cobalt, 25% calcium. Limestone Products Corporation, Newton, N. J.

[3] Trace mineral premix (mg. per kg. of diet): $MnSO_4$ (27% Mn) 222, ZnO (72% Zn) 78, ethylene diamine dihydroiodide (80.4% I) 0.625.

[4] Vitamin mixture provides the following in mg. per kg. of diet: vitamin A (250,000 I.U./g.) 32, vitamin AD_3 (250,000 I.U. each of vitamins A and D per g.) 8, vitamin E acetate (275 I.U./g.) 72, vitamin K (35 mg. MSBC/g.) 86, riboflavin 8, *d*-calcium pantothenate 20, niacin 30, vitamin B_{12} (0.1% trituration on mannitol) 20, thiamine·HCl 4, pyridoxine·HCl 4, folic acid 2, and sucrose 46.

[5] Vitamin mixture provides the following in mg. per kg. of diet: vitamin A (30,000 I.U./g.) 258.5, vitamin D_3 (30,000 I.U./g.) 68, vitamin E acetate (275 I.U./g.) 78.5, vitamin K (35 mg. MSBC/g.) 71.1, riboflavin (35 mg./g.) 173, *d*-calcium pantothenate (70 mg./g.) 147.8, niacin (50%) 150, and choline chloride (50%) 1750.

TABLE 5 *Influence of added biotin[1] in breeder and growing diets on performance during growing period*

Measurement (weeks)	Added biotin	Males[2]				Females[3]			
	Breeder:	−	−	+	+	−	−	+	+
	Growing:	−	+	−	+	−	+	−	+
Body weight (kg.)									
1		0.122	0.120	0.121	0.121	0.116	0.113	0.113	0.115
3		0.470	0.386	0.471	0.391	0.493	0.364	0.410	0.367
6		1.47	1.36	1.49	1.36	1.22	1.14	1.23	1.16
11		3.97	3.80	4.00	3.78	3.10	3.02	3.13	3.04
18		7.36	6.98	7.29	6.95	5.42	5.28	5.46	5.28
Condition of hocks, score[4]									
3		1.74	2.82	1.74	2.57	1.28	2.29	1.48	2.23
6		2.02	2.47	2.02	2.40	1.19	1.44	1.27	1.51
11		2.23	2.17	2.20	2.33	1.04	1.04	1.08	1.09
Condition of toes, score[5]									
3		1.52	2.13	1.64	2.34	2.13	1.77	1.93	1.64
6		1.25	1.42	1.34	1.39	1.70	1.37	1.52	1.28
11		1.20	1.42	1.46	1.43	1.42	1.24	1.28	1.18
Condition of pads, score[6]									
3		1.30	1.59	1.19	1.63	1.48	1.44	1.32	1.39
6		1.88	1.86	1.72	1.90	2.44	2.26	2.27	2.12
11		2.06	2.20	1.95	2.15	2.49	2.55	2.21	2.39
Condition of feathers, score[7]									
3		1.57	1.55	1.64	1.63	1.58	1.62	1.56	1.63
6		1.43	1.57	1.42	1.54	1.35	1.45	1.52	1.47
11		1.80	1.99	1.83	1.94	1.56	1.65	1.61	1.70

[1] Levels of added biotin in breeding and starting diets were 300 and 100 mcg./kg. respectively.
[2] The number of males at 1, 3, 6, 11 and 18 weeks was 695, 695, 686, 649 and 605, respectively.
[3] The number of females at 1, 3, 6, 11 and 18 weeks was 679, 679, 674, 656 and 637, respectively.
[4] Condition of hocks scored as follows: 1, normal; 2, questionable enlargement; 3, definite enlargement; 4, enlarged and flattened or twisted; 5, slipped tendon. The more severely affected leg served as basis for score.
[5] Toes were scored as follows: number of phalanges with fissures were added together with an added point if blood was present.
[6] Foot pads scores: 1, normal; 2, rough; 3, calloused; 4, calloused and bloody.
[7] Scoring of flight feathers: 1, normal; 2, barbs missing and/or shaft broken; 3, feathers missing; 4, combination of 2 and 3.

Several studies on insects have been reported in which an excess intake of biotin (60 ppm-2.0% of the diet) has been found to cause severe degenerative effects on the ovary, resulting in sterility (107, 106, 206, 1084).

Local Irritation

Crittenden reported that biotin produced no signs of local irritation upon application to the cornea of rabbits (0.1% d-biotin, pH 7.3), intradermal injection into the abdominal skin of guinea pigs (0.1 ml), or intramuscular injection into rabbits (0.5 or 1.0 ml-0.1% d-biotin) (285).

Hypersensitivity

The case of a female lab worker who developed hypersensitivity to biotin was outlined in a report by Keller. Whenever mixing a particular dry, dusty diet containing butter yellow and known members of the vitamin B complex, this individual developed red, itching patches on her arms and hands. There were slightly raised areas in the center of the red patches which lasted about half an hour. A series of skin patch tests were employed to ascertain the cause of this reaction, and, as suspected, biotin was implicated. It was noted that this individual did not get a

reaction to biotin unless the skin had previously been cleansed with 70 per cent alcohol or unless nicotinic acid was present in the mixture; nor was itching noted at the site of reaction when biotin alone was applied to the cleansed skin surface. However, in conjunction with nicotinic acid, itching was present. It was further observed that biotin, in conjunction with nicotinic acid, applied to the uncleansed skin produced a reaction of the wheal and erythema type accompanied by itching. This, however, was not observed if the individual previously had applied a commercial cream to the exposed area (683).

Therapeutic Application

The treatment of a 5-month-old infant who presented beta-hydroxyisovalericaciduria with metabolic acidosis and ketosis associated with beta-methylcrotonylglycinuria was reported by Gompertz et al. The child was treated, orally, with 10 mg per day for about 3 weeks. He had tended to be constipated, but after biotin administration he developed diarrhea which treatened to be troublesome before it settled after 4 or 5 days. The child's clinical condition improved immediately after biotin treatment. No adverse effects, other than diarrhea, were reported (488).

Using both normal persons and patients with atherosclerosis and hypertonic illness, Braginskii et al. examined the effect of biotin on the content in blood serum of cholesterol and lecithin, the ratio of lipoprotein fractions, overall content of albumin and its fractions, activity of blood coagulation - anticoagulation systems (recalcification time of oxalate plasma, prothrombin index, proconvertin index, plasma tolerance to heparin, fibrinogen content, fibrinolytic activity, free heparin), as well as thromboelastrogram indices. Studies were carried out before and after administration of biotin, which was given in doses of 1, 3, and 5 mg every 24 hours, internally, over 7 and 14 days. In patients, but not normals, there was a significant decrease in serum beta-lipoproteins following biotin administration and in cases of initial hyperfibrinogenemia (over 400 mg%), biotin administration (1 or 3 mg, but not 5 mg) caused a decrease in serum fibrinogen content. No adverse effects of biotin administration were observed (166).

In studying the effect of different doses of biotin on the ascorbic acid allowance of man, Braginskii and Kalkun treated 12 normals and 140 patients suffering from atherosclerosis and hypertensive disease with 1, 3 and 5 mg doses of biotin daily for periods of 7 and 14 days. Doses of 1 mg/day, but not 3 or 5 mg/day, were found to reduce urinary ascorbic acid excretion and decrease its level in the blood, indicating increased ascorbic acid utilization. No contraindications of biotin administration were reported (164).

In the treatment of a 2-year-old child suffering with propionicacidemia and secondary ketotic hyperglycinemia, Barnes et al. administered orally, 5 mg biotin twice daily for 5 days without the appearance of any adverse effects (83).

Nisenson successfully treated nine infants with seborrheic dermatitis and two with Leiner's disease with biotin. The total dosage per infant

ranged from 3 mg within a 2-week period to 10 mg injected within a 4-day period. One 4-month-old infant was given a total of 40 mg injected within an approximate 2-month period - 20 mg of which were given within 4 days. No adverse effects from the biotin treatment were reported (986).

Biotin therapy (0.1 mg/day i.m., or 20 mg p.o.) for 3 to 5 weeks was reported by Svejcar and Homolka to result in significant improvement in 28 infants ill with Leiner's disease or other kinds of dermatitis. No adverse effects from the biotin treatment were reported (1333).

In the treatment of 8 patients suffering from seborrhea or other forms of dermatitis, Oppel administered oral doses of 250 mcg of biotin daily for 4-8 weeks. In a few instances larger doses were administered. No consistent, beneficial effects nor any harmful effects of biotin administration were observed (1023).

In a report of case studies, Chavarria et al. mentioned no adverse effects resultant from the therapeutic, oral administration of 0.25 mg of biotin to alopecic children 2 or 3 times per day for 3 months (234).

Daily doses (in combination treatment every 2 days) of 1-2 ampuls containing 5 mg biotin administered i.m. or 1-4 tablets containing 5 mg biotin administered p.o., or occasionally higher doses, were utilized in the treatment of 211 erythrodermia cases and 192 cases of seborrhoid dermatitis. Treatment generally lasted for 1-3 weeks. Biotin was always tolerated very well both in oral and intramuscular application (daily doses of up to 100 mg). Even on prolonged administration neither subjective nor objective side effects were observed (954).

In the treatment of diffuse alopecia, Pawlowski and Kostanecki treated 28 women, ranging in age from 17 to 51 years, with daily doses of 10 mg biotin for 28 days. No beneficial or deleterious effects were observed (1060).

Weirich reported that 5 patients suffering from onychodystrophy were given 120 mg biotin/day orally for a period of 40 days. The treatment was observed to partially alleviate the patients disorders, without the production of any harmful side effects (1459).

In the treatment of 30 infants with dermatitis seborrhoides, Gautier et al. administered daily doses of 5 mg biotin intramuscularly for 6-10 days and then orally until about the 10th or 15th day. Favorable results were observed in 90% of the cases. The authors mentioned no adverse effects (467).

Biochemical Aspects

I. Breakdown

According to Borenstein, the available evidence indicates that biotin shows good stability in foods. Evaporated and powdered milk incur losses of 10-15% biotin during processing. Biotin is stable during home cooking of frozen baby lima beans and does not leach in cooking water. Stability of crystalline biotin in dry products is excellent and work with pharmaceutical liquid products indicates losses of 0-8% at pH 5-8, 6-12% at pH 4.1-4.3 and 15-20% at pH 3.3-3.6 after one year at room temperature (456).

Elvehjem reported that approximately 23% of the biotin content of meat is lost in cooking (384).

II. Absorption-Distribution

The absorption of biotin was studied by Spencer and Brody by using everted intestinal sacs from a number of animals. At a concentration of 1×10^{-5} M, biotin-H^3 and biotin-C^{14} were transported against a concentration gradient by hamster intestine, principally in the first 1/3 to 1/2 of the small intestine. Similar transport was shown by the small intestine of the white mouse, Mongolian gerbil, chinchilla, flying squirrel, roundtail squirrel, Malayan black squirrel and chipmunk. Transport under these conditions was not shown by the small intestine of the rat, rabbit, guinea pig, ferret, or carp. No correlation was found between the need for biotin as a vitamin in a species and the presence of this transport system. Biocytin, biotin methyl ester, diaminobiotin, desthiobiotin, and thioctic acid, but not amino acids, urea, biotin-d-sulfoxide, or biotin-l-sulfonide, inhibited biotin transport (1289).

III. Metabolism and Excretion

The metabolic fate of biotin was studied by Lee et al. through i.p. injection of ureido carbonyl-labeled ^{14}C-biotin (1 mg, 4 micrograms, or 0.5 micrograms/100 g body weight) into young adult male rats. Following injection of milligram quantities of biotin, more than 90% of the injected dose was excreted in the urine within 12 hours, and almost 100% within 24 hours; no significant radioactivity was excreted in the expired CO_2 and only a trace was excreted in the feces. The initial excretion rate was found to decrease with decreasing doses of biotin, the decrease being even more marked with biotin-depleted rats. To study the metabolites of biotin, biotin-depleted rats, treated with succinyl sulfathiazole and penicillin, were given a single injection of radioactive biotin (0.5 microgram/100 g body weight). Fractionation of the urine revealed 4 radioactive metabolites - d- and l-sulfoxides of biotin, bisnorbiotin and a neutral ketone. No radioactive CO_2 or urea could be detected. In further experiments, rat liver homogenates were incubated with carbonyl-labeled biotin at 37 degrees for 40 or 60 minutes, with or without aeration. Under oxygen-limiting conditions, the same 4 biotin metabolites were also found, but in different proportions. With aeration, the amount of biotin

sulfoxides increased; also, trace amounts of radioactive CO_2 and urea were detected. It was concluded that the rat has the ability to metabolize biotin via at least partial beta-oxidation of the side chain and to effect some oxidation of the thioether sulfur to sulfoxides. Under physiological conditions, however, the metabolism of biotin in rats does not include extensive cleavage of the ring structure of the molecule (797).

Both biotin-sufficient and biotin-deficient male, Sprague-Dawley rats (250-310 g) were observed by Lee et al. to excrete, in the urine, approximately 95% of an i.p. dose of 0.5 mg/100 g body weight of ^{14}C-labeled biotin within 24 hours after injection; 84% was excreted within the first 3 hours. No significant radioactivity was excreted through the feces or expired CO_2 (796).

Following the i.p. injection of 0.01-0.02 mg of ^{14}C-labeled biotin to rats, Fraenkel-Conrat and Fraenkel-Conrat observed that 85% of the radioactivity was excreted in the urine within the first 24 hours. About half of the excreted radioactivity represented free biotin while the rest was found to be in a microbiologically inactive form. Tissue analysis indicated that 5-8% of the administered radioactivity remained in the liver after 3-6 days, apparently largely as biotin. Considerably less radioactivity was found in the kidneys and spleen and no radioactivity was detected in the adrenals, mesentary, lungs, blood, thyroid or muscle (444).

Dakshinamurti and Mistry determined the tissue and intracellular distribution of biotin-$^{14}COOH$ of biotin-depleted rats and chicks following an i.p. injection of 0.01 mg of labeled biotin. Their results are summarized in Tables 1 and 2 (303).

TABLE 1

Biotin-$C^{14}OOH$ distribution in chicks and rats

Chicks and rats were on the biotin-deficient raw egg white diet for 5 and 6 weeks, respectively. After deprivation of food overnight, a single dose of labeled biotin, divided into two equal parts, was administered as mentioned in the text. Chicks weighed 210 and 304 g; rats, 164 and 171 g.

Tissue	Dose recovered after hours			
	Chicks		Rats	
	1	3	1	4
	%	%	%	%
Liver	5.2	14.0	7.0	15.6
Kidney	9.0	17.0	4.6	6.4
Injected muscle	27.3	7.3	37.9	11.8
Remaining muscle	3.3	7.3	5.5	7.9
Spleen	0.1	0.3	0.2	0.1
Viscera	5.1	3.3	6.8	4.3
Lungs	0.2	0.7	0.3	0.3
Heart	0.2	0.3	0.3	0.6
Excreta	31.4	30.7	16.0	30.1
Expired CO_2	0.2	0.7	1.6	3.9
Total	82.0	81.6	80.2	81.0

TABLE 2

Biotin content of cell fractions of normal and biotin-deficient rat and chick livers

Rats and chicks were on the biotin-deficient raw egg white diet for 6 and 5 weeks, respectively. Normal animals received, in addition, biotin once a week intraperitoneally as described in the text.

Fraction	Rat			Chick	
	Normal				
	Total biotin	Bound biotin as per cent of total in each fraction	Deficient: total biotin	Normal: total biotin	Deficient: total biotin
	%	%	%	%	%
Homogenate	100[a]	92	100[b]	100[c]	100[d]
Nuclei	37	99	75	38	49
Mitochondria I	9	89	13	24	14
Mitochondria II	1	65	9	4	1
Microsomes	2	23	0	3	6
Supernatant	47	91	0	24	16
Recovery	96		97	93	86

[a] Equal to 1980 mμg of biotin per g of fresh liver.
[b] Equal to 200 mμg of biotin per g of fresh liver.
[c] Equal to 875 mμg of biotin per g of fresh liver.
[d] Equal to 291 mμg of biotin per g of fresh liver.

In a study by Tuszynska, large doses of biotin (4 mg administered over a 2-week period) were given orally to both biotin-depleted and biotin-sufficient Wistar rats. Analytical investigations of the biotin level in the tissues and systemic fluids indicated that excess biotin is not stored in the body (1403).

Several researchers have reported that the amount of biotin excreted by man in the feces often greatly exceeds the amount ingested and is relatively unaffected by the level of intake. This phenomenon is attributed to biotin synthesis by the intestinal flora and is most pronounced with a low biotin intake. Urinary biotin excretion, however, is reported to be significantly influenced by the level of intake, with biotin supplements leading to increased urinary excretion (331, 330, 465, 463, 1022).

IV. Effects on Enzymes and Other Biochemical Parameters

The pharmacology of biotin was investigated by Crittenden through a number of parameters. The addition of dl-biotin (10^{-4}) or d-biotin (10^{-3}) to modified Ringer-Locke solution had no effect on the contractions of the isolated duodenal strip or isolated uterus of the normal rat. The sensitivity of the nerve endings in the depilated skin of the lower abdomen of the normal rat following the oral administration of 0.1 mg of d-biotin 6 times per week for approximately 3 weeks did not differ from that of the control group receiving saline. The rectal temperature of these animals was not altered by the biotin administered, nor was the blood fibrinogen affected. No changes were observed in the blood pressure,

heart rate or intestinal circulation of 8 anesthetized normal rats (150 g) given 1 or 5 mg of dl- or d-biotin intravenously. The secretion of free HCl by the gastric glands appeared to be depressed by the i.v. administration of 1 mg d-biotin to anesthetized rats. A single oral dose of 0.1 mg of d-biotin to rats had no effect on the diuretic action of 10 ml of physiological saline per 100 g body weight. A single oral dose of 0.1-0.2 mg of d-biotin had no significant action on the metabolic rate of normal rats. Oral and topical applications of biotin were found to have no effect on skin regeneration or healing of scalded skin in the normal rat (285).

Schmidt and Landy reported that biotin failed to produce any changes in the following physiological functions: blood pressure, heart rate and respiration of anesthetized cats following rapid i.v. injection of 0.25 mg/kg; excised guinea pig and rabbit uterus and rabbit intestine suspended in concentrations up to 1:40,000; and the frog heart perfused in situ with 0.2 mg of biotin (1209).

In a study by Steiger-Wald and Boehle, 55 patients of various ages with basal metabolic conditions were given either 10 mg biotin in injection form or 100 mg in infusion form within a period of 1 hour. Blood samples taken before, and every half hour up to 4 hours later, were examined for total lipids, lipid fractions, acetone bodies, and pyruvic, oxalacetic, and ketoglutaric acids. The data obtained is presented in Figures 1-3. The workers concluded that biotin stimulates the production of oxalacetic acid from pyruvic acid or lactic acid, and that it increases the oxidation of fat (1299).

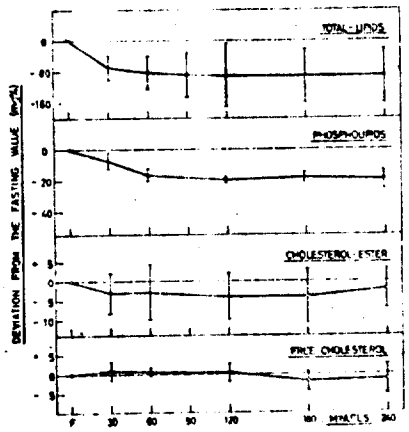


Fig 1. Mean value curves of the metabolites taken from 15 test persons after infusion of 100 mg Biotin.

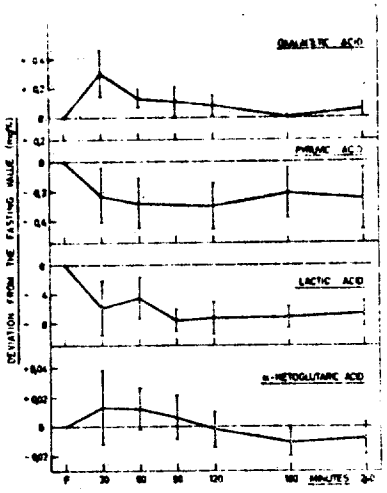


Fig. 2. Same as Fig. 1.

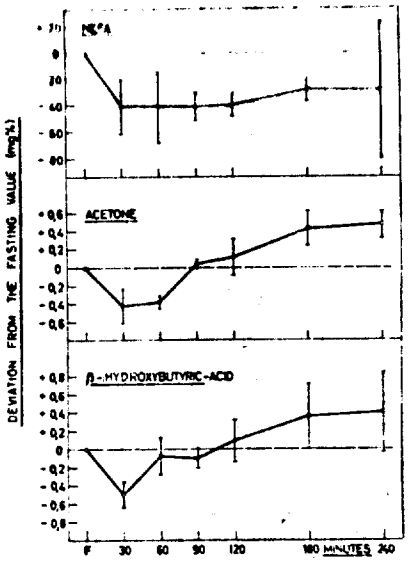


Fig. 3. Same as Fig. 1.

Petrelli and Farabollini studied the effect of biotin administration on the blood sugar level of normal rats through endoperitoneal injection of biotin in aqueous solution in single doses of 0.5, 1 and 2 mg per rat (200 g). As shown in Figure 4, the biotin administration led to a rapid increase in the blood sugar level. The magnitude of the hyperglycemia was not great and was not proportional to the dose of biotin administered. In a further study, the administration of biotin was found to have no effect on the hypoglycemic action of insulin (1067).

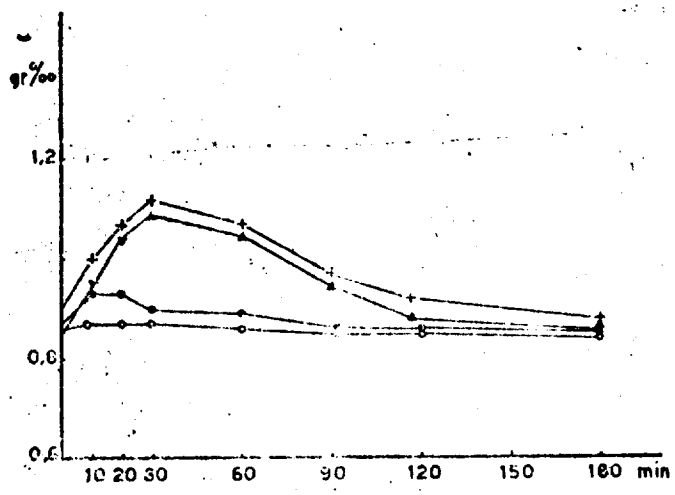


Fig. 4 Mean values of the blood sugar level of groups of 12 rats treated with biotin and of 12 control rats.

Abscissae: time from the beginning of the experiment.
Ordinates: blood sugar expressed as g./1000 ml.
○ = control
● = 0.5 mg. biotin
▲ = 1 mg. biotin
+ = 2 mg. biotin

The subcutaneous administration of biotin to normal and hypercholesterolemic rats in a dose of 0.3 micrograms/100 g body weight/day for 20 days was found by Ivanov to significantly increase tissue respiration, as measured by O_2 consumption, in the heart, liver, brain, kidneys, spleen and muscle tissues. In the normal rats, tissue respiration increased 2-5 times during the first hour of observation and 3-7 times during the second hour. The hypercholesterolemic rats exhibited a somewhat lower response to the biotin (627).

Wilson and Leduc observed a slight stimulation of mitotic activity in the livers of normal mice (104-106 days old) following s.c. injection of biotin (2 micrograms/day). This effect, which appeared on the third day of injection was fleeting and did not recur with subsequent daily injections (1492).

The concentration of cholesterol in the serum, carcass, and liver of young male albino rats (30-40 g) was found by Rajalakshmi et al. to be increased by the s.c. administration of biotin at a level of 0.1 mg/day for 8 weeks. Studies on the incorporation of radioactive acetate and mevalonic acid into cholesterol revealed that biotin enhances the level of tissue cholesterol by stimulating its synthesis from acetate. It was suggested that the mechanism by which biotin accentuates cholesterol synthesis may be by facilitating the utilization of C-5 acids towards cholesterol synthesis. Cholesterol in the tissues of rats treated with biotin was found to remain at the normal level when additional supplements of inositol or inosithin (1% of diet) were given (1127).

The oral administration of 1 mg biotin/kg body weight/day for 9 days to normal rats was reported to cause no significant changes in serum free fatty acids, cholesterol or triglycerides (see Table 3). No adverse effects were reported (954).

Table 3. Effect of Biotin* on Lipid Metabolism in the Rat

Treatment	Serum FFA μEq/L ± S. E.	Serum Cholesterol mg/100 ml ± S. E.	Serum Triglycerides mg/100 ml ± S. E.
Control	776 ± 45	73.1 ± 1.4	87.4 ± 4.8
Biotin	724 ± 23	71.8 ± 3.2	70.6 ± 6.9

* Biotin was administered orally at 1 mg/kg for 9 days.

V. Drug Interaction

It has long been known that on a well balanced diet to which relatively large quantities of raw egg white have been added, rats lose their hair and develop a severe dermatitis and skin hemorrhages; these symptoms are accompanied by nervous disorders and loss of weight. A spasticity develops which in the later stages of the disease causes the rats to assume a typical kangaroo-like posture, and unless the condition is remedied, death ensues. The component of egg white responsible for this reaction is avidin, which exerts its effect by forming a stable, unabsorbable complex with biotin. Thus dietary biotin is excreted in the feces as an avidin-biotin complex, resulting in biotin deficiency. This condition can be countered by the administration of supplementary biotin, either orally or parenterally (576, 1226, 1487).

The addition of sulfa drugs (sulfaguanidine, succinylsulfathiazole) and antibiotics (terramycin) to purified diets of rats has been reported to result in the retardation of growth and the production of symptoms similar to those of egg white injury. These effects have been attributed to the bacteriostatic and bactericidal action of these drugs which results in inhibition of biotin synthesis by the bacterial flora and, consequently, biotin deficiency, since much of the biotin utilized by the rat is obtained through such synthesis. It is reasonable to assume that any substance exhibiting bacteriostatic or bactericidal action could produce similar effects. These effects are easily cured through biotin supplementation (292, 389, 550, 963, 972, 1461).

Orlandi and Rabaglia reported that the simultaneous oral or i.v. administration of sodium salicylate (1 g) and biotin (0.2 g) to children enhanced the action of the salicylate and slowed its elimination (1024).

VI. Consumer Exposure

A table of the biotin levels found in a wide variety of foods (Table 4) was compiled by Hardinge and Crooks (560).

Tables 5-7 were compiled in 1972 from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Table 5 reports the usage of biotin in infant formulas or baby foods. Table 6 reports the annual poundage data for biotin. Table 7 reports the possible daily intake per food category and total dietary based on food consumption by total sample.

TABLE 4 Vitamin B_6, pantothenic acid, biotin, folic acid, choline, and inositol content of foods, edible portion

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
			Cereals and Bread Products											
		gm.	mcg.		mg.		mcg.		mcg		mg.		mg.	
Cereals, cooked, whole														
Barley, whole	½ c.	30(dry)	167.1	557	0.219	0.73	9.3	31.0	15.	50.0	41.7	139.0	117.6	392.0
Corn, yellow	½ c.	20(dry)	93.4	467	0.128	0.64	4.2	21.0	5.3	26.5	12.2	61.0	10.0	50.0
Oats	½ c.	20(dry)	41.0	205	0.300	1.50	4.8	24.0	6.6	33.0	31.2	156.0	53.8	269.0
Rice, brown	½ c.	30(dry)	186.0	620	0.456	1.52	3.6	12.0	6.0	20.0	33.6	112.0	35.7	119.0
Wheat, whole	½ c.	30(dry)	158.4	528	0.411	1.37	4.8	16.0	14.7	49.0	28.2	94.0	110.0	370.0
Cereals, refined, cooked														
Cornmeal														
White	½ c.	20(dry)	(12.2)*	(61)	0.116	0.58	1.3	6.6	1.8	9.0	—†	—	10.2	51
Yellow	½ c.	20(dry)	—	—	0.138	0.69	—	—	1.8	9.0	2.0	10.0	—	—
Hominy, grits	½ c.	20(dry)	(1.2)	(6)	0.068	0.34	0.1	0.7	0.9	4.5	—	—	0.6	3
Rice														
Converted	½ c.	30(dry)	20.4	68	0.393	1.31	2.4	8.0	6.0	20.0	26.7	89.0	6.0	20
Parboiled	½ c.	30(dry)	30.0	100	0.411	1.37	3.0	10.0	5.7	19.0	20.4	98.0	7.5	25
White	½ c.	30(dry)	11.1	37	0.225	0.75	1.5	5.0	4.8	16.0	17.7	59.0	3.0	10
Cereal concentrates, raw														
Rice														
Bran	1 oz.	30(dry)	750.0	2500	0.831	2.77	18.0	60.0	43.8	146.0	51.0	170.0	138.9	463
Germ	1 oz.	30(dry)	480.0	1600	0.900	3.00	17.4	58.0	120.0	430.0	90.0	300.0	111.6	372
Polishings	1 oz.	30(dry)	600.0	2000	1.000	3.33	17.1	57.0	57.6	192.0	30.6	102.0	136.2	454
Wheat														
Bran	1 oz.	30(dry)	414.0	1380	0.900	3.00	4.2	14.0	58.5	195.0	42.9	143.0	—	—
Germ	1 oz.	30(dry)	275.4	918	0.660	2.20	—	—	91.5	305.0	121.8	406.0	231.0	770
Ready-to-eat, dark cereal														
Corn soya	1 c.	30	—	—	0.273	0.91	—	—	24.0	80.0	—	—	—	—
Oats	1 c.	25	—	—	0.230	0.92	—	—	5.6	22.4	—	—	—	—
Wheat														
Bran	1 c.	35	—	—	1.015	2.90	—	—	35.0	100.0	—	—	—	—
Flakes	1 c.	35	—	—	0.304	0.87	—	—	16.4	47.0	—	—	—	—
Shredded Wheat	1 biscuit	30	—	—	0.210	0.70	—	—	16.5	55.0	—	—	—	—
Ready-to-eat white cereal														
Corn flakes	1 c.	25	—	—	0.047	0.19	—	—	1.4	5.5	—	—	—	—
Rice	1 c.	30	(14.4)	(48)	0.114	0.38	0.4	1.3	2.3	7.6	—	—	5.7	19
Bread														
White	1 sl.	23	23.0	100	0.101	0.44	0.3	1.1	3.4	15.0	—	—	11.7	51
Whole wheat	1 sl.	23	96.0	420	0.182	0.79	0.4	1.9	6.9	30.0	—	—	15.4	67
Flour														
White	1 c.	110	363.0	330	0.594	0.54	1.1	1.0	8.8	8.0	57.2	52	51.7	47
Whole wheat	1 c.	120	1116.0	930	1.296	1.08	10.8	9.0	45.6	38.0	—	—	132.0	110
			Dairy Products											
Cheese														
Cheddar	1 oz.	30	(18.7)	(66)	0.114	0.40	1.0	3.6	4.5	16.0	13.6	48	7.0	25
Cottage	1 oz.	30	—	—	0.080	0.28	—	—	8.8	31.0	—	—	—	—
Processed	1 oz.	30	—	—	0.136	0.48	1.3	4.6	3.1	11.0	—	—	—	—
Egg														
Whole	1 med.	50	126.0	252	0.795	1.59	11.2	22.5	2.5	5.1	252.0	504	16.5	33
Yolk	1 med.	17	52.4	308	0.719	4.23	8.8	52.0	2.2	12.9	253.3	1490	—	—
White	1 med.	31	67.3	217	0.043	0.14	2.2	7.0	0.2	0.6	0.6	2	—	—
Milk														
Whole	1 c.	244	87.8	36	0.756	0.31	11.5	4.7	1.5	0.6	36.6	15	31.7	13
Evaporated reconstituted	1 c.	244	53.7	22	0.805	0.33	11.0	4.5	1.7	0.7	36.6	15	—	—
Non-fat dry reconstituted	1 c.	244	80.5	33	0.830	0.34	8.3	3.4	0.5	0.2	25.4	10.4	—	—
			Fats, Oils, and Oily Foods											
Butter	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Lard	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Margarine	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Vegetable oils	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—

*Data from Chichelli and Williams (26), as only data available, even though they may not be comparable to figures obtained by other methods. Figures which are appreciably lower than other values available for similar types of foods are enclosed in parentheses.

†Dashes indicate that no representative value was found in the literature for particular constituent.

TABLE 4 continued

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Fruits														
		gm.	mcg.		mg.		mcg.		mcg.		mg.		mg.	
Fruits, fresh or frozen														
Apples, medium	1	130	30.0	30	0.130	0.10	1.2	0.9	2.6	2.0	—	—	31.2	24
Apricots	3 med.	100	71.0	71	0.290	0.29	—	—	3.3	3.3	—	—	—	—
Bananas	1 med.	100	320.0	320	0.310	0.31	4.4	4.4	9.7	9.7	—	—	34.0	34
Berries														
Blackberry	⅔ c.	100	—	—	0.260	0.26	—	—	13.7	13.7	—	—	—	—
Blueberry	⅔ c.	100	91.0	91	0.120	0.12	—	—	8.0	8.0	—	—	—	—
Raspberry, sweetened	¾ c.	100	38.0	38	0.210	0.21	—	—	5.0	5.0	—	—	—	—
Strawberry, sweetened	⅔ c.	100	61.0	61	0.160	0.16	4.0	4.0	9.0	9.0	—	—	60.0	60
Cantaloupe, diced	½ c.	100	36.0	36	0.260	0.26	3.1	3.1	6.8	6.8	—	—	120.0	120
Cherries, sour red, pitted	⅔ c.	100	85.0	85	0.070	0.07	—	—	6.0	6.0	—	—	—	—
Figs, small	3	114	148.2	130	0.388	0.34	—	—	16.0	14.0	—	—	—	—
Grapefruit, small	½	120	25.0	21	0.288	0.24	3.6	3.0	3.4	2.8	—	—	180.0	150
Grapes, 1 bunch	3½ oz.	100	85.0	85	0.050	0.05	1.6	1.6	5.2	5.2	—	—	—	—
Oranges, small	1	100	31.0	31	0.220	0.22	1.9	1.9	5.1	5.1	—	—	210.0	210
Peaches, sweetened	½ c.	100	20.0	20	0.120	0.12	1.7	1.7	4.0	4.0	—	—	96.0	96
Pineapple	3½ oz.	100	75.0	75	0.170	0.17	—	—	6.0	6.0	—	—	—	—
Rhubarb, sweetened	3½ oz.	100	29.0	29	0.070	0.07	—	—	4.0	4.0	—	—	—	—
Watermelon, diced	1 c.	150	49.5	33	0.450	0.30	5.4	3.6	0.9	0.6	—	—	96.0	64
Fruits, canned														
Apricots	½ c.	125	67.5	54	0.125	0.10	—	—	0.6	0.5	—	—	—	—
Blackberries	½ c.	125	30.0	24	0.100	0.08	—	—	17.5	14.0	—	—	—	—
Blueberries	½ c.	125	48.7	39	0.087	0.07	—	—	5.2	4.2	—	—	—	—
Cherries, sweet	½ c.	125	196.2	157	0.150	0.12	—	—	3.7	3.0	—	—	—	—
Peaches	½ c.	125	28.8	23	0.088	0.07	0.3	0.2	0.6	0.5	—	—	—	—
Pineapple	½ c.	125	88.7	71	—	—	—	—	1.0	0.8	—	—	—	—
Plums, purple	½ c.	125	33.7	27	0.100	0.08	—	—	1.2	1.0	—	—	—	—
Fruit, dried														
Apricots, small	6 halves	30	—	—	0.225	0.75	—	—	1.4	4.7	—	—	—	—
Dates, pitted	5	45	—	—	0.351	0.78	—	—	11.1	24.7	—	—	—	—
Figs, large	2	40	128.0	320	0.208	0.52	—	—	12.8	32.0	—	—	—	—
Prunes	5 med.	35	—	—	0.126	0.36	—	—	1.9	5.4	—	—	—	—
Raisins, seedless	3 Tbsp.	30	98.0	327	0.030	0.10	1.4	4.5	3.0	10.0	—	—	36.0	120
Fruit Juices														
Canned fruit juice														
Apple	½ c.	120	42.0	35	—	—	—	—	0.2	0.2	—	—	—	—
Grapefruit	½ c.	120	15.6	13	0.204	0.17	1.0	0.8	2.3	1.9	—	—	120.0	100
Lemon	½ c.	120	61.2	51	—	—	—	—	0.2	0.2	—	—	—	—
Orange	½ c.	120	28.8	24	0.156	0.13	1.0	0.8	2.8	2.3	—	—	169.0	141
Pineapple	½ c.	120	115.2	96	0.120	0.10	—	—	1.1	0.9	—	—	—	—
Frozen or fresh														
Apple	½ c.	120	36.0	30	0.024	0.02	0.5	0.4	1.8	1.5	0.7	0.6	28.8	24
Grape	½ c.	120	25.2	21	0.048	0.04	0.4	0.3	3.6	3.0	—	—	—	—
Grapefruit	½ c.	120	16.8	14	0.192	0.16	0.8	0.7	1.6	1.3	—	—	120.0	100
Lemon, single	½ c.	120	46.8	39	0.108	0.09	—	—	1.2	1.0	—	—	—	—
Orange, fresh	½ c.	120	31.2	26	0.168	0.14	0.4	0.3	2.6	2.2	14.4	12	141.6	118
Orange, frozen	½ c.	120	26.4	22	0.168	0.14	0.4	0.3	2.6	2.2	14.4	12	110.4	92
Pineapple	½ c.	120	88.8	74	0.144	0.12	—	—	1.2	1.0	—	—	—	—
Legumes														
	cooked	dry												
Mung bean	½ c.	32	182.4	570	0.800	2.50	2.4	7.5	46.4	145	66.9	209	22.4	70
Cowpeas	½ c.	32	(67.2)	(210)	0.397	1.24	6.7	21.0	140.5	439	82.2	257	76.8	240
Garbanzos	½ c.	32	172.8	540	0.400	1.25	3.2	10.0	40.0	125	78.4	245	76.8	240
Lentils	½ c.	32	156.8	490	0.480	1.50	4.2	13.2	34.2	107	71.4	223	41.6	130
Lima beans	½ c.	32	176.0	550	0.416	1.30	3.1	9.8	41.0	128	—	—	54.4	170
Navy beans	½ c.	32	—	—	0.387	1.21	—	—	40.0	125	—	—	160.0	500
Kidney beans	½ c.	32	—	—	0.208	0.65	—	—	57.6	180	—	—	—	—
Split peas	½ c.	32	105.0	328	0.700	2.18	5.9	18.4	16.3	51	64.3	201	48.0	150
Soy beans	½ c.	32	204.8	640	0.538	1.68	19.5	61.0	71.7	224	108.8	340	64.0	200
Soy flour	1 c.	90	590.4	656	1.512	1.68	63.0	70.0	383.4	426	202.5	225	184.5	205
Meat and Poultry‡														
Beef														
Ground	3½ oz.	100	460.0#	460	0.440#	0.44	—	—	6.9	6.9	—	—	—	—
Liver	2 oz.	57	378.5	664	5.324	9.34	54.7	96.0	167.6	294.0	290.7	510	29.1	51
Rib roast, lean	3 oz.	100	480.0#	480	0.600#	0.60	3.4	3.4	—	—	82.0	82	—	—
Round	3 oz.	100	495.0#	495	0.520#	0.52	2.6	2.6	10.5	10.5	68.0	68	11.5	11.5

‡Raw values, except where indicated. #Cooked values

TABLE 4 continued

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Meat and Poultry, concluded‡														
		gm.	mcg.		mg.		mcg.		mcg.		mg.		mg.	
Lamb														
Leg	3 oz.	100	320.0f	320	0.620f	0.62	5.9	5.9	3.3	3.3	84.0	84	58.0	58
Liver	2 oz.	57	—	—	4.047	7.10	72.4	127.0	157.3	276.0	—	—	—	—
Loin chop	3 oz.	100	330.0f	330	0.590f	0.59	—	—	—	—	76.0	76	—	—
Veal														
Chop	3½ oz.	100	430.0f	430	0.550f	0.50	2.0	2.0	—	—	96.0	96	33.0	33
Leg	3½ oz.	100	200.0	200	0.914	0.914	—	—	—	—	132.0	132	—	—
Shoulder	3½ oz.	100	300.0	300	—	—	—	—	—	—	93.0	93	—	—
Stew meat	3½ oz.	100	330.0	330	0.832	0.852	—	—	4.6	4.6	96.0	96	—	—
Pork														
Bacon	2 sl.	16	88.0f	550	0.070f	0.440	1.2	7.6	—	—	12.8	80	6.9	43
Ham	3½ oz.	100	440.0f	440	0.640f	0.640	5.0	5.0	10.6	10.6	122.0	122	31.0	31
Liver	2 oz.	57	—	—	3.994	7.008	57.0	100.0	126.0	221.0	314.6	552	—	—
Loin	3½ oz.	100	480.0f	480	0.400f	0.400	5.2	5.2	2.4	2.4	77.0	77	45.0	45
Poultry														
Chicken														
Dark meat	3½ oz.	100	(25.0)	(25)	0.692	0.692	10.0	10.0	2.8	2.8	—	—	47.0	47
White meat	3½ oz.	100	(130.0)	(130)	0.804	0.804	11.3	11.3	3.0	3.0	—	—	48.0	48
Turkey	3½ oz.	100	—	—	0.748	0.748	—	—	7.5	7.5	—	—	—	—
Bologna, A.P.	2 oz.	57	—	—	—	—	—	—	—	—	34.2	60	—	—
Frankfurter, A.P.	1	50	65.0	130	0.212	0.425	—	—	—	—	28.5	57	—	—
Sausage, pork, A.P.	2 oz.	57	108.3	190	0.321	0.563	—	—	6.5	11.5	27.4	48	—	—
Fish														
Halibut, canned	3½ oz.	100	(110.0)	(110)	0.150	0.150	8.0	8.0	—	—	—	—	17.0	17
Mackerel, Pacific, canned	3½ oz.	100	270.0	270	0.470	0.470	18.0	18.0	0.6	0.6	—	—	—	—
Salmon, canned	3½ oz.	100	450.0	450	0.580	0.580	15.0	15.0	0.5	0.5	—	—	17.0	17
Tuna, canned	3½ oz.	100	670.0	670	0.420	0.420	3.0	3.0	1.8	1.8	—	—	—	—
Sardines, Pacific, canned	3½ oz.	100	280.0	280	0.600	0.600	24.0	24.0	0.5	0.5	—	—	—	—
Shellfish, canned														
Clams	3½ oz.	100	83.0	83	0.590	0.590	—	—	2.0	2.0	—	—	—	—
Crabs	3½ oz.	100	364.0	364	—	—	—	—	0.4	0.4	—	—	—	—
Oysters	3½ oz.	100	37.0	37	0.490	0.490	8.7	8.7	11.3	11.3	—	—	44.0	44
Shrimp	3½ oz.	100	111.0	111	0.210	0.210	—	—	1.8	1.8	—	—	—	—
Nuts and Oily Fruits														
Nuts, raw														
Almonds	12-15	15	15.0	100	0.087	0.578	2.7	18.0	6.7	45.0	—	—	—	—
Brazil nuts	2 med.	15	—	—	0.035	0.231	—	—	0.7	4.5	—	—	—	—
Cashews	6-8	15	—	—	0.174	1.62	—	—	—	—	—	—	—	—
Coconut, fresh	½ oz.	15	—	—	0.049	0.330	—	—	4.1	27.6	—	—	—	—
Filbert	10-12	15	—	—	0.172	1.146	—	—	10.0	66.6	—	—	—	—
Peanuts, roasted	15-17	15	45.0	300	0.320	2.137	5.1	34.0	8.5	56.5	24.3	162	27.0	180
Pecan halves	12	15	—	—	0.256	1.707	4.0	27.0	4.0	27.0	7.5	50	—	—
Walnut halves	8-15	15	144.0	960	0.146	0.970	5.5	37.0	11.5	77.0	—	—	—	—
Peanut butter	1 Tbsp.	16	48.0	300	0.400	2.500	6.2	39.0	8.5	56.5	23.2	145	28.8	180
Fruits, oily														
Avocado, Fuerte (cubed)	½ c.	75	457.5	610	0.675	0.90	4.1	5.5	22.5	30.0	—	—	—	—
Olive, ripe, mammoth	6	40	6.4	16	0.008	0.02	—	—	0.3	0.7	—	—	—	—
Vegetables														
Vegetables, fresh or frozen														
Asparagus	3½ oz.	100	136.0	136	0.620	0.62	—	—	109.0	109.0	10.0	10.0	—	—
Beans, Lima	3½ oz.	100	170.0	170	0.450	0.45	—	—	34.0	34.0	—	—	—	—
Beans, snap, green	3½ oz.	100	63.0	63	0.200	0.20	—	—	27.5	27.5	42.0	42.0	—	—
Beets (diced)	⅓ c.	100	(37.0)	(37)	0.170	0.17	1.9	1.9	13.5	13.5	—	—	21.0	21
Broccoli	⅓ c.	100	171.0	171	1.290	1.29	—	—	53.5	53.5	—	—	—	—
Brussels sprouts	3½ oz.	100	162.0	162	0.720	0.72	—	—	49.0	49.0	—	—	—	—
Cabbage (fine shreds)	1 c.	100	120.0	120	0.260	0.26	2.4	2.4	32.3	32.3	23.0	23.0	95.0	95
Carrots (grated)	½ c.	55	66.0	120	0.150	0.27	1.4	2.5	4.4	8.0	7.0	13.4	26.4	48
Cauliflower (buds)	1 c.	100	177.0	177	1.010	1.01	17.0	17.0	22.2	22.2	—	—	95.0	95
Celery (diced)	1 c.	100	—	—	0.430	0.43	—	—	7.0	7.0	—	—	—	—
Corn	3½ oz.	100	222.0	222	0.890	0.89	6.0	6.0	28.0	28.0	—	—	—	—
Lettuce	½ hd.	100	(71.0)	(71)	0.360	0.36	3.1	3.1	21.0	21.0	—	—	55.0	55
Mixed vegetables	3½ oz.	100	122.0	122	0.310	0.31	—	—	16.0	16.0	—	—	—	—
Onion (2½ in.)	1	110	(69.3)	(63)	0.187	0.17	3.8	3.5	11.0	10.0	—	—	96.8	88
Peas, cow	3½ oz.	100	118.0	118	0.400	0.40	21.0	21.0	41.0	41.0	97.0	97.0	240.0	240
Peas, green	3½ oz.	100	150.0	150	0.820	0.82	9.4	9.4	25.0	25.0	75.0	75.0	162.0	162
Potatoes, peeled	1 med.	100	220.0	220	0.400	0.40	—	—	6.8	6.8	20.0	20.0	29.0	29
Potatoes, sweet	½ med.	100	320.0	320	0.930	0.93	4.3	4.3	12.0	12.0	11.5	11.5	66.0	66

‡Raw values, except as indicated. f Cooked.

TABLE 4 concluded

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Vegetables, Concluded														
		gm.	mcg.		mg.		mcg.		mcg.		mg.		mg.	
Squash, winter	3½ oz.	100	91.0	91	0.490	0.49	—	—	12.0	12.0	—	—	—	—
Squash, yellow	3½ oz.	100	63.0	63	0.390	0.39	—	—	17.0	17.0	—	—	—	—
Tomato	1 small	110	—	—	0.341	0.31	4.4	4.0	8.8	8.0	—	—	50.6	46
Mushrooms, fresh	2 oz.	60	(27.0)	(45)	1.626	2.71	9.6	16.0	14.4	24.0	—	—	10.2	17
Greens														
Beet	3½ oz.	100	—	—	0.260	0.26	2.7	2.7	60.0	60.0	—	—	21.0	21
Kale	3½ oz.	100	185.0	185	1.290	1.29	—	—	70.0	70.0	—	—	—	—
Mustard	3½ oz.	100	133.0	133	0.250	0.25	—	—	60.0	60.0	22.0	22.0	—	—
Spinach	3½ oz.	100	198.0	198	0.310	0.31	6.9	6.9	75.0	75.0	22.0	22.0	27.0	27
Turnip	3½ oz.	100	98.0	98	0.380	0.38	—	—	42.0	42.0	27.0	27.0	46.0	46
Vegetables, canned with liquid														
Asparagus, green	½ c.	120	90.0	75	0.240	0.20	2.0	1.7	32.4	27.0	—	—	—	—
Beans														
Lima	½ c.	120	97.2	81	0.132	0.11	—	—	15.6	13.0	—	—	—	—
Green string	½ c.	120	51.6	43	0.084	0.07	1.5	1.3	14.4	12.0	—	—	—	—
Beets (diced)	½ c.	125	67.5	54	0.125	0.10	—	—	3.5	2.8	—	—	—	—
Carrots (diced)	½ c.	125	51.2	41	0.137	0.11	1.9	1.5	4.1	3.3	—	—	—	—
Corn	½ c.	125	270.0	216	0.275	0.22	2.7	2.2	9.6	7.7	—	—	—	—
Mushrooms	½ c.	120	75.6	63	1.128	0.94	8.7	7.3	4.7	3.9	—	—	20.4	17
Peas														
Cow	½ c.	125	66.2	53	—	—	—	—	32.5	26.0	—	—	—	—
Green,	½ c.	125	55.0	44	0.212	0.17	2.6	2.1	12.9	10.3	—	—	—	—
Spinach	½ c.	120	114.0	95	0.071	0.059	2.8	2.3	58.8	49.0	—	—	—	—
Tomatoes	½ c.	120	181.2	151	0.276	0.23	2.2	1.8	4.4	3.7	—	—	—	—
Tomato juice	½ c.	120	230.4	192	0.360	0.30	—	—	8.0	6.7	—	—	—	—
Miscellaneous														
Chocolate	1 oz.	28.4	(6.5)	(23)	0.054	0.19	9.0	32.0	28.1	99.0	—	—	24.1	85
Molasses	1 Tbsp.	20	(54.0)	(270)	0.092	0.46	1.8	9.0	1.9	9.5	17.2	86	30.0	150
Yeast, brewer's	1 Tbsp.	8	193.5	2419	0.880	11.0	16.0	200.0	161.8	2022.0	19.2	240	—	—
Food yeast, torula	1 Tbsp.	8	280.0	3500	0.800	10.0	8.0	100.0	240.0	3000.0	20.0	250	21.6	270
Honey	1 Tbsp.	20	2.0	10	0.012	0.06	—	—	0.6	3.0	—	—	—	—
Pollen, flower	—	1	9.0	900	0.027	2.70	0.3	25.0	—	—	—	—	—	—
Royal jelly	—	1	10.2	1020	0.320	32.00	4.1	410.0	0.5	50.0	—	—	0.1	10
Dairy Food Substitutes														
Soybean milk, commercial type														
Soyagen: infant, all-purpose, or malt-flavored powder	—	30	52.5	185.0	0.25	0.870	3.4	11.90	6.6	23.34	—	—	32.2	11.30
Reconstituted liquid	1 c.	240	57.5	24.0	0.27	0.112	3.7	1.54	7.2	3.00	—	—	35.3	1.47
Soyalac, infant, concentrated, liquid, reconstituted	1 c.	240	72.5	30.0	0.178	0.074	3.28	1.37	27.50	11.46	—	—	22.75	9.48
Prepared Plant Protein														
Gluten-type entrées														
Chopjets	2 oz.	60	54.0	90.0	0.048	0.080	7.7	12.8	17.0	28.5	6.0	10.0	3.5	5.8
Dinner Cuts	2 oz.	60	36.0	60.0	0.032	0.054	3.0	5.0	13.2	22.0	9.5	12.5	3.0	5
Vegeburger	2 oz.	60	53.4	89.0	0.036	0.060	3.6	6.0	15.0	25.0	6.0	10.0	3.0	5
Weighted average‡	2 oz.	60	46.2	77.0	0.036	0.060	4.2	7.0	14.7	24.5	6.6	11.0	3.2	5.3
Nutmeat-type entrées														
Linketts	2 oz.	60	55.8	93.0	0.100	0.164	5.7	9.5	22.2	37.0	7.2	12.0	6.0	10
Nuteena	2 oz.	60	25.8	43.0	0.121	0.202	22.8	38.0	52.8	88.0	11.4	19.0	6.0	10
Patties	2 oz.	60	39.6	66.0	0.021	0.035	2.4	4.0	76.2	127.0	48.0	80.0	40.8	68
Proteena	2 oz.	60	43.2	72.0	0.125	0.208	13.2	22.0	19.2	32.0	6.9	11.5	6.6	11
Vegalinks	2 oz.	60	42.0	70.0	0.016	0.026	4.2	7.0	42.3	70.5	18.0	30.0	16.8	28
Weighted average‡	2 oz.	60	45.4	76.0	0.112	0.186	9.3	15.3	34.2	57.0	12.3	20.5	10.0	16.8

‡Weighted on the basis of sales per product per year.

TABLE 5 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP III) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0030	BIOTIN	03	FORMULAS(B)	*	.00012	.00030

TABLE 6, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
BIOTIN NAS 0030	*/ *	33	43	43			43

TABLE 7, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
BIOTIN NAS 0030	03	FORMULAS(B)	*	0-5 MO.	.402840	.738000	1.007100
				6-11 MO.	.082080	.391080	.205200
				12-23 MO.	.026400	.007440	.016000
BIOTIN NAS 0030	ALL CATEGORIES		*	0-5 MO.	.402840	.738000	1.007100
	**********			6-11 MO.	.082080	.391080	.205200
	**********			12-23 MO.	.026400	.007440	.016000

BIOTIN

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Barnes-'70
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Lancet 2: 244-245, 1970.

BIOTIN-RESPONSIVE PROPIONICACIDÆMIA

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Summary A child with propionicacidæmia and secondary ketotic hyperglycinæmia showed a greatly modified biochemical response to isoleucine loading after treatment with biotin.

INTRODUCTION

PROPIONICACIDÆMIA and methylmalonicacidæmia may both be associated with secondary ketotic hyperglycinæmia.[1,2] These conditions are due to the failure of the activity of propionyl-CoA carboxylase and methylmalonyl-CoA mutase, respectively. Methylmalonyl-CoA mutase is a coenzyme-B_{12} dependent enzyme, and some cases of methylmalonicacidæmia respond to high doses of vitamin or coenzyme B_{12}.[3,4] Some children with propionicacidæmia may respond to treatment with biotin, the coenzyme for propionyl-CoA carboxylase.

We have investigated a child with ketotic hyperglycinæmia, and found a raised plasma-propionate. The effect of biotin on this child's response to isoleucine, a precursor of propionyl CoA, is reported here.

CASE-REPORT

This boy born to unrelated Italian parents 2 years ago developed tachypnœa from the second day of life. He then became drowsy, hypotonic, and showed athetoid movements. Metabolic acidosis, ketosis and hyperglycinæmia were demonstrated. Although he has been maintained on a low-protein diet, frequent episodes of ketosis and acidosis have occurred. He is retarded in growth and development and remains extremely hypotonic. Of the previous five children of this marriage, three are well but two children, both male, died at the age of 4 days with unexplained respiratory distress.

METHODS

Plasma-propionate and urinary ketones were measured as described previously.[1]

The child was stabilised on a diet containing only 0·1 g. protein/ per kg. body-weight and then an oral load of L-isoleucine (0·1 g. per kg. body-weight) was given. Blood-samples were taken 0, 4, 12, and 24 hours after the isoleucine load, and urine was collected for 24 hours. This test was repeated after treatment with biotin, 5 mg. twice daily orally for 5 days.

RESULTS

Plasma-propionate concentrations are shown in fig. 1. The initial isoleucine-loading test produced a rise in plasma-propionate which was maintained for 12 hours after the test. Treatment with biotin reduced both the resting plasma-propionate concentration and the response to isoleucine. The height of the response and its duration were reduced, the plasma-propionate returning to the resting level less than 12 hours after the isoeucine load.

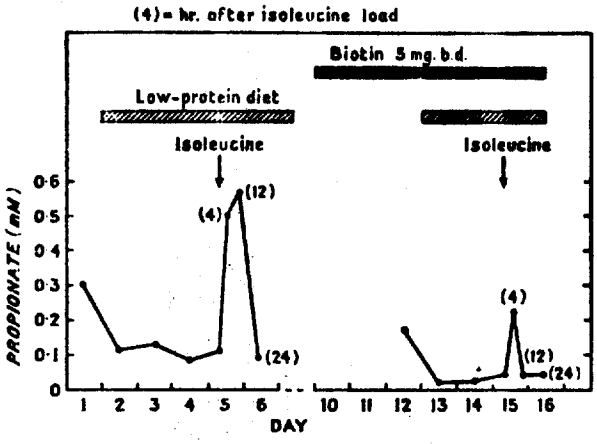

Fig. 1—Effect of biotin on plasma-propionate response to an isoleucine load.
Arrow shows injection point.

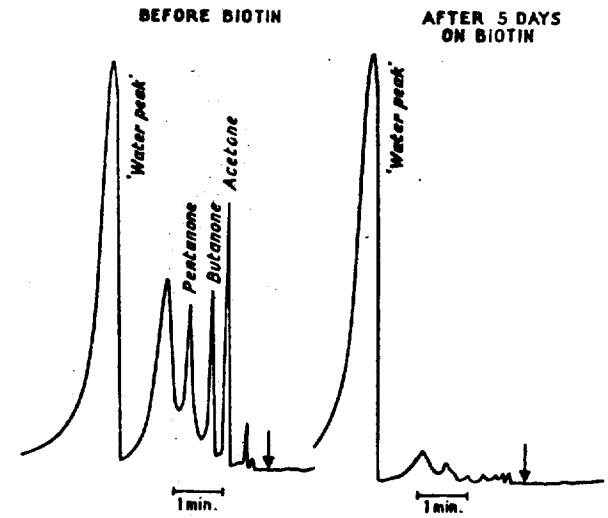

Fig. 2—Gas-chromatogram tracings of urinary ketone excretion after an isoleucine load.
Arrow shows injection point.

Aminoacid analysis on the first day of the study confirmed that there was a raised plasma-glycine (1·03 m*M*). Ketonuria was demonstrated only during the first loading test, and analysis of the urinary ketones during this period by gas-liquid chromatography showed abnormal excretion of the long-chain ketones, butanone and pentanone (fig. 2).

There was no significant change in the patient's clinical state during either isoleucine-loading test. However, there seemed to be a slight improvement in the patient's alertness and muscle tone on biotin.

DISCUSSION

Biotin is linked to the apoenzyme of propionyl-CoA carboxylase through an ε-amino group of lysine and another enzyme, apoenzyme-biotin ligase, is responsible for this attachment. Alteration of the patient's response to an isoleucine-loading test by treatment with biotin suggests that there is a failure to attach biotin to propionyl-CoA carboxylase. The defect in this patient might be due either to an altered propionyl-CoA carboxylase apoenzyme or to an altered ligase. Treatment with high doses of biotin could overcome the metabolic block if either of these enzymes were modified in such a way that they had a low affinity for biotin.

Treatment with biotin abolished the long-chain ketonuria seen during the first loading test. The very high butanone excretion is consistent with the suggestion that butanone is derived from an intermediate formed during the isoleucine degradation.[5]

The previous patient with propionicacidæmia studied by us [1] was given a comparable dose of biotin parenterally on two occasions, but showed no clinical or biochemical improvement. It seems that there may be both vitamin-responsive and non-responsive variants of propionicacidæmia as described for methylmalonicacidæmia.[3,4]

Further observations will demonstrate whether the child's protein tolerance or clinical condition are improved by biotin.

We thank Prof. C. C. Booth for advice and encouragement and the National Fund for Research into Poliomyelitis and other Crippling Diseases and the Medical Research Council for financial support. We also thank Dr. D. Brenton of University College Hospital Medical School for plasma-aminoacid analyses.

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Benschoter-'67
Bib No. 106

J. Econ. Entomol. 60(5):1326-1328. 1967.

Effect of Dietary Biotin on Reproduction of the House Fly[1, 2, 3]

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ABSTRACT

Sterilizing activity of biotin was demonstrated with adults of the house fly, *Musca domestica* L. Diets containing as little as 0.25% biotin reduced egg hatch by about 50%, but continuous feeding on 2% biotin in food was necessary to effect complete sterility of females. Male flies were only slightly affected at a 2% dosage, and morphological changes in the testes were not apparent. Mortality increased with time but had not reached 50% in either sex at the end of a 15-day period. Females fed this medium for short intervals of 2–10 days were able to produce small numbers of viable eggs when returned to standard diet and mated with untreated males. Flies found normal food more palatable than biotin-treated medium. However, indications were that the sodium hydroxide solution used as the solvent for biotin was the principal cause of this response.

Sterilizing effects obtained by Benschoter and Paniagua (1966) by feeding biotin (Nutritional Biochemicals Corp., Cleveland, Ohio)[3] to Mexican fruit flies, *Anastrepha ludens* (Loew), aroused curiosity as to whether this vitamin would affect other insects in the same manner. Adults of the house fly, *Musca domestica* L., were selected for testing, because biotin can easily be incorporated into their food medium. Mitlin et al. (1957) and Mitlin and Baroody (1958) demonstrated that house fly fertility was inhibited when the adults were fed diets containing several chemical substances classified as mitotic poisons and antimetabolites. LaBrecque et al. (1960) also did extensive work in this direction with the house fly.

Experiments were designed to determine the effect of this vitamin on the reproductive capacity of the house fly. Biotin was dissolved in 5 ml of a 1%

[1] Diptera: Muscidae.
[2] Accepted for publication April 26, 1967.
[3] Mention of a proprietary product does not necessarily imply its endorsement by the USDA.

aqueous solution of NaOH and added to 5 g of fly food composed of a 6:6:1 dry mixture of powdered milk, sugar, and powdered egg. Sodium hydroxide alone had no measurable effect on house fly fecundity and fertility and was used in check lots. However, it did reduce longevity slightly. Food plates were dried and offered to flies as a solid homogeneous cake. Effects were evaluated on the basis of fly mortality, egg fecundity and fertility, and condition of the gonads.

To determine dosage effect, biotin concentrations of 0.25, 0.5, 1.0, and 2.0% by weight were tested in food against 25 pairs of randomly selected insects. Induced feeding was continuous from time of fly emergence. Six egg samples for fecundity and fertility determinations were collected over a period of 15 days. Mortality was recorded at 5-day intervals throughout the same period (Table 1). Both egg fecundity and hatchability were negatively correlated with dose. Mortality increased with dose for both sexes but it was greater for males.

When sexes were fed separately on 2% biotin in food for periods of 2, 6, 10, and 14 days and then returned to the standard diet, males were found to be only slightly affected by extended feeding as measured by egg eclosion of untreated females with which they were mated. Morphological changes in testes were not apparent. Biotin-fed females were exposed to untreated males after each feeding period and egged 5 times at 2-day intervals. Fecundity ranged from normal after 2 days of feeding to 0 after 14 days of feeding. The corresponding egg eclosion declined from 77 to 0%. Results indicate that female flies must feed on this vitamin continually if reproductivity is to be strongly inhibited. The effect of continual feeding by females on this compound is illustrated in Fig. 1.

An experiment was designed to compare biotin-feeding effects on both sexually immature and sexually mature flies and to determine whether the sterilizing action could be reversed when the vitamin was removed from the diet. A concentration of 2% biotin in food was used. Feeding was initiated with 50 pairs of newly emerged flies per treatment and progressed as shown in Table 2. The results indicate that both young and old flies can be sterilized by continual feeding on this compound. However, the sterilizing action of biotin in this instance appeared to be permanent, since a return to standard diet did not restore female fecundity. It may be that the treatment intervals were too long to accurately measure this parameter. Mortality again was consistently higher for males.

To determine the palatability of this vitamin, flies were allowed to feed ad libitum on both biotin-treated and standard food. In separate tests, 2 doses of biotin (1 and 2%), as well as NaOH alone (4 ml of a 1% aqueous solution), in food were compared with untreated food. Based on 21 consecutive observations of feeding response, the following conclusions were drawn: (1) mortality was positively correlated with concentration of biotin in the food; (2) feeding frequencies on standard food were 2.3, 1.2, and 2.8 times greater than on foods treated with 2% biotin, 1% biotin, and NaOH alone, respectively. These data suggest that NaOH is the principal repellent factor in the food mixtures. The use of more dilute concentrations of NaOH or some less caustic solvent in biotin diets might reduce feeding inhibition.

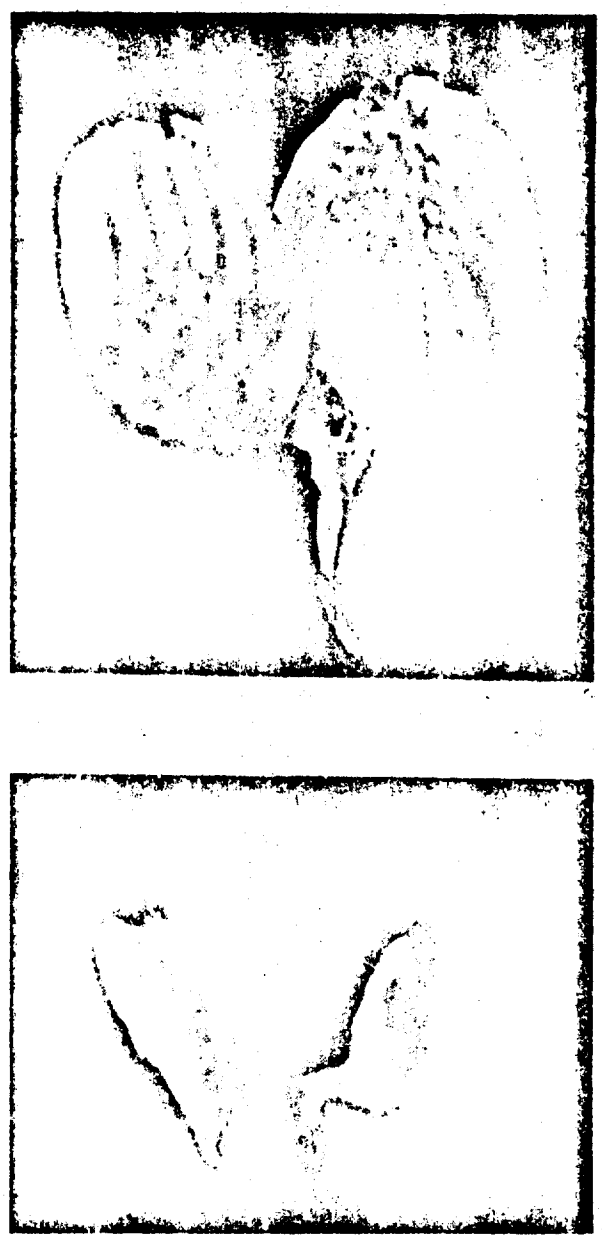

FIG. 1.—Ovaries of house fly (control above and biotin-fed flies below).

Table 1.—Relation of dietary biotin to sexual development and longevity of house flies. (Continual feeding initiated with newly emerged adults.)

% biotin in food	Total no. eggs produced (5 eggings)	% eclosion[a]	% mortality[a] of adults after 15 days ♀	♂
0.25	428	49.5	12	20
.5	187	25.1	12	28
1.0	99	12.1	24	36
2.0	85	0.0	32	44

[a] Corrected from control lots. (Hatch in controls > 85%.)

Table 2.—Chronological record of house fly response to untreated food vs food with biotin added as measured by adult mortality and egg fecundity and hatchability.

Egg collection dates	Age of flies (days)	Cage no. 1				Cage no. 2			
		Cumulative % mortality		No. eggs collected	% eclosion	Cumulative % mortality		No. eggs collected	% eclosion
		♂	♀			♂	♀		
		Standard food				*Standard food + biotin*			
5/30				0				0	
6/1	4			100+	92			0	
3				100+	92			0	
5	8	4	0	100+	84	6	14	0	
		Standard food + biotin				*Standard food*			
6/7				100+	82			0	
9	12			52	65			0	
11		10	4	0		46	20	0	
13	16	20		0		86	48	21	0
15[a]				0				0	

[a] Diets were again switched on 6/16, but subsequent egg collections through 6/23 were negative. All males dead on 6/23.

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Benschoter-'66
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Reproduction and Longevity of Mexican Fruit Flies, *Anastrepha ludens* (Diptera: Tephritidae), Fed Biotin in the Diet[1]

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ABSTRACT

Tests conducted at Mexico City during 1962 showed that continuous feeding of both sexually immature and mature adult Mexican fruit flies, *Anastrepha ludens* (Loew), on biotin at concentrations of 0.1%–2.0% in food resulted in loss of reproductive capacity in females. When biotin was removed from the diet, females recovered their ability to produce eggs. Treated male flies produced viable spermatozoa even though morphological aberrations of the gonads resulted. Mating frequency was generally low, and the higher doses caused mortality. Short-period feedings (12 days or less) on 2% biotin, followed by a return to standard diet, did not permanently suppress the reproductive capacity of either sex. Gonad development, mating behavior, and longevity were normal. Flies given a choice of food with or without biotin showed no preference.

Vitamins constitute a group of chemical substances generally known to be essential to vigorous animal growth, but some produce metabolic disturbances when administered in large doses. In Mexico City during 1962 we tested biotin[3] in the diet of the adult Mexican fruit fly, *Anastrepha ludens* (Loew), to determine its effect on the reproductive capacity of this insect. Woolley (1952) stated that no extensive study of the effect of biotin and its analogs on animals had been recorded. Gilmour (1961) cited studies establishing the fact that this vitamin in trace amounts is an essential metabolite for some insects.

[1] Accepted for publication June 23, 1965.
[2] Now at Brownsville, Texas, Box 1033.
[3] Available from Nutritional Biochemicals Corp., under this name. Mention of a company name does not necessarily imply endorsement of this product by the USDA.

PROCEDURES AND RESULTS

Biotin was dissolved in 5 ml of a 1% aqueous solution of NaOH, then added to 5 g of fly food composed of a 4:1 mixture of granulated sugar and orange juice crystals. Sodium hydroxide alone in the food had no measurable effect on the flies and was used in control lots. Food plates were dried, then offered to flies as a solid homogeneous cake. Effects were evaluated on the basis of fly mortality, egg fecundity and fertility, mating frequency, and condition of the gonads.

Experiment 1.—Dosages of 0.1%–2.0% of biotin in food were tested against sexually immature adult flies, 50 pairs to a cage. Two groups were tested at each dose. Feeding began as soon as the flies emerged and was continuous for 24 days. Egg samples for

Table 1.—Fecundity, fertility, and longevity of Mexican fruit flies continuously fed biotin beginning when they were sexually immature and up to 24 days of age (50 pairs of flies/cage, 2 replicates of each test).

Biotin in food (%)	Total eggs produced	Hatch (%)[a]	Mortality[a] after 24 days (%)	
			♀	♂
0.1	150	25.4	0	0
.25	51	0	0	0.5
.5	0	0	0	20.8
1.0	0	0	27.9	56.1
2.0	0	0	63.2	73.0

[a] Corrected after comparison with that in control lots.

fertility and fecundity determinations were collected when flies were 12 and 19 days old. Mortality counts were made twice a week for the duration of the experiment. Egg production was completely inhibited with 0.5% biotin in the food. Slight mortality resulted at the end of 24 days at this same concentration but increased with dose, the effect being greater for males than for females (Table 1).

Examination of female flies revealed that ovaries were undeveloped (Fig. 1). Although the effect on male gonads was less pronounced, the testes appeared shriveled and changed from normal yellow to deep orange (Fig. 1). The number of pairs of flies in copula was observed daily at 5:30 PM over a 15-day period. All biotin dosages produced marked reduction in matings as compared with those in the control (Fig. 2).

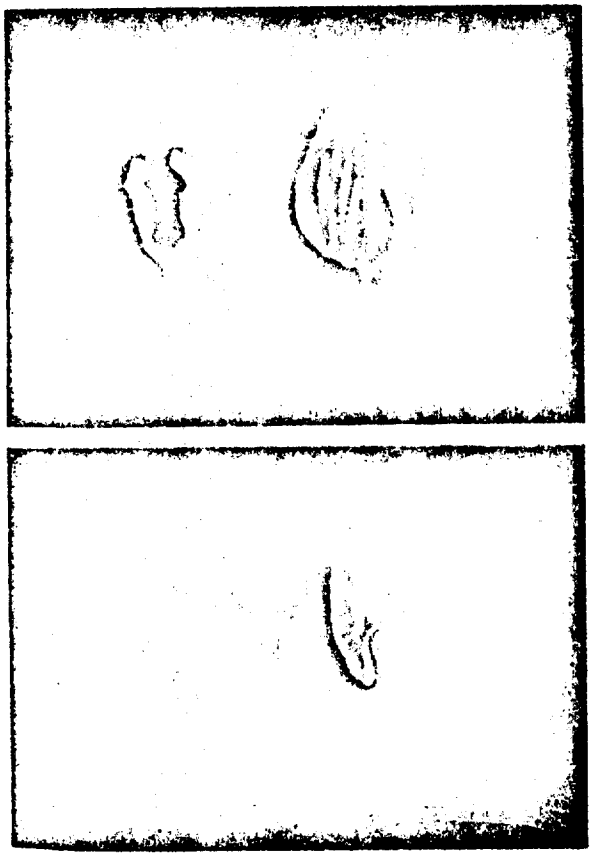

FIG. 1.—Ovaries (top) and testes (bottom) of Mexican fruit fly, abnormal in biotin-fed flies on left and normal on right. (Greatly enlarged.)

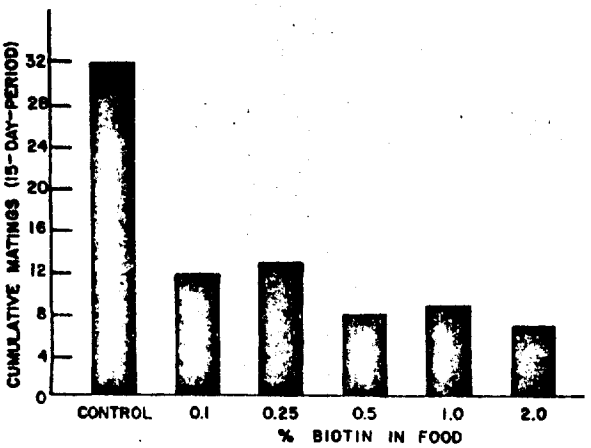

FIG. 2.—Comparison of mating frequency of Mexican fruit flies fed standard and biotin-supplemented diets.

A biotin analog, desthiobiotin,[3] was tested in the manner just described. Fly longevity and fecundity were normal, but a 2% dose reduced egg fertility by about 60%.

Experiment 2.—Groups of 30 pairs of sexually mature flies (14 days old) were fed continuously on food containing 0.5% biotin in duplicated tests (Table 2). A gradual reduction of fly fecundity and fertility was noted. Female longevity was normal, but male mortality averaged 35%. Mating frequency in both replicates was slightly less than that in the control. Examination of gonads at termination of the experiment revealed ovaries which appeared normal but were devoid of eggs and testes slightly shriveled and darker in hue.

Experiment 3.—Short-period feeding tests with sexually immature flies were conducted to determine to what extent each sex was affected, how long biotin

Table 2.—Fecundity and fertility of Mexican fruit flies fed for 30 days on a 0.5% concentration of biotin beginning when they were sexually mature (2 replicates of 30 pairs flies/treatment).

Egg collection date (1962)	Standard food (control)[a]		0.5% biotin in food[a]	
	Eggs produced	Hatch (%)	Eggs produced	Hatch (%)
July 7	323	89.2	223	80.0
16	208	73.6	45	8.4
23	139	75.5	10	0
30	153	74.5	10	0
Aug. 6	62	54.8	0	0
Totals:	885		288	

[a] Mean values for 2 replicates.

Table 3.—Fertility of Mexican fruit flies fed as sexually immature adults on 2% biotin for varying periods of time. (Sexes fed separately in lots of 50/treatment).

Test group[a]	Egg hatch (%) when feeding period was—[b]			
	2 days	4 days	8 days	12 days
T ♂ × UT ♀	98.7	90.4	93.6	100.0
T ♀ × UT ♂	94.7	100.0	75.1	73.1

[a] T = treated, UT = untreated.
[b] Mean values for 4 eggings, corrected from control lots.

had to be consumed to disrupt reproductive processes, and whether the effects were reversible. A dose of 2% biotin was used for feeding periods of 2, 4, 8, and 12 days. Sexes were fed separately in lots of 50, then placed over standard food and water with an equal number of untreated flies of the opposite sex. Data are shown in Table 3. Female flies were egged 1 week after close of each feeding period. Male fertility was not reduced as measured by egg eclosion of normal females with which they were mated. The slight reduction in egg eclosion of treated females indicated that the effects of biotin were not permanent. Gonads of both sexes subjected to biotin feeding were normal in appearance in all tests, and mating frequency was comparable with that in control lots.

Experiment 4.—Flies in the previous experiments were forced to feed on biotin. In this experiment lots of 100 pairs were offered food with and without biotin in separate containers to determine whether this vitamin was repellent to the flies. Feeding was continuous with food plates in each cage reversed every other day to preclude positional effect. Dosages of 0.5%, 1.0%, and 2.0% were tested with both young and old flies. Feeding responses were observed and recorded daily at 8:30 AM for 15 days. Food containing biotin, regardless of dosage, and standard food were equally palatable.

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Booth-'67
Bib No. 151

FEDERATION PROCEEDINGS
Vol. 26, No. 6, November-December, 1967
Printed in U.S.A.

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INTERSOCIETY SYMPOSIUM

GASTROENTEROLOGY

Sites of absorption in the small intestine

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UNTIL RECENTLY LITTLE WAS KNOWN of the functions of different segments of the small intestine. There are no obvious differences in mucosal cell structure in the proximal or distal parts of the small intestine and this made the concept of differential function difficult for some physiologists to accept. As long ago as 1887, however, Tappeiner (37) showed in experimental animals that the bile salts are not absorbed in the duodenum, but that absorption is maximal in the ileum. More recently, the discovery that vitamin B_{12} is specifically absorbed in the ileum has stimulated greater interest in sites of absorption. A great deal of work has been carried out in experimental animals but very much less attention has been paid to sites of absorption in man.

This paper first describes some of the methods which have been used to study sites of absorption in man, and indicates the principal factors that determine the site of absorption of a substance. The sites of absorption of the different dietary constituents are then considered separately.

METHODS

Modern techniques usually involve some method of intestinal intubation. The double lumen tube was introduced in 1934 by Miller and Abbott (26); it was passed by mouth and a test substance was introduced into the intestine through one lumen of the tube. The unabsorbed material was then aspirated through the other lumen from the distal site. For accuracy this technique required complete recovery of the unabsorbed material from the distal end of the segment under study and this is virtually impossible. Recently, the introduction of nonabsorbable markers has greatly improved the value of intubation techniques of this sort. Using such markers, substances can be perfused through a limited segment of either the proximal or the distal small intestine, and absorption is determined by measuring the ratio of the concentration of the test substance to that of the unabsorbed marker (34). This is a considerable advance because it is not necessary to recover all of the unabsorbed material. These methods indicate the capacity of a given segment of the gut to absorb and are best used for kinetic studies. They do not necessarily demonstrate the normal site of absorption. A more physiological method was suggested by Blankenhorn, Hirsch and Ahrens (2) and this has been used particularly by Borgström and others (8). A thin polyvinyl tube is swallowed and allowed to pass through the entire gastrointestinal tract. A meal, mixed with a nonabsorbable marker, is given and the intestinal contents are sampled through the tube at various levels. From analysis of the ratio of the various food substances to the concentration of the unabsorbed marker, absorption at different levels can be calculated. These methods, however, cannot be used for fat-soluble substances, since water-soluble markers are unsatisfactory for the study of fat-soluble material (39).

One of the most widely quoted methods of assessing sites of absorption is to measure absorption after resection of various segments of the small intestine. Such observations must be interpreted with considerable caution, for compensatory changes may occur in the gut after extensive resection. Other methods have included measurement of the active uptake of a test substance by the intestinal mucosa freshly taken from different areas, and in the case of B_{12}, assessment of the distribution of radioactivity at laparotomy after oral administration of a labeled test dose.

When sites of absorption in man are considered, it will be seen that for many substances there is relatively little evidence as to the site of absorption, the observations often being limited to the results of only one method at most.

From the Intersociety Symposium on "Gastroenterology" presented at the 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill., April 18, 1967.

[1] Professor Booth's attendance at this Symposium was made possible by a grant-in-aid from Roche Laboratories, division of Hoffmann-La Roche, Nutley, New Jersey (RKC).

FACTORS INFLUENCING SITES OF ABSORPTION

The factors which influence the site of absorption are first the relationship between the rate of absorption and the rate of transit through the small intestine. Since the jejunum is a highly motile organ, only substances which are absorbed fairly rapidly will be completely absorbed there. The slower the absorption rate, the more the substance will be propelled on into the distal intestine before absorption is complete. Many absorption mechanisms are in fact less active in the ileum than in the jejunum, but the motility of the ileum is sluggish and transit slow, so that even with relatively inactive transport systems in the ileum effective absorption can occur there.

The second factor is the mechanism of absorption, whether by passive diffusion or by an active process, and in the case of absorption by an active transport process, the distribution of this mechanism in the intestinal mucosa is important. Finally, the form in which the substance exists in food and the rate at which it is released from complex or bound forms may determine its site of absorption.

If a substance is absorbed by passive diffusion, whether through the water or the lipid phase of the intestinal cell membrane, it will clearly be absorbed by the proximal part of the intestine after oral feeding, and how far it travels down the gut during absorption will depend on its rate of diffusion. Furthermore, since the rate of diffusion is directly related to the concentration of the substance, the site of absorption will remain the same regardless of the amount of the test substance given. If, however, absorption occurs by active transport, absorption will again occur in the proximal intestine if the transport mechanism is present there; but as the oral load is increased the active mechanism may become saturated; the site of absorption may then become more distal, the distal intestine absorbing what has overflowed from the proximal. Under these circumstances the ileum acts as a functional intestinal reserve. But that is not the only function of the ileum, because if a substance is absorbed by an active transport mechanism which is only present in the ileum, absorption can only occur there and will not take place proximally under physiological circumstances.

CARBOHYDRATES

There is good evidence that sugars are absorbed from the proximal part of the small intestine. Borgström and his colleagues (8), using the Blankenhorn tube technique, studied the site of absorption of glucose from a mixed meal of corn oil, lactose, and protein and demonstrated that glucose was virtually entirely absorbed by the time the meal had passed through the duodenum and proximal 100 cm of jejunum.

On theoretical grounds, sugars which are not actively transported should be absorbed from both proximal and distal small intestine, but after oral feeding the proximal part of the intestine should be the main site of absorption. In man, indirect evidence has suggested that rhamnose, arabinose, and xylose are absorbed in the jejunum (13), and more recently direct studies by Fordtran, Soergal and Ingelfinger (16) have indicated that xylose is normally absorbed from the proximal small intestine. The ileum is also capable of absorbing xylose but only about one-sixth as effectively as the jejunum.

Much of the dietary carbohydrate is absorbed as disaccharide, and the final stage of digestion, hydrolysis of the disaccharide, takes place in the intestinal mucosal cells through the activity of the small intestinal disaccharidases. The sites of absorption of the disaccharides are, therefore, likely to be related to the sites in the small intestine of maximal disaccharidase activity. In man, there are low levels of sucrase, isomaltase, and lactase in the first part of the duodenum, but all the disaccharidases are present in the jejunum as well as in the ileum (1). The site of absorption of sucrose, maltose, and lactose was studied directly in man by Dahlqvist and Borgström (12) using an intubation technique and they found that although lactose and maltose were absorbed proximally, sucrose appeared to be absorbed in the ileum, a surprising finding since sucrase is present in the jejunal mucosa. This was reinvestigated by Gray and Ingelfinger (17), however, and these workers showed that sucrose absorption can occur from the jejunum.

PROTEIN

The most widely accepted view on the site of protein absorption in man is derived from the study of Borgström et al. (8) already referred to. In addition to studying glucose, these workers measured protein absorption at different levels in the small intestine and absorption appeared to occur from the proximal part of the gut. In these experiments, however, it was not protein that was measured but the absorption of a marker of ^{131}I labeled human serum albumin. This is unsatisfactory, since the ^{131}I label may become detached from the protein during absorption and this may give erroneous results (20). The site of absorption of dietary protein must therefore remain sub judice. Studies of nitrogen balance and of the absorption of tritium-labeled albumin in patients with extensive gut resections suggest a proximal absorption site for protein (21); but this is not yet proved and the extent to which the ileum participates in protein absorption is unknown.

FAT

There has been considerable controversy about the site of fat absorption in recent years. Some workers have claimed that the ileum is the main site of fat absorption, whereas others have suggested the jejunum. In experimental animals there is evidence that the jejunum is the major site of fat absorption. In man, histological and electron-microscopic studies have shown fat in the jejunal epithelial cells during absorption and these observations would suggest a proximal absorption site. Other evidence has been derived from intestinal intubation techniques

and from studies of patients with small intestinal resections. The intestinal intubation evidence, however, is of uncertain significance since it depends on the use of water-soluble markers. As already indicated, the results of intubation studies which have relied on water-soluble markers are not satisfactory for when a water-soluble marker is mixed with fat, there is separation of the two phases (39).

The evidence provided by studies of absorption in patients subjected to intestinal resections must therefore be considered, but the interpretation of such data in patients with gut resections is difficult. If the absorption of fat by patients with jejunal or ileal remnants is compared, it appears superficially that the ileum may be important in fat absorption, for ileal resection certainly can cause steatorrhea. But although frequently there is steatorrhea when the distal gut is resected, much of the dietary fat can still be absorbed even when there is only 2 feet of jejunum remaining—and some absorption occurs when there is as little as 8 inches (4). These findings support the idea of a proximal absorption site for fat in man and have suggested that the ileum is normally only necessary for completing the last stages of fat absorption, mopping up what the jejunum has missed as it were, and acting as a reserve when dietary intakes are high. The ileum can absorb fat well after the main absorbing area in the proximal intestine has been resected. A resection of 8 feet of the jejunum, for example, causes no steatorrhea, and even though there is some steatorrhea after resection of all the small gut but for the distal 18 inches of the ileum, much of the dietary fat can still be absorbed (4). These data have been used to indicate the sites of fat absorption but there are a number of snags. When there is ileal resection, for instance, there may be a reduction of bile salt reabsorption in the small intestine, and the steatorrhea in distal resection patients may therefore be more than can be explained by the resection alone (18, 19). Furthermore, compensatory changes may occur after resection.

RESPONSE OF RESIDUAL INTESTINE AFTER PARTIAL RESECTION

It was said many years ago that the intestinal villi become longer after gut resection (15), but a recent paper has failed to confirm this (30). After distal resection, however, the villi in man can show hypertrophy. The mean villus height in jejunal biopsy samples obtained by Dr. R. H. Dowling from a group of patients with distal gut resection is compared with that of control subjects in Table 1. There is a significant increase in villus height in the resection patients. This structural change is accompanied by an increase in function. Figure 1 shows the amount of glucose absorbed by a 25-cm segment of jejunum in control subjects and in patients with distal gut resection (13). The observations were made using a double-lumen tube perfusion technique. There is a significant but not great increase in glucose absorption after distal gut resection.

TABLE 1. *Mean villus height in control subjects compared with distal intestinal resection*

	No. of Subjects	Villus Height, μ*	Statistical Significance
Control subjects	11	382±20	$P < 0.05$
Patients with intestinal resection	12	440±16	

* Mean ± SEM.

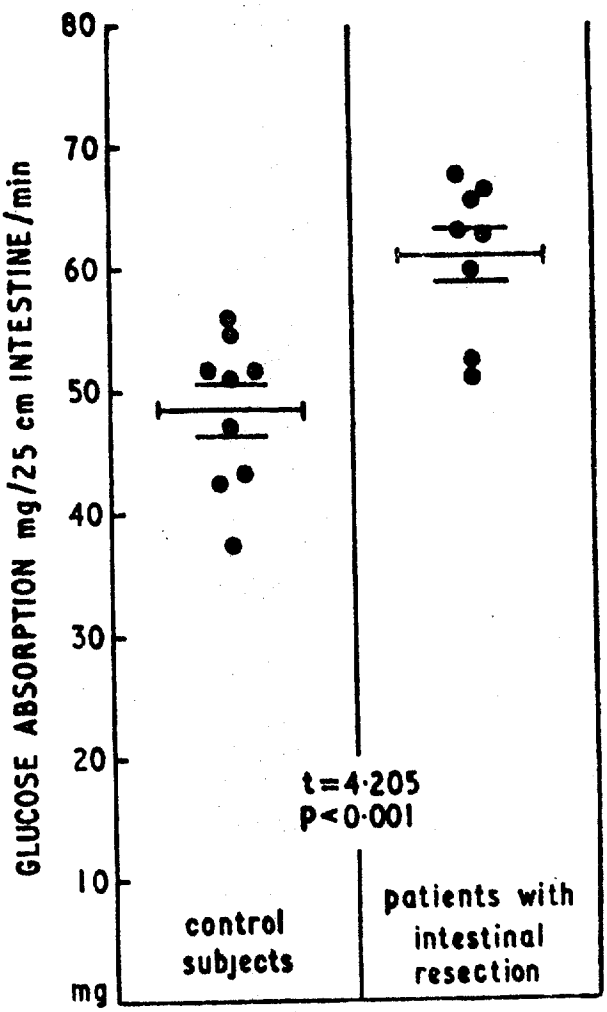

FIG. 1. Results of glucose absorption in milligrams (mean ± SEM) using the segmental-perfusion technique (13).

It is possible, however, that the functional change may be much more striking in the ileum after a proximal resection, for studies in experimental animals have shown a difference in the functional response of jejunum and ileum after resection. Groups of rats were subjected to resection of two-thirds of either the proximal or the distal small intestine (14). Table 2 shows the villus heights in control rat jejunum and ileum, in the jejunum after distal resection, and in the ileum after proximal resection. As in man, there is a slight but significant increase in jejunal villus height after distal resection.

But the changes in the ileal villi (which are normally shorter than those in the jejunum) are very much more striking. This anatomical change is accompanied by an increase in function. Figure 2 shows the amount of glucose absorbed by rat jejunum or ileum; the results were obtained using a recirculation perfusion technique. In the jejunum there was a significant increase in the absorption of glucose after distal resection, but (as in man) this increase was not gross. In the ileum, on the other hand, the increase was much more marked, the absorption of glucose from the ileum becoming virtually as effective as in the jejunum after the proximal intestine was resected. It is, therefore, clear that absorption sites may change under the unphysiological conditions of intestinal resection. It is not yet known whether the same change in absorptive capacity occurs in the ileum in man after a proximal resection, but the clinical observation that absorption is so much better after a proximal resection than after a distal resection may well be due to a similar mechanism, and data obtained after gut resection, particularly proximal resection, do not necessarily provide a valid method for determining sites of absorption. Since these are virtually the only data available on the site of fat absorption in man, with the exception of Borgström's observations using the Blankenhorn tube, it is clear that further information is required on the sites of fat absorption, and this necessitates the development of a fat-soluble, nonabsorbable marker.

FAT-SOLUBLE VITAMINS

There is even less information on the sites of absorption of the fat-soluble vitamins for there are no direct data available. But supposing these vitamins are absorbed by passive diffusion through the lipid phase of the intestinal cell membrane, it would be expected that they would be absorbed proximally in the gut. Direct observations on the site of absorption of vitamin A in animals suggest that vitamin A is absorbed in the jejunum (9). As far as vitamin D is concerned, Norman and de Luca (28) and Kodicek (22) have favored the unlikely idea that the ileum is the site of vitamin D absorption. On the other hand, Schachter, Finkelstein and Kowarski (33), using tied intestinal loops in the rat, have suggested that the jejunum is the main site of absorption of vitamin D. In man, the only data available are derived from absorption studies after gut resection. Figure 3 shows the plasma radioactivity in a patient with only 4 feet of jejunum remaining after being given an oral dose of 1 mg of ^{3}H-labeled vitamin D_3. Although the plasma radioactivity here was slightly subnormal, 60% was absorbed from this dose, which is normal (38). Subject to the criticism that this is a resection situation, it seems likely that vitamin D can be absorbed in the proximal small intestine in man.

TABLE 2. *Villus height after resection of two-thirds of either proximal or distal small intestine compared with villus height in control rats**

	No. of Rats	Mean Villus Height†	Statistical Significance
Control jejunum	24	323±11	$t = 3.18$
Jejunum after distal resection	23	369±9	$P < 0.01$
Control ileum	27	232±7	$t = 8.78$
Ileum after proximal resection	28	354±12	$P < 0.001$

* From Dowling and Booth (14). † Mean ± SEM.

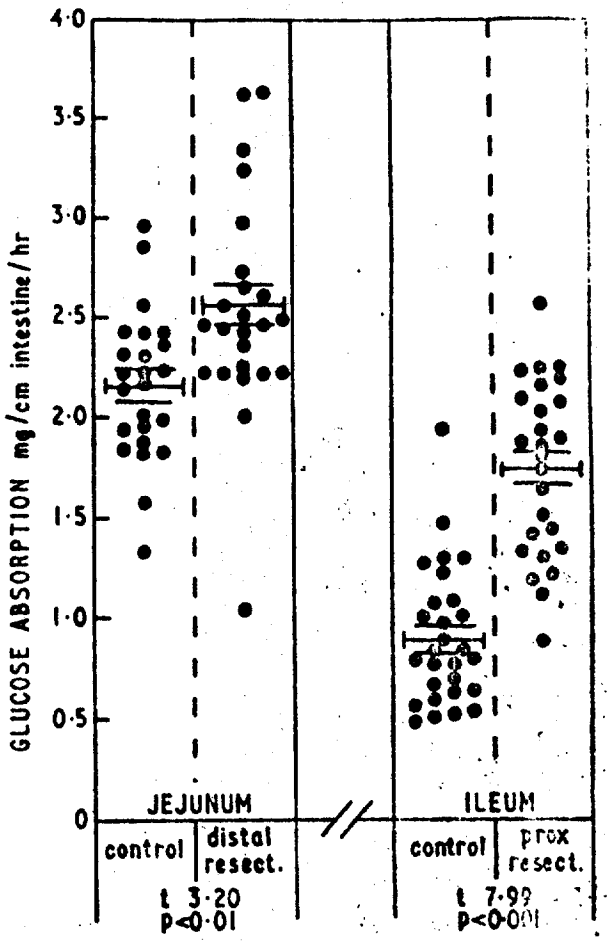

FIG. 2. Mean glucose absorption (± SEM) irrespective of time after operation, from the jejunal and ileal remnants after resection, compared with absorption from corresponding lengths of control intestine in the rat. Concentration of glucose in the infused fluid was 380 mg/100 ml. Absorption is expressed as milligrams per centimeter per hour (14).

WATER-SOLUBLE VITAMINS

As regards the water-soluble vitamins, there is again relatively little direct evidence on sites of absorption in man. No active transport mechanisms have yet been defined for ascorbic acid or for most of the B group vitamins. If their absorption is passive, as has been claimed for riboflavin (36), vitamin C (35), and pyridoxine (10), then absorption should occur proximally. Direct studies were carried out for vitamin C by Nichol-

son and Chomock (27) using the Miller Abbott tube, and these showed rapid absorption of vitamin C from the jejunum. The mechanism of absorption of folic acid is not yet known, but it may be that some form of facilitated diffusion is involved. Intubation studies demonstrate that the jejunum can absorb folic acid and, as with xylose, the ileum absorbs less (Hepner and Hoffbrand, unpublished data). There are many clinical observations in resection patients which suggest that folic acid is absorbed in the jejunum (11), and similar data are also available for pyridoxine (9), so that the available evidence suggests a proximal absorption site for vitamin C and many of the B group vitamins.

But vitamin B_{12}, as is well known, behaves quite differently. Vitamin B_{12} is unique in requiring an intrinsic factor secreted by the stomach to ensure its absorption; it is also unique in that there is a curious delay in the transport of B_{12} across the intestinal mucosa during absorption (5, 6); and it has a specific absorption site in the ileum (7). This was first suggested by clinical studies of B_{12} absorption in resection patients by McIntyre et al. (25), and their observation that B_{12} cannot be absorbed in the absence of the ileum has been repeatedly confirmed. In man, the first direct data on absorption in the ileum were obtained by studying the distribution of radioactivity in the gut at laparotomy, after oral administration of labeled B_{12} (7). Since then ileal absorption of vitamin B_{12} has been demonstrated in man using the double-lumen tube technique by Rønnov-Jessen and Hansen (31). In addition, Rosenberg, Lau and Herbert (32) have shown that homogenates of intestinal mucosa from human jejunum cannot take up B_{12} bound to intrinsic factor, whereas the vitamin can be effectively taken up by homogenates of the ileum. Data from at least four different methods of approach, therefore, indicate that the ileum is the site of absorption of B_{12} in man. A recent paper, however, has suggested that the jejunum can absorb B_{12} (24). This observation was made by a surgeon who studied B_{12} absorption from loops of intestine isolated between clamps at laparotomy and there was apparently no difference between jejunum and ileum. Whether such studies have physiological significance, however, is uncertain for McColl et al. (24) also found that intrinsic factor did not seem to be necessary for the B_{12} absorption that was demonstrated.

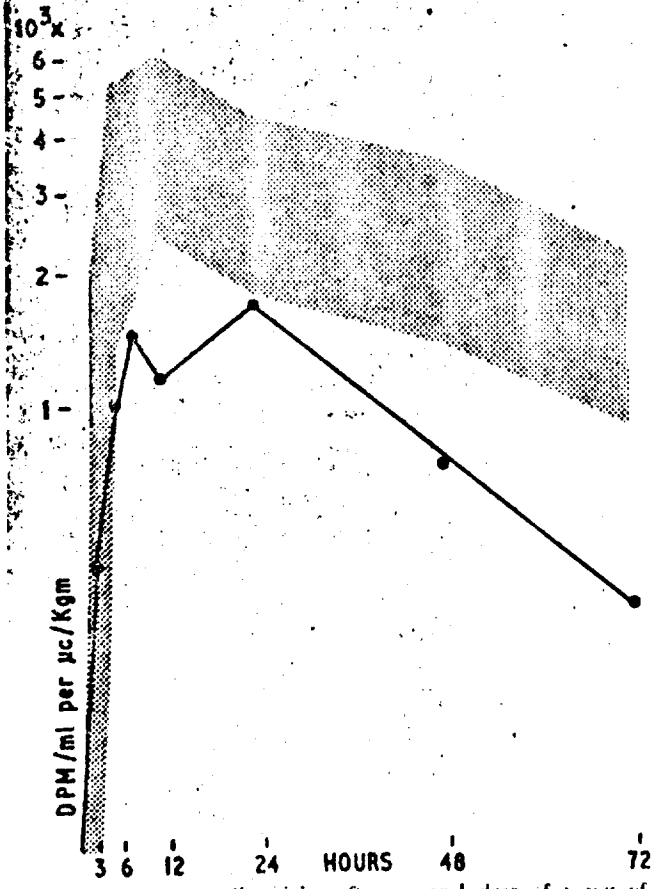

FIG. 3. Plasma radioactivity after an oral dose of 1 mg of H-labeled vitamin D_3 in a patient who had undergone resection of all the small intestine but for the proximal 4 feet of the jejunum. Hatched area indicates the normal range (38).

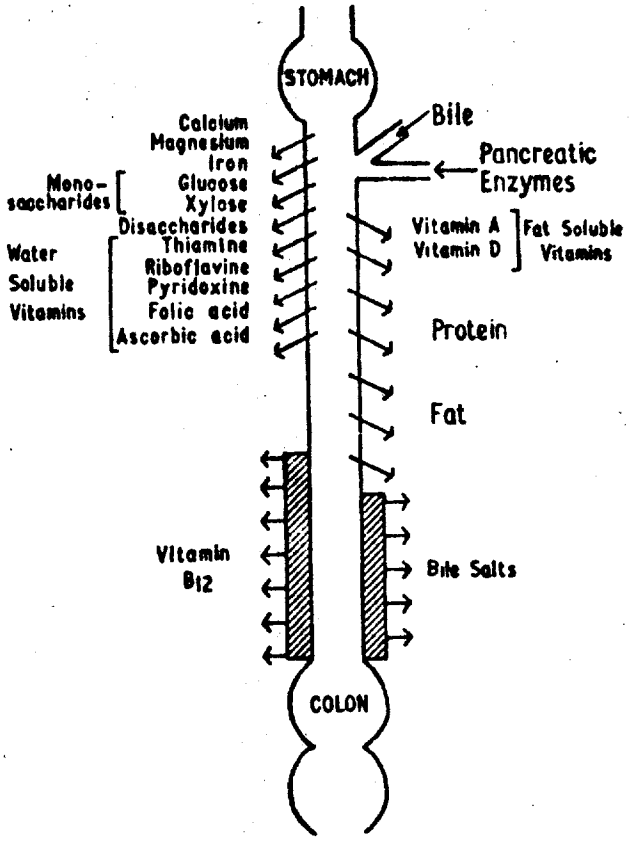

FIG. 4. Sites of absorption in the small intestine (3).

MINERALS

Finally, the sites of absorption of minerals should be considered. The first point is that although in vitro

studies in animals suggest that iron is specifically absorbed in the duodenum, this is certainly not true in vivo. In man, absorption can occur from the ileum if iron is put there through a tube. Similarly with calcium and magnesium, although absorption may take place proximally, distal absorption can also occur and it is not yet known how far the different regions of the gut normally contribute to the absorption of these divalent cations.

CONCLUSION

Figure 4 summarizes diagrammatically what seems to be true about sites of absorption in the small intestine (3). The diagram represents the small intestine as a simple tube, and shows that the proximal part of the small intestine is the major area of absorption of dietary constituents. However, the ileum can also absorb virtually all the substances normally absorbed proximally and it is therefore a reserve area for the absorption of substances that have escaped absorption proximally, as when a proximal transport mechanism has been saturated, or after resection of the proximal small gut. Also, although absorption is in general less active in the ileum, its sluggish motility favors absorption of those substances that reach this region. The ileum, therefore, not only absorbs B_{12} and the bile salts, but also acts as an admirable functional reserve.

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Vopr. Pitan. 31(3): 47-50. 1972.

Braginskii-'72
Bib No. 164

EFFECT OF DIFFERENT DOSES OF BIOTIN ON ASCORBIC ACID ALLOWANCE IN PATIENTS WITH ATHEROSCLEROSIS AND HYPERTONIC ILLNESS

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Abstract. The effect was studied of different doses of biotin on the organism's allowance of ascorbic acid in healthy persons and patients with atherosclerosis and hypertonic illness. The ascorbic acid yield in urine and its content in blood plasma on an empty stomach were determined, as well as its content in oxidized form (dehydroascorbic and diketogulonic acids). It is shown that giving patients 1 mg biotin a day for 7 and 14 days decreased its yield in urine and its content in blood, which may be considered as an increase in ascorbic acid utilization in the organism. 3- and 5-mg doses of biotin do not reveal such an effect.

In a preceding article we showed that in patients with atherosclerosis and hypertonic illness, a deficit in ascorbic acid in the organism is caused by disruption of its utilization. In this connection, it is necessary to increase ascorbic acid utilization in the organism in order to decrease or eliminate the deficit.

In our clinic, the positive effect of biotin was shown for indices of lipid exchange in patients with atherosclerosis and hypertonic illness. Under the influence of biotin, cholesterol content decreases in blood serum, the lecithin/cholesterol coefficient increases, and the relative and absolute β-lipoprotein content increase (1).

In the present investigation, data are presented on the effect of biotin on indices characterizing the ascorbic acid allowance in patients with atherosclerosis and hypertonic illness.

Ascorbic acid allowance was determined on the basis of its yield in daily urine samples over 3 days (the mean value was taken) and its content in blood plasma taken on an empty stomach. Ascorbic acid content was measured by A. N. Klimov's method (2), permitting the determination as well of its oxidized forms (dehydroascorbic and diketogulonic acids).

Biotin was given in 1-, 3-, and 5-mg doses per day, internally, for 7 and 14 days.

In all, 140 patients and 12 healthy subjects (serving as a control) were studied. Of the 140 patients studied, 51 suffered from atherosclerosis and 89 from hypertonic illness.

In the control group (Table 1) which received biotin once a day over 7 days, ascorbic acid yield in urine increased significantly, as did its content in blood. In addition, the content of the oxidized forms of ascorbic acid increased in the urine, but did not change in the blood. In this connection, it may be proposed that under the influence of biotin ascorbic acid yield from the organism increased due to its liberation from tissues.

As is evident from Table 1, biotin in 1-mg doses given over 7 days reflected a monotypic effect in patients with atherosclerosis (19) and with hypertonic illness (33). A fall in ascorbic acid yield in urine is noted after a single dose and still more after 7 days. Moreover, ascorbic acid content in the blood decreased in both groups, as well as did the absolute content of oxidized forms of ascorbic acid. Such a biotin effect may be considered an increase in ascorbic acid utilization in the organism, which proceeds due to an increased intake in tissues.

Table 1.

Effect of Biotin on Indices Characterizing Ascorbic Acid Allowance in Healthy Subjects and Patients with Atherosclerosis and Hypertonic Illness (M + or - m)

Group Studied	AA* Yield in Urine (in mg)								AA Content in Blood (in mg%)			
	Initial		After 1 day		After 7 days		After end of exper.		Initial		After intake	
	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG
	After 7 days in doses of 1 mg per day											
Healthy	22,7±0,9	6,9±0,2	28,2±3,5	8,4±1,3	35,1±2,9	10,2±0,8	23,9±2,5	7,1±0,6	0,98±0,07	0,23±0,04	1,2±0,07	0,42±0,03
Atherosc. patients	21,9±1,2	6,1±0,8	17,7±1,1	4,2±0,6	15,9±1,2	3,6±0,6	15,1±1,3	4,8±0,5	1,1±0,06	0,25±0,03	0,79±0,04	0,16±0,02
Hypertonic patients	24,4±1,5	6,6±0,8	19,9±1,3	5,2±0,7	15,8±1,2	3,9±0,6	18,4±1,3	5,6±0,6	0,99±0,05		0,22±0,03	0,76±0,04	0,17±0,02
	After 14 days in 1-mg doses per day											
Atherosc. patients	19,8±1,0	4,4±0,5	16,8±0,8	4,0±0,4	13,8±0,8	3,2±0,3	14,6±0,9	3,6±0,4	1,02±0,08	0,29±0,03	0,67±0,07	0,15±0,02
Hypertonic patients	22,3±1,0	6,7±0,6	18,6±1,2	5,6±0,5	12,9±0,8	4,4±0,3	12,1±0,7	3,4±0,3	1,11±0,06	0,40±0,05	0,70±0,07	0,20±0,02

* Here and in Table 2: AA = ascorbic acid, DAA = dehydroascorbic acid, DKG = diketogulonic acid

Table 2.

Effect of Biotin on Indices Characterizing Ascorbic Acid Allowance in Patients with Atherosclerosis and Hypertonic Illness (M + or - m)

Group Studied	AA* Yield in Urine (in mg) Initial		After 1 day		After 7 days		After end of exper.		AA Content in Blood (in mg%) Initial		After intake	
	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG	Over-all AA	DAA + DKG
After 7 days in doses of 3 mg per day												
Atherosclerotic patients	19,4±1,1	6,8±0,5	18,1±1,7	5,9±0,7	24,2±2,1	7,8±0,2	18,6±2,5	6,5±1,3	0,98±0,05	0,39±0,04	1,20±0,07	0,56±0,08
Hypertonic patients	17,7±1,2	5,5±0,6	18,4±1,6	6,5±1,0	23,4±1,3	8,5±0,6	16,8±1,3	6,3±0,9	0,87±0,05	0,32±0,03	1,2±0,07	0,6±0,04
After 7 days in 5 mg doses per day												
Atherosclerotic patients	21,6±1,3	8,1±0,8	24,4±2,8	9,4±1,3	19,1±2,7	6,9±1,5	18,3±1,8	6,4±0,9	0,9±0,04	0,23±0,02	0,76±0,03	0,22±0,02
Hypertonic patients	22,1±1,4	8,3±0,8	24,2±1,6	10,4±1,0	23,0±1,4	9,1±0,9	20,1±1,2	7,8±0,8	0,77±0,05	0,28±0,03	0,79±0,04	0,3±0,03

Still more obvious is the decrease in ascorbic acid yield in urine and the fall in content in blood noted after 14 days of biotin intake at the same dosage (see Table 1).

The data obtained are evidence that biotin contributes to an increase in ascorbic acid utilization in an initial deficit and increases its yield in the absence of a deficit (control).

In 11 patients with atherosclerosis and 18 with hypertonic illness, the effect of biotin was studied for 3-mg doses per day, given internally, for the same indices (Table 2). As is evident, in patients with atherosclerosis and hypertonic illness, after a single administration of biotin, ascorbic acid yield in urine do not change significantly. After 7 days, ascorbic acid yield in urine increases significantly. On the second day after cessation of biotin dosage, ascorbic acid yield decreases and approaches the initial value. Ascorbic acid content in blood after biotin intake also increases.

Thus, for 3-mg doses daily, biotin shoes an opposite effect compared to that for 1-mg doses.

In 31 patients (10 with atherosclerotic coronary sclerosis and 21 with hypertonic illness), the same indices were studied for 5-mg doses of biotin (see Table 2). In atherosclerotic patients under the effect of single and 7-day administration of biotin, ascorbic acid yield in urine does not change significantly, but its content in blood decreases. In hypertonic patients after biotin administration, ascorbic acid yield in urine and its content in the blood do not change significantly either.

Thus, there is a definite relationship between biotin and ascorbic acid, which depends on biotin dose and the initial ascorbic acid allowance. In atherosclerotic and hypertonic patients, a biotin dose of 1 mg per day, given internally, contributes to ascorbic acid utilization in the organism due to a decrease in its content in the blood.

Considering the positve lipotropic effect of 1-mg biotin doses per day and the nature of its influence on ascorbic acid allowance, this dose may be recommended for complex therapy in atherosclerosis and Stage 2 and 3 hypertonic illness.

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Braginskii-'72
Vopr. Pitan. 31 (3): 47-50. 1972. Bib No. 164

УДК 616.13-004.6+616.12-008.331.1]-085.356:577.164.18]-07:616-008.934.752-074

ВЛИЯНИЕ РАЗЛИЧНЫХ ДОЗ БИОТИНА НА ОБЕСПЕЧЕННОСТЬ АСКОРБИНОВОЙ КИСЛОТОЙ БОЛЬНЫХ АТЕРОСКЛЕРОЗОМ И ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНЬЮ

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Изучено влияние различных доз биотина на обеспеченность организма аскорбиновой кислотой у здоровых людей и больных атеросклерозом и гипертонической болезнью. Определяли выделение аскорбиновой кислоты с мочой и содержание ее в плазме крови натощак, а также содержание ее окисленных форм (дегидроаскорбиновой и дикетогулоновой). Показано, что при приеме 1 мг биотина в день в течение 7 и 14 дней у больных уменьшается выделение аскорбиновой кислоты с мочой и содержание ее в крови, что может расцениваться как повышение утилизации аскорбиновой кислоты в организме больных. Доза биотина в 3 и 5 мг в день такого действия не оказывает.

В предыдущем сообщении нами было показано, что у больных атеросклерозом и гипертонической болезнью дефицит аскорбиновой кислоты в организме обусловлен нарушением утилизации ее. В связи с этим необходимо усилить утилизацию аскорбиновой кислоты в организме для уменьшения или ликвидации дефицита ее.

В нашей клинике было отмечено положительное влияние биотина на показатели липидного обмена у больных атеросклерозом и гипертонической болезнью. Под влиянием биотина уменьшается содержание холестерина в сыворотке крови, возрастает коэффициент лецитин/холестерин, уменьшается относительное и абсолютное содержание β-липопротеидов [1].

В настоящем исследовании представлены данные о влиянии биотина на показатели, характеризующие обеспеченность аскорбиновой кислотой больных атеросклерозом и гипертонической болезнью.

Об обеспеченности аскорбиновой кислотой судили на основании выделения ее с суточной мочой в течение 3 суток (брали среднее значение) и содержания ее в плазме крови натощак. Содержание аскорбиновой кислоты измеряли по методу А. Н. Климова [2], позволяющему определять также и ее окисленные формы (дегидроаскорбиновую и дикетогулоновую).

Биотин применяли в дозах 1, 3 и 5 мг в сутки внутрь в течение 7 и 14 дней.

Всего обследовано 140 больных и 12 здоровых (служивших контролем). Из 140 обследованных больных 51 больной атеросклерозом и 89 гипертонической болезнью II и IIIА стадий.

У лиц контрольной группы (табл. 1), принимавших биотин 1 раз в сутки в течение 7 дней, статистически достоверно увеличивается выделение аскорбиновой кислоты с мочой и возрастает содержание ее в крови. При этом повышается содержание окисленных форм аскорбиновой кислоты в моче и не меняется их содержание в крови. В связи с этим можно предположить, что под влиянием биотина увеличивается выделение аскорбиновой кислоты из организма за счет высвобождения ее из тканей.

Как видно из табл. 1, биотин в дозе 1 мг при приеме его в течение 7 дней оказал однотипное влияние как у больных атеросклерозом (19), так и у больных гипертонической болезнью (33). Отмечено падение выделения аскорбиновой кислоты с мочой как после однократного приема, так и еще более выраженное после 7-дневного приема. При этом уменьшается содержание аскорбиновой кислоты в крови в обеих группах, а также абсолютное содержание окисленных форм аскорбиновой кислоты. Такое влияние биотина можно расценить как усиление утилизации аскорбиновой кислоты в организме, которое происходит благодаря повышенному поступлению ее в ткани.

Таблица 1

Влияние биотина на показатели, характеризующие обеспеченность аскорбиновой кислотой здоровых, больных атеросклерозом и гипертонической болезнью ($M \pm m$)

Группа обследуемых	Выделение АК с мочой (в *мг*)								Содержание АК в крови (в *мг*%)			
	исходное		после 1-го дня приема		после 7-го дня приема		после прекращения приема		исходное		после приема	
	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК
После 7-дневного приема в дозе 1 мг в сутки												
Здоровые лица	22,7±0,9	6,9±0,2	28,2±3,5	8,4±1,3	35,1±2,9	10,2±0,8	23,9±2,5	7,1±0,6	0,98±0,07	0.23±0,04	1,2±0,07	0,42±0,06
Больные атеросклерозом	21,9±1,2	6,1±0,8	17,7±1,1	4,2±0,6	15,9±1,2	3,6±0,6	15,1±1,3	4,8±0,5	1,1±0,06	0,25±0,03	0,79±0,04	0,16±0,02
Больные гипертонической болезнью	24,4±1,5	6,6±0,8	19,9±1,3	5,2±0,7	15,8±1,2	3,9±0,6	18,4±1,3	5,6±0,6	0,99±0,05	0.22±0,03	0,76±0,04	0,17±0,02
После 14-дневного приема в дозе 1 мг в сутки												
Больные атеросклерозом	19,8±1,0	4,4±0,5	16,8±0,8	4,0±0,4	13,8±0,8	3,2±0,3	14,6±0,9	3,6±0,4	1,02±0,08	0,29±0,03	0,67±0,07	0,15±0,02
Больные гипертонической болезнью	22,3±1,0	6,7±0,6	18,6±1,2	5,6±0,5	12,9±0,8	4,4±0,3	12,1±0,7	3,4±0,3	1,11±0,06	0,40±0,05	0,70±0,07	0,20±0,02

Обозначения здесь и в табл. 2: АК — аскорбиновая кислота, ДАК — дегидроаскорбиновая кислота, ДКГК — дикетогулоновая кислота.

Таблица 2

Влияние биотина на показатели, характеризующие обеспеченность аскорбиновой кислотой больных атеросклерозом и гипертонической болезнью (M±m)

Группа обследуемых	Выделение АК с мочой (в *мг*)								Содержание в крови (в *мг%*)			
	исходное		после 1-го дня приема		после 7-го дня приема		после прекращения приема		исходное		после приема	
	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК	общая АК	ДАК+ДКГК
После 7-дневного приема в дозе 3 мг в сутки												
Больные атеросклерозом	19,4±1,1	6,8±0,5	18,1±1,7	5,9±0,7	24,2±2,1	7,8±0,2	18,6±2,5	6,5±1,3	0,98±0,05	0,39±0,04	1,20±0,07	0,56±0,08
Больные гипертонической болезнью	17,7±1,2	5,5±0,6	18,4±1,0	6,5±1,0	23,4±1,3	8,5±0,6	16,8±1,3	6,3±0,9	0,87±0,05	0,32±0,03	1,2±0,07	0,6±0,04
После 7-дневного приема в дозе 5 мг в сутки												
Больные атеросклерозом	21,6±1,3	8,1±0,8	24,4±2,8	9,4±1,3	19,1±2,7	6,9±1,5	18,3±1,8	6,4±0,9	0,9±0,04	0,28±0,02	0,76±0,06	0,22±0,02
Больные гипертонической болезнью	22,1±1,4	8,3±0,8	24,2±1,6	10,4±1,0	23,0±1,4	9,1±0,9	20,1±1,2	7,8±0,8	0,77±0,05	0,28±0,03	0,79±0,04	0,3±0,03

Еще более выраженное уменьшение выделения аскорбиновой кислоты с мочой и падение содержания ее в крови отмечены после 14-дневного приема биотина в той же дозе (см. табл. 1).

Полученные данные свидетельствуют, что биотин способствует повышению утилизации аскорбиновой кислоты при исходном дефиците ее и усиливает выделение ее при отсутствии дефицита (контроль).

У 11 больных атеросклерозом и 18 больных гипертонической болезнью изучали влияние биотина при приеме в дозе 3 мг в сутки внутрь на те же показатели (табл. 2). Как видно, у больных атеросклерозом и гипертонической болезнью после однократного приема биотина выделение аскорбиновой кислоты с мочой статистически достоверно не меняется. После 7-дневного приема статистически достоверно увеличивается выделение аскорбиновой кислоты с мочой. На 2-й день после прекращения приема биотина выделение аскорбиновой кислоты снижается и приближается к исходному. Содержание аскорбиновой кислоты в крови после приема биотина также возрастает.

Таким образом, в дозе 3 мг в сутки биотин оказывает противоположный эффект по сравнению с дозой в 1 мг.

У 31 больного (10 атеросклеротическим коронаросклерозом и 21 гипертонической болезнью) изучали те же показатели при назначении им биотина в дозе 5 мг (см. табл. 1). У больных атеросклерозом под влиянием однократного и 7-дневного приема биотина выделение аскорбиновой кислоты с мочой статистически достоверно не меняется, содержание ее в крови снижается. У больных гипертонической болезнью после приема биотина выделение аскорбиновой кислоты с мочой и содержание ее в крови достоверно не изменяются.

Таким образом, между биотином и аскорбиновой кислотой имеется определенная взаимосвязь, зависящая от дозы биотина и от исходной обеспеченности аскорбиновой кислотой. У больных атеросклерозом и гипертонической болезнью биотин в дозе 1 мг в сутки внутрь способствует утилизации аскорбиновой кислоты в организме за счет уменьшения выделения ее с мочой и падения содержания в крови.

Учитывая положительное липотропное влияние биотина в дозе 1 мг в день и характер его влияния на обеспеченность аскорбиновой кислотой, его можно рекомендовать в этой дозе для комплексной терапии атеросклероза и гипертонической болезни II и III стадий.

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Поступила 5/II 1971 г.

B. M. Braginsky, D. P. Kalkun — THE EFFECT OF DIFFERENT DOSES OF BIOTIN ON THE ASCORBIC ACID ALLOWANCE IN PATIENTS WITH ATHEROSCLEROSIS AND HYPERTENSIVE DISEASE

Summary. The influence exerted by different doses of biotin in 12 normals, serving as controls, and 140 patients suffering from atherosclerosis and hypertensive disease of the II and III stages was investigated. Patients receiving 1 mg of biotin a day for 7 and 14 days showed a reduced excretion of ascorbic acid with the urine and a fall of its level in the blood, which may be interpreted as an increased utilization of ascorbic acid in the patients' body. Biotin doses of 3 and 5 mg failed to produce such an action.

Braginskii-'70
Bib No. 166

Kardiologiya 10(9): 149-150, 1970.

EFFECT OF BIOTIN ON CERTAIN INDICES OF PROTEIN AND LIPID EXCHANGE ON THE STATE OF THE COAGULATION SYSTEM AND BLOOD ANTICOAGULATION AND ON THE THROMBOELASTOGRAM IN PATIENTS WITH ATHEROSCLEROSIS AND HYPERTONIC ILLNESS

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In the use of vitamins in the complicated treatment of atherosclerosis, it is necessary not only to consider the lipotropic effect but also to study from all sides their effect on the final state of organs and systems, primarily the cardiovascular and blood coagulation-anticoagulation systems, since the disruption of the latter's function leads to most serious complications in this illness.

In the present work, data are presented on the effect of biotin on the content in blood serum of cholesterol and lecithin, the ratio of lipoprotein fractions, over-all content of albumin and its fractions, activity of blood coagulation-anticoagulation systems (recalcification time of oxalate plasma, prothrombin index, proconvertin index, plasma tolerance to heparin, fibrinogen content, fibrinolithic activity, free heparin), as well as thromboelastogram indices.

The use of dietetic treatment and hypotensive (reserpine) and vasodilatational (papazol, daukarin, validol) substances caused no changes over a period of 7 days in the blood coagulation-anticoagulation system, thromboelastogram indices, or lipid and protein exchange.

All the studies were carried out before and after administration of biotin, which was given in doses of 1, 3, and 5 mg every 24 hours, internally, over 7 and 14 days.

In healthy persons (control group) and in 50 patients with atherosclerosis and hypertonic illness, a 7-day administration of biotin in 1-mg doses per day did not evoke any significant changes in EKG indices or arterial pressure. Analogous data was noted for 20 healthy persons and 20 patients who received biotin in the same dosage for 14 days.

The effect of biotin (1 mg) on lipid and protein exchange indices was studied in 64 patients, of whom 36 suffered from stage II and III atherosclerosis (according to the terminology of A. L. Myasnikova) and 28 from stage IIB and IIIA hypertonic illness (according to the same authority). The control group consisted of 12 haethy persons. In anamnesis, myocardium infarct was present in 12 patients, the majority of whom (8) were examined over a 1-2 year period after the acute stage, and 4 were in the acute stage (after 3-4 weeks). Since the nature of the effect of biotin in these patients was monotypic, we put them in the same group. The mean values of indices before and after biotin administration are presented in the table.

As is evident from the table, biotin did not evoke statistically significant changes in healthy persons in the contents of cholesterol, lecithin, and over-all albumin, or in the ratio of the lipoprotein, albumin, or globulin fractions. No changes were noted in the ratio of globulin fractions either (data are not presented in the table).

In the patients, a significant decrease in over-all cholesterol

in blood serum occurred, but the amount of lecithin did not change, which led to a decrease in the cholesterol-lecithin coefficient. Under biotin influence in the patients, the ratio of α - and β -lipoproteins increased due to a decrease in the β -fraction. The content of over-all albumin, the ratio of albumins and globulins, as well as globulin fractions in blood serum did not change.

Biotin was administered to 20 patients in 1-mg doses every 24 hours over the course of 14 days. In general, the nature of changes in protein and lipid exchange indices was the same. The growth of relative content of the β -lipoprotein fraction was more obvious.

The effect of biotin over 7 days in 1-mg doses on the state of the blood coagulation-anticoagulation systems and thromboelastogram indices was studied in 16 patients with stage II and III atherosclerosis and in 34 patients with stage IIB and IIIA hypertonic illness; 20 healthy persons made up the control group.

In the given study, there were no people with acute myocardium infarct.

Indices for the blood coagulation-anticoagulation system and the thromboelastogram after biotin administration did not undergo statistically significant changes. Fibrinogen content in blood plasma changes in relation to the initial level.

In all cases of initial hyperfibrinogenemia (over 400 mg%), biotin caused a decrease in fibrinogen content.

The same indices were studied before and after biotin administration over 14 days in 40 patients. Its action was analogous.

The effect of biotin in 3- and 5-mg doses per day over 7 days was studied accordingly in patients of the 2 groups (with 15 persons in each). Indices for the coagulation-anticoagulation systems and

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Effect of Biotin in 1-mg Doses, Administered in the Course of 7 Days, on Certain Indices of Protein and Lipid Exchange in Patients with Atherosclerosis and Hypertonic Illness (M + or - m)

Index	Healthy		Ill	
	Initial Level	After Biotin Admin.	Initial Level	After Biotin Admin.
Over-all Cholesterol (in mg%)	[illegible]	161.2±3.1	[illegible]	187.[illegible]
P		>0.05		<0.05
Lecithin (in mg%)	[illegible]	205.[illegible]	[illegible]	210.[illegible]
P		>0.05		>0.05
Cholesterol-lecithin coefficient	[illegible]	0.78±0.02	[illegible]	0.89±0.02
P		>0.05		<0.05
Over-all albumin (in g%)	[illegible]	7.4±0.7	7.2±0.8	7.[illegible]
P		>0.05		<0.05
Globulins (in %)	[illegible]	46.9±1.4	[illegible]	55.8±1.8
P		>0.05		>0.05
Albumin-globulin coefficient	[illegible]	1.11±0.12	[illegible]	0.78±1.2
P		>0.05		>0.05
β -lipoproteins (in %)	[illegible]	67.7±0.6	74.4±0.3	70.[illegible]
P		>0.05		<0.05

the thromboelastogram did not change with biotin dosage increase. Higher dosages of biotin affected fibrinogen content in the blood differently. While the 3-mg dose decreased fibrinogen content in the blood in cases with initial hyperfibrinogenemia, the 5-mg dose turned out to be indifferent.

Thus, biotin has an influence on independent indices of lipid exchange and fibrinogen content, and does not show a secondary negative effect, which permits its use to be recommended in the complicated therapy of atherosclerosis and late-stage hypertonic illness.

Braginskii-'70
Bib No. 166

Kardiologiya 10(9): 149-150. 1970.

УДК 616.13-004.6+616.12-008.331.1]-008.836.077.164.19]-07:[616-008.9+616.151.5

ВЛИЯНИЕ БИОТИНА НА НЕКОТОРЫЕ ПОКАЗАТЕЛИ БЕЛКОВОГО И ЛИПИДНОГО ОБМЕНА, СОСТОЯНИЕ СИСТЕМЫ СВЕРТЫВАНИЯ И АНТИСВЕРТЫВАНИЯ КРОВИ И ТРОМБОЭЛАСТОГРАММУ У БОЛЬНЫХ АТЕРОСКЛЕРОЗОМ И ГИПЕРТОНИЧЕСКОЙ БОЛЕЗНЬЮ

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Поступила 30/III 1970 г.

При применении витаминов в комплексном лечении атеросклероза необходимо не только учитывать липотропный эффект, но и всесторонне исследовать их влияние на функциональное состояние органов и систем, в первую очередь сердечно-сосудистой и свертывающей и антисвертывающей системы крови, так как нарушение функции последних приводит к наиболее грозным осложнениям этого заболевания.

В настоящей работе представлены данные о влиянии биотина на содержание в сыворотке крови холестерина и лецитина, соотношение фракций липопротеидов, содержание общего белка и его фракций, активность свертывающей и антисвертывающей системы крови (время рекальцификации оксалатной плазмы, протромбиновый индекс, активность проконвертина, толерантность плазмы к гепарину, содержание фибриногена, фибринолитическая активность, свободный гепарин), а также на показатели тромбоэластограммы.

Применение диетического лечения, гипотензивных (резерпин) и сосудорасширяющих (папазол, даукарин, валидол) средств в течение 7 дней не вызывало изменений со стороны свертывающей и антисвертывающей системы крови и показателей тромбоэластограммы, а также липидного и белкового обмена.

Все исследования проводили до и после применения биотина, который назначали в дозах 1, 3 и 5 мг в сутки внутрь в течение 7 и 14 дней.

У здоровых (контрольная группа) и у 50 больных атеросклерозом и гипертонической болезнью 7-дневный прием биотина в дозе 1 мг в сутки не вызвал каких-либо существенных изменений показателей ЭКГ и артериального давления. Аналогичные данные отмечены у 20 здоровых и 20 больных, получавших биотин в той же дозе в течение 14 дней.

Влияние биотина (1 мг) на показатели липидного и белкового обмена исследовали у 64 больных, из которых 36 страдали атеросклерозом II и III стадий (по А. Л. Мясникову) и 28 — гипертонической болезнью IIБ и IIIА стадий (по А. Л. Мясникову). Контрольную группу составили 12 здоровых лиц. В анамнезе инфаркт миокарда был у 12 больных, большинство из которых (8) обследованы через 1—2 года после острого периода, а 4 — вне острого периода (спустя 3—4 недели). Поскольку характер влияния биотина у этих больных был однотипным, мы объединили их в одну группу. Средние значения показателей до и после применения биотина представлены в таблице.

Как видно из таблицы, у здоровых лиц биотин не вызывал статистически достоверных изменений в содержании холестерина, лецитина, общего белка и в соотношении фракций липопротеидов, альбуминов и глобулинов. Не отмечено изменений и в соотношении глобулиновых фракций (данные в таблице не представлены).

У больных произошло достоверное снижение содержания в сыворотке крови общего холестерина, а количество лецитина не изменилось, что привело к снижению холестерин-лецитинового коэффициента. Под влиянием биотина у больных повысилось соотношение α- и β-липопротеидов за счет снижения β-фракции. Не изменилось содержание общего белка, соотношение альбуминов и глобулинов, а также глобулиновых фракций в сыворотке крови.

У 20 больных биотин применялся в дозе 1 мг в сутки в течение 14 дней. В основном характер изменений показателей белкового и липидного обмена был таким же. Отмечен более выраженный рост относительного содержания α-фракции липопротеидов.

Влияние 7-дневного приема биотина в дозе 1 мг на состояние свертывающей и антисвертывающей системы крови и показатели тромбоэластограммы исследовали у 16 больных атеросклерозом II и III стадий и у 24 больных гипертонической болезнью IIБ и IIIА стадий; 20 здоровых лиц составили контрольную группу.

В данное исследование не вошли больные острым инфарктом миокарда.

Показатели свертывающей, антисвертывающей системы крови и тромбоэластограмма после приема биотина не претерпели статистически достоверных изменений. Содержание фибриногена в плазме крови изменялось в зависимости от исходного уровня.

Во всех случаях исходной гиперфибриногенемии (выше 400 мг%) биотин вызвал снижение содержания фибриногена.

Те же показатели исследовали до и после приема биотина в течение 14 дней у 40 больных. Действие его было аналогичным.

Влияние биотина в дозах 3 и 5 мг в день в течение 7 дней изучали соответственно у больных 2 групп (по 15 человек в каждой). Показатели свертывающей, антисверты-

Влияние биотина в дозе 1 мг, принимавшего в течение 7 дней, на некоторые показатели белкового и липидного обмена у больных атеросклерозом и гипертонической болезнью ($M \pm m$)

Показатель		Здоровые		Больные	
		исходный уровень	после приема биотина	исходный уровень	после приема биотина
Общий холестерин (в мг%)		158,2±2,4	161,3±3,1	215,7±6,4	187,5±5,3
	P		>0,05		<0,05
Лецитин (в мг%)		210,4±3,4	205,2±2,8	208,7±4,2	210,5±3,8
	P		>0,05		>0,05
Холестерин-лецитиновый коэффициент		0,75±0,01	0,78±0,02	1,03±0,08	0,80±0,02
	P		>0,05		<0,05
Общий белок (в г%)		7,6±0,5	7,4±0,7	7,2±0,8	7,0±0,9
	P		>0,05		<0,05
Глобулины (в %)		46,4±1,3	46,9±1,4	56,2±1,3	55,8±1,6
	P		>0,05		>0,05
Альбумино-глобулиновый коэффициент		1,15±0,11	1,11±0,12	0,78±1,1	0,78±1,2
	P		>0,05		>0,05
β-Липопротеиды (в %)		68,3±0,5	67,7±0,6	74,4±0,3	70,3±0,4
	P		>0,05		<0,05

вающей системы и тромбоэластограммы при повышении дозы биотина не изменялись. В более высоких дозах биотин по-разному влиял на содержание в крови фибриногена. В то время как в дозе 3 мг он снижал содержание фибриногена крови в случаях с исходной гиперфибриногенемией, доза 5 мг оказалась индифферентной.

Таким образом, биотин обладает эффективным действием на отдельные показатели липидного обмена, содержание фибриногена и не оказывает побочного отрицательного влияния, что позволяет рекомендовать его применение в комплексной терапии атеросклероза и поздних стадий гипертонической болезни.

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CANITIES AND ALOPECIA IN CHILDREN ASSOCIATED WITH AVITAMINOSIS

Preliminary Report

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In order to understand the so-called minimal or subclinical nutritional deficiency reactions observed in the United States, it is well to consider the severe or extreme reactions which may be observed elsewhere and especially in tropical America. At the Hospital San Juan de Dios in San José, Costa Rica, special attention has been given in the past three years to the study of changes in the hair of infants and young children with severe avitaminosis. Hair disturbances have been noted especially in the severe forms of vitamin A deficiencies, pellagra, riboflavin deficiency (cheilosis), nutritional edemas, beriberi and in the mixed forms of these general types. Such extreme hair disturbances have not been seen in the nutritional disturbances in the United States according to Blankenhorn,[1] Spies[2] and Vilter.[3] In the Medical Survey of Nutrition in Newfoundland,[4] note was made of a form of change in the hair, namely a dry, lackluster hair, so-called "staring hair." A number of South African investigators (Trowell,[5] Kark,[6] Williams,[7] the Gillmans[8] and their associates[9]) have described changes in the hair associated with severe nutritional diseases in children. According to Gillman and Gillman,[8a] Trowell[5] was the first to suggest that this nutritional deficiency syndrome observed by the various authors was probably infantile pellagra. Gillman and Gillman[8a] in Johannesburg, Union of South Africa, have done extensive work in that syndrome which they also call infantile pellagra. Clinically, this is "characterized by severe edema, typical cutaneous lesions, labial and angular stomatitis, glossitis, diarrhea or steatorrhea, peculiar depigmentation of the skin over the entire body and a graying of the hair of the scalp with varying degrees of alopecia." Williams[7] in work in the Gold Coast colony has emphasized that the changes of the hair are more noticeable when the child is older, "say of 4 years in whom the condition may have become chronic; or perhaps owing to occasional improvements in the diet, and the child has had a series of relapses and recoveries." The changes in the hair which all these authors describe are similar to those which we have observed not only in the syndrome described herein, which we, too, believe to be a form of pellagra, but also in children who presented decided follicular keratoses and angular stomatitis and occasionally glossitis with no other changes as well as in children who presented generalized edema with normal blood proteins, and with no other cutaneous or mucosal reactions. True beriberi in children has been observed in Costa Rica by Peña Chavarría and his co-workers, and these changes in the hair have been observed in such children. It is possible that the changes are similar to but more severe than the dry "staring" hair observed in Newfoundland. It is our opinion, then, that canities and alopecia may occur in children with vitamin deficiency syndromes other than that described by Trowell, Kark, Williams and Gillman and Gillman. This occurrence of canities and alopecia in children is a manifestation of deficiency disease. As Williams indicated, the more striking changes of pigmentation were observed in those children with chronic conditions. However, the syndrome, from a clinical aspect, is not caused by a deficiency of any of the well known vitamins such as nicotinic acid, thiamine, riboflavin or vitamin A. According to Vilter,[3] it may be related to a deficiency of one of the less well known members of the vitamin B complex. It is curious that no similar severe reactions of the hair have been observed in adult vitamin deficiency syndromes even of the extreme types which are found so easily in Central America. For adults, presumably, Savill[10] has indicated that "diseases of the intestinal canal in particular have in many cases hastened the onset of canities." She has reported, in a few instances, gradual improvement of the hair after improvement of the systemic condition.

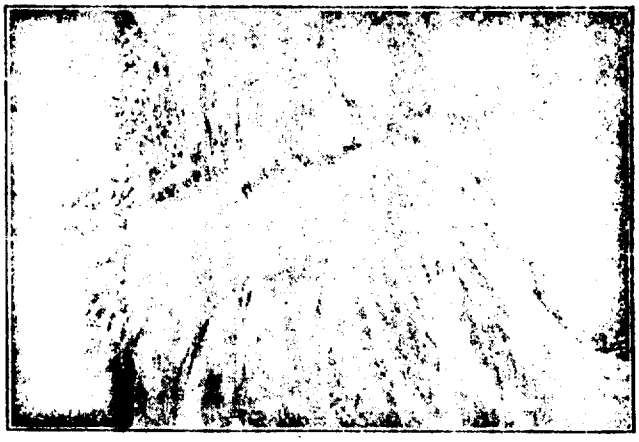

Fig. 1.—Return of pigment to the hair at proximal ends of shafts in a young girl previously ill of diarrhea and edema.

The changes in Costa Rica are observed for the most part in the hair of white and mestizo children.

These disturbances in the hair of children follow a similar pattern of development. The hair becomes dry, loses color, usually in toto, and is more fragile. It becomes loosened and is easily pulled out of the scalp. While talking to the child one can remove a handful of

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Biotin furnished by Merck & Co., Inc.

Dr. Marion Blankenhorn, Dr. Tom Spies and Dr. Richard Vilter made a critical survey of the preliminary study.

1. Blankenhorn, M.: Personal communication to the authors.
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hair without any pain or discomfort to the child. In some instances, the spontaneous loss of hair has been more pronounced over the frontal area and has produced on the sides the so-called "professor's corners"

Fig. 2.—More advanced stage of return of growth and pigment in the hair of a young boy who had had follicular keratoses, angular stomatitis and glossitis.

and in front the widened forehead, both characteristic of the idiopathic baldness in the adult.

The more interesting changes are those of depigmentation varying from slight graying of the hair to intense

Fig. 3.—Frontal alopecia with slight canities in a child with cirrhosis and generalized edema.

whiteness. This color change is diffusely spread throughout the scalp. With gradual improvement over a period of weeks to months, the pigment of the hair returns first at the scalp attachment and rapidly spreads through the shaft of the hair. The return of pigment in attached hairs may be more rapid than the return of growth in bald areas. Selected hairs were mounted and studied microscopically. The most striking feature was the depigmentation and hypopigmentation of the distal portions of the hairs. The change was gradual and was associated with spotty absence and lessened intensity of the medulla with diminishing color of the cortex, so that the distal portions of the hair presented a pale yellow, waxy appearance with only a faint trace of the medulla, only a few recognizable granules of pigment and many air spaces. The diameter, unmeasured, of the distal portions appeared lessened. The proximal portions of the hair appeared normal. Subsequent examinations of these hairs under the polarizing microscope were negative. For comparison, microscopic studies were made of the hair of a 6 year old white child with premature canities of familial origin. Biotin therapy over a period of three months had no effect on the pigment. The hair, grossly, was coarse, thin and inelastic and was entirely white. Some hairs showed bits of distal pigmentation. Under the microscope the hair showed a few scattered granules of pigment at the roots and cortex. The medulla was colorless. In general, the chief differences between these hairs and the hairs associated with the nutritional defects were that the former were coarser and harder. Sample

Fig. 4.—Hair of boy in figure 2 adjacent to the zone of depigmentation; the diameter had diminished, the medulla was indistinct and absent and the pigment granules in the cortex were sparser and lighter; × 10.

Fig. 5.—Hair of boy in figure 2 at the zone of depigmentation; the medulla is seen to be less pronounced, lighter in color and absent in portions, the pigment granules are less intense in color and more scattered. Some air spaces are also present. × 160.

hair from children in Costa Rica with the hair disturbances has been taken now and then to illustrate the progress of the return of pigment, but no controlled time measurements of growth were taken. Except for microscopic examinations, no special studies thus far have been made of these hairs. The scalp revealed slight pityriasis but no seborrheic dermatitis. Changes were observed sometimes in the eyebrows and eyelashes. As mentioned previously, associated cutaneous changes varied from normal skin to severe follicular keratoses and severe pellagrous dermatitis.

In general, records of the food intake of the children who exhibited such hair disturbances indicated a diet that consisted chiefly of bananas and molasses. Detailed analyses of the diets of these children will be published in subsequent reports.

In an effort to understand the background of these changes in the hair which would recede with improvement in the patient's general condition, various fractions of vitamin B complex were given in an uncontrolled fashion to the children. It had been observed previously that the hospital diet had effected a cure, unless the patient died shortly after admission or presented such severe reactions as diarrhea, edema, hepatosis or cirrhosis when the parenteral use of the vitamin fractions was necessary. Originally it had been planned to attempt to study the addition of such specific substances as inositol, calcium pantothenate, para-aminobenzoic acid and choline, alone and together with other fractions of the B complex. However, in addition to usual routine therapies biotin, without assay studies, was given orally, usually in doses of 0.25 mg. two or three times a day. With the addition of biotin, the return of both pigment and growth of the hair appeared to be accelerated definitely over that observed on a hospital diet or a hospital diet plus thiamine plus nicotinamide and other vitamins. These studies have been wholly uncontrolled; hence, the assumption of the more rapid action of biotin is purely an opinion and not a proved fact. Since similar cases of interesting changes in the hair are still available for additional studies, an effort will be made to determine, with due allowance for individual variations, the comparative accelerative effect of the various fractions of the B complex group.

CONCLUSIONS

In Costa Rica infants and young children with severe avitaminosis, especially vitamin A deficiency, pellagra, riboflavin deficiency, beriberi, nutritional edemas or mixed avitaminosis syndromes, have been observed to exhibit characteristic associated changes in the hair, both loss, diffuse or especially pronounced in the frontal area, and depigmentation. These changes in the hair resemble those reported previously by Trowell, Kark, Williams, Gillman and Gillman and others in the syndrome of infantile pellagra in South Africa. These changes are easily reversible if the patient survives the severe vitamin deficiency state. Improvement may be produced by a general, adequate diet if it is utilized alone or together with polyvitamin mixtures of the B complex.

The addition of biotin is thought, but not proved, to accelerate over other therapies the return of normal growth and pigmentation to the hair. Similar severe changes were not observed in the hair of adults with decided vitamin deficiency syndromes.

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Experientia 24(4):367-368. 1968.

Disrupted Fertility of the Hidebeetle *Dermestes maculatus* (Deg.) due to Dietary Overdosage of Biotin

It has been known for some time that growth and reproduction of several insect species become inhibited upon excessive intake of certain vitamins. According to LEVINSON and BERGMANN 1959[1], fatal hypervitaminosis in pyridoxine, pantothenic, nicotinic or folic acid may be caused to housefly larvae, and AKOV and GUGGENHEIM[2] observed toxic effects due to dietary surplus in riboflavin on *Aedes aegypti*. Moreover, BENSCHOTER and PANIAGUA[3] demonstrated recently impeded reproduction of Mexican fruitflies (*Anastrepha ludens*) due to large biotin doses. Mortality in larvae of carpetbeetles was induced also by overdosage of vitamin K_1, K_2 or K_3[4]. This communication deals with a study on the influence of oversupply of B vitamins on growth and reproduction of the hidebeetle *Dermestes maculatus*. The nutritional requirements of larvae and adults are similar in this species and can be met by a semisynthetic diet[5].

Individual larvae as well as pairs of male and female beetles were maintained at 29 ± 0.5 °C and 60–70% relative humidity on diets[5] containing additions of 0.01, 0.1, 0.5 and 1.0% of single B vitamins. 15–20 larvae and adults of either sex were employed for testing each vitamin and dietary level. Rates of food consumption, development, emergence, fecundity and fertility were the criteria of these experiments.

As normal development was evident in presence of all vitamin levels, dietary excess of either nicotinic, pantothenic or folic acid, pyridoxine, biotin, thiamine, riboflavine, choline chloride or inositol has certainly no detrimental effect on growth of hidebeetle larvae. It is noteworthy, that the 5 vitamins listed first are indispensable for development of *D. maculatus*, whilst the 2 vitamins listed subsequently are needed only partly for this process[5].

However, adults were found to differ from larvae in their response to biotin overdosage. Although addition of 0.5% biotin to the control diet hardly affected the number of eggs laid, it permitted only a negligible proportion of larvae to hatch from the latter. Moreover, those larvae

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died within 24 h after hatching. In presence of 1.0% dietary biotin, fecundity became lowered considerably and larval hatch was completely wanting (see Figure). Mortality occurred during embryogenesis and the eggs, which appeared to have their normal shape at the time of oviposition, shrunk gradually due to dessication.

The amounts of fecal pellets produced by beetles receiving diets with or without excessive biotin reveal normal food consumption in presence of biotin surplus (Table I). It is interesting that the effects mentioned above could not be duplicated by dietary administration of 1.0% of desthiobiotin or pimelic acid to adult *D. maculatus*.

In order to clarify the influence of excessive biotin, beetles of either sex were overdosed with dietary biotin and crossmated with an equal number of untreated beetles of the opposite sex. It becomes evident from Table II that mating females receiving 1.0% dietary biotin with normally fed males results in the production of non-viable eggs, whereas matings between normally fed females and males dosed with 1.0% biotin have no adverse effect on fecundity. Those results are consistent with the observation that the spermathecae of biotin-overdosed beetles invariably contain mobile spermatozoa. The sterilizing effect of biotin surplus is therefore specific for female hidebeetles and resembles that of certain antimetabolites for the females of other insect species[6]. Since sterility can also be produced by excessive feeding of this vitamin to female *Ceratitis capitata*, the observed phenomenon is certainly not limited to one order of insects.

It is interesting that biotin-induced sterility could be reversed readily upon replacing the disbalanced diet by one containing 0.2 µg biotin/g. Yet, sterility could be promptly restored by returning the above females to diets containing 1.0% biotin. On the other hand, feeding biotin excess to larvae does not upset the reproductive capacity of the adults developing from them.

It may be concluded that feeding superfluous biotin to *D. maculatus* results in detrimental effects on oogenesis and embryogenesis, but appears to be harmless to the larvae and adults of this insect. It should be recalled, however, that the larvae of other species, e.g. *Tribolium confusum* and *Oryzaephilus surinamensis*, are notably less tolerant than hidebeetle larvae to excessive biotin in the diet[7]. The tendency of biotin to form complexes with animal proteins including those of eggs[8] may be related to the inhibitory influence of surplus biotin on embryonic development[9].

Table I. Production of feces by hidebeetles maintained on diets with different biotin levels

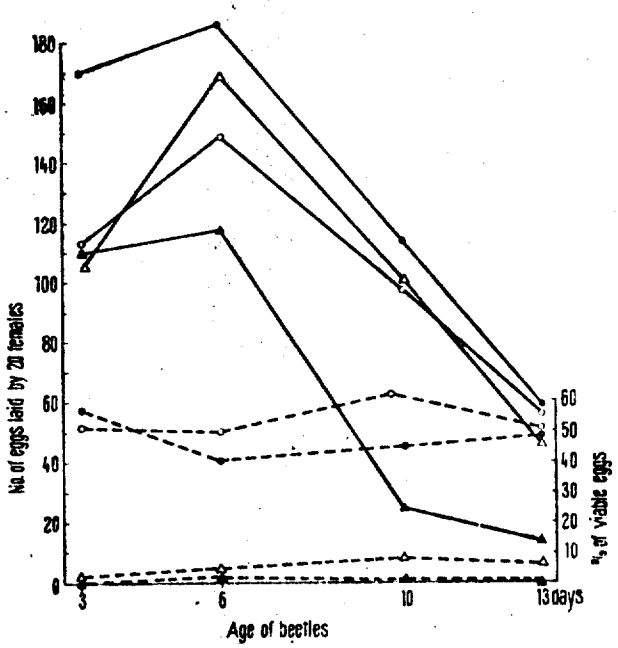
Concentration of biotin in diet, %	Volume of fecal pellets, ml
0.00002 (control)	0.70
0.01	0.90
0.1	0.65
1.0	0.70

The amount of fecal pellets passed by a total of 15 male and 15 female *D. maculatus* during 13 days is recorded for each diet.

Table II. Influence of dietary biotin overdosage on the reproductive capacity of male and female *D. maculatus*

Experimental group	Crossmatings[a]	No. of eggs[b]	% hatch
1	♀*b* × ♂*b*	217	3.2
2	♀*b* × ♂	201	3.5
3	♀ × ♂*b*	282	65.6
4	♀ × ♂	252	56.7

Each experimental group consisted of 15 male and 15 female beetles kept on test diets during 14 days. The beetles were allowed to copulate for approximately 3 h at intervals of 4 days. [a] The postscript *b* indicates insects receiving continuously 1.0% dietary biotin. [b] Total of 4 egg batches laid by 15 females.



Fecundity (solid lines) and fertility (broken lines) of *Dermestes maculatus* maintained on diets containing different biotin levels. ○— —○, 0.00002% biotin (control); ●— —●, 0.1% biotin; △— —△, 0.5% biotin; ▲— —▲, 1.0% biotin.

Zusammenfassung. Verabreichung übermässiger Biotindosen (0,5 und 1,0% in synthetischer Diät) an Speckkäfer (*Dermestes maculatus* Deg.) erzeugt Sterilisation der Weibchen und nicht der Männchen, ohne die Kopulation und Lebensdauer der Insekten negativ zu beeinflussen. Die embryozide Wirkung des Biotinüberschusses kann durch starke Herabsetzung des Vitaminspiegels in der Diät aufgehoben werden. Bei anderen B-Vitaminen, Desthiobiotin und Pimelinsäure konnte ein derartiger Einfluss nicht festgestellt werden. Andererseits schadet Verfütterung obgenannter Biotinmengen der Larvenentwicklung in keiner Weise.

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Crittenden-'48
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FROM THE MERCK INSTITUTE FOR THERAPEUTIC RESEARCH
RAHWAY, N. J.

STUDIES ON THE PHARMACOLOGY OF BIOTIN

BY

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(*Received for publication* 22-3-1948).

Prior to the synthesis of biotin by HARRIS, et al. (1), the amounts of this vitamin available for pharmacological studies were too small to permit an extensive investigation of its pharmacodynamic effects. The early experimental findings (2, 3, 4, 5) obtained with relatively small doses of natural biotin would seem to indicate that no striking effect could be expected. The studies to be reported here were undertaken in order to furnish information on the action of massive single or multiple doses of synthetic *dl*-or *d*-biotin, as the sodium salt, when administered to *normal* animals.

TOXICITY

Mice. Mice weighing an average of 20 grams tolerated single intravenous injections of 1 gram of *dl*-biotin per kg. body weight without signs of toxic effects.

Two groups of mice of approximately seven and fourteen weeks of age were treated for 60 days as follows : fifteen of the younger group and 10 of the older group were given daily oral doses of 1 mg. of *dl*-biotin in 0.2 ml. saline, an equal number 1 mg. of *d*-biotin and 10 of each group 0.2 ml. of physiological saline only. Toxic signs

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attributable to biotin were not noted. The number of deaths for the groups receiving *dl*- or *d*-biotin did not differ significantly from those of the controls.

Rats. Five male rats, weighing an average of 141 grams, received 50 mg. of *d*-biotin daily for 10 days by stomach tube. No toxic signs were observed. There were no changes in the number of erythrocytes, leucocytes, or amount of hemoglobin. No gross changes were noted at autopsy.

Two groups of 5 male rats each were given per os daily for 120 days 5 mg. of *dl*- or *d*-biotin. An equal number of animals was given physiological saline (1 ml.). At the end of the experiment the average body weight of the control group was 48 grams greater than that of the group receiving *dl*-biotin, and 35 grams greater than that of the group receiving *d*-biotin. In general, the animals appeared normal throughout the experiment. There were no significant changes in the number of blood cells or amount of hemoglobin. The hepatic function determined by the modified bromsulfalein procedure of SEELER and KUNA (6) was normal. Renal function, tested by ability to excrete 10 ml. of physiological saline per 100 grams of body weight, showed no deviation from the normal.

Dogs. Four dogs weighing from 6 - 10.8 kg. were injected intravenously for 10 successive days with 10 mg. of *d*-biotin. No changes were observed in any of the following: general behaviour; body weight; rectal temperature; condition of the fur, skin, buccal membranes, tongue or teeth; corneal reflex; scratch reflex; pupillary light reflex; heart rate; and respiratory rate. The urine showed no deviation from the normal with regard to volume, pH, specific gravity, sediment (microscopic examination), and presence of albumen or blood. There were no changes in the number of erythrocytes, leucocytes, or amount of hemoglobin. No signs of thrombosis in the injected vein were observed. No gross tissue changes were noted in the one animal sacrificed.

PHARMACODYNAMIC EFFECTS

The addition of *dl*-biotin (10^{-4}) or *d*-biotin (10^{-3}) to modified Ringer-Locke solution had no effect on the contractions of the isolated duodenal strip or isolated uterus of the normal rat. These concentrations of biotin did not influence the response of these isolated organs to acetyl-β-methyl-choline (1 or 2 $\times$ 10^{-7}) or epinephrine (1 or 2 $\times$

10^{-7}), nor the isolated uterus to ergotamine tartrate (10^{-4}) or pituitrin (1 I. U. per 100 ml.).

The local effects of biotin were examined by instillation of a 0.1 % solution of *d*-biotin (pH 7.3) into the eyes of rabbits. With the exception of an occasional slight and transient irritation, no abnormal signs were observed. The pupillary light reflex was unchanged. There was no indication of a loss of sensitivity of the cornea. Furthermore, intradermal injection of 0.1 cc. into the abdominal skin of guinea pigs produced neither irritation nor an anesthetic action at the site of the injection. No indications of irritation were found on the 2nd, 4th or 7th day following the intramuscular injection of 0.5 or 1 ml. of 0.1 % *d*-biotin in rabbits.

To study the irritability of the nerve endings in the depilated skin of the lower abdomen of the normal rat, the instrument devised by KNIAZUK (7) was used. The sensitivity of the nerve endings following the oral administration of 100 micrograms of *d*-biotin six times per week for approximately three weeks, did not differ from that of the control group receiving saline. In these animals the mid-afternoon rectal temperature remained normal. Likewise blood fibrinogen, determined according to the method of CULLEN and VAN SLYKE (8), was the same, 255 mg. per cent, in animals given either *d*-biotin or saline.

The effects of 1 or 5 mg. of *dl*- or *d*-biotin intravenously on the carotid blood pressure, heart rate and intestinal circulation was observed in 8 anesthetized normal rats weighing approximately 150 grams. No changes were observed.

Three groups of five anesthetized (nembutal) normal rats each were used for the determination of the effects of biotin on the secretion of free hydrochloric acid by the gastric glands. The animals were deprived of food but not water for 18 hours prior to the test. An incision in the abdomen was made and a metal catheter was tied into the stomach for withdrawal of the gastric secreta. After a 30 minute control period during which the amount of free hydrochloric acid was noted at ten minute intervals, one group received 1 mg. (0.2 ml.) of *d*-biotin intravenously; the second group, physiological saline (0.2 ml.); and the third group served as the non-injected controls. After the control period the amount of free hydrochloric acid secreted was noted at 10 minute intervals for one hour.

The results showed a great variation in the secretion of free hydrochloric acid among the individual animals. Two animals in the group given biotin and in the non-injected control group showed no secretion

or only minute amounts of free hydrochloric acid. With the three animals which did secrete free acid, however, biotin produced a decrease in acid secretion to approximately half of the control values. Our findings of an *achlorhydria gastrica* do not support the statement of FRIEDMAN (9) that the rat has a continuous secretion of free hydrochloric acid.

A single oral dose of 100 micrograms of *d*-biotin to rats had no effect on the diuretic action of 10 cc. of physiological saline per 100 grams of body weight. A single oral dose of 100 or 200 micrograms of *d*-biotin had no significant action on the metabolic rate (ml. of O_2 per 100 grams body weight per 5 minutes) of normal rats.

EFFECT ON WOUND HEALING

A circular area of skin 1 cm. in diameter was removed from the right scapular region on the back of 31 normal anesthetized rats. A like area on the left side was scalded with boiling water. These injured areas were treated 6 times weekly with gauze moistened with 0.01 % *dl*- or *d*-biotin (1 ml.) or saline (1 ml.). The gauze was held in place with adhesive tape. The time necessary for the regeneration of the skin was 38 days (*dl*-biotin), 25 days (*d*-biotin), and 24 days (saline). The *dl*-biotin may have delayed the regeneration of the skin. For the healing of the scalded areas the times were respectively 18, 20, and 18 days.

In a second group of rats injured and treated as above, animals were sacrificed each day for the first *seven* days, and on the *ninth*, *twelfth* and *fourteenth* day after injury. The areas under treatment were removed and studied histologically to determine if any difference in the healing process attributable to treatment could be observed. No differences were noted.

In a third group of rats skin was removed or scalded as above. No dressings were applied to the injured areas. Twenty-five animals received 100 micrograms of *d*-biotin (1 ml.) per os 6 times per week and 23 animals, 1 ml. of saline. The time necessary for skin regeneration was 18 days for both the biotin treated and the controls. The healing time for the scalded areas was 18 and 19 days respectively.

Discussion

That the administration of biotin either in a single massive dose or multiple large doses produces essentially no striking pharmacodynamic action is not surprising. This is in keeping with observations made on other vitamins (10-17). Our observation on the blood pressure, heart rate, respiration, isolated uterus and intestine confirm the findings of Schmidt and Landy (2). Since the doses of biotin used by us are much larger, our results accentuate the fact that it is without pharmacological action in these instances.

The role of biotin in the regeneration of tissue has been studied by several investigators (18-25). West and Woglom (18) found no difference in the biotin content of regenerating or hyperplastic tissue as compared to these tissues in either the non-regenerating or non-hyperplastic state. While no biotin assays were done in our experiments, the results support the thought that in normal animals additional natural biotin appears to produce no changes from the normal in the regeneration or healing of tissue.

Conclusions

1) A single intravenous administration of 1 gram of *dl*-biotin per kg. produces no toxic signs in mice. Multiple large doses of biotin given intravenously to dogs or per os to mice and normal rats cause no noteworthy signs of toxicity.

2) Relatively high concentrations of biotin do not alter the contractions of the isolated uterus or intestine of the normal rat.

3) Biotin produces no significant signs of irritation following local application to the cornea (rabbit), intradermal injection (guinea pigs) or intramuscular injection (rabbit).

4) In the anesthetized normal rat large doses of biotin intravenously produce no effect on blood pressure, respiration, or intestinal circulation. The secretion of free hydrochloric acid by the gastric glands appeared to be depressed by *d*-biotin.

5) A large single oral dose of *d*-biotin to normal rats produces no deviation from the normal renal function or metabolic rate. Repeated oral administration of large doses of biotin has no effect on hepatic

function, renal function, blood fibrinogen, rectal temperature, sensitivity of nerve endings, skin regeneration or healing of scalded skin in the normal rat.

The author is indebted to Dr. C. W. MUSHETT for pathological observations and determination of prothrombin and hemoglobin values, and blood counts; to Dr. R. H. SILBER for the determination of blood fibrinogen levels; and to Mr. S. KUNA for certain of the pharmacological studies.

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Daft-'42
Bib No. 292

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.

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[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.

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Dakshinamurti-'63
Bib No. 303

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 238, No. 1, January 1963
Printed in U.S.A.

Tissue and Intracellular Distribution of Biotin-C^{14}OOH in Rats and Chicks*

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(Received for publication, July 23, 1962)

Relatively little is known about the tissue and intracellular distribution of biotin. Fraenkel-Conrat and Fraenkel-Conrat (3) studied the excretion of biotin in rats after parenteral administration of biotin labeled in the ureido carbon. In a preliminary study with biotin-C^{14}OOH, Mistry and Leuthardt (1) reported the distribution of radioactivity in the tissues of biotin-deficient chicks. The results of a detailed investigation of the tissue and cellular distribution of labeled biotin in rats and chicks are reported in this paper. The results of a study of conditions for maximal incorporation of labeled biotin and subsequent fractionation of the liver supernatant are also presented.

EXPERIMENTAL PROCEDURE

By use of 1-day-old chicks and weanling male albino rats, biotin deficiency was produced in 5 to 6 weeks on a 20% raw egg white diet complete in all nutrients except biotin. Control rats and chicks received intraperitoneally 100 and 200 μg of biotin per week, respectively. After an overnight deprivation of food, normal and deficient animals received injections of 1 μc (10 μg) of biotin-C^{14}OOH per 100 g of body weight per day in two equal doses into the right and left breast muscle of birds and the hind leg muscle of rats. The animals were killed after the duration of the experiment as indicated in Tables I and II. The organs were removed, the colon was washed, and the washings were combined with the excreta.

Cell Fractionation—A 10% homogenate in 0.25 M sucrose, containing 0.01 M Versene at pH 7.8, was prepared from the livers of biotin-deficient chicks and rats that had received labeled biotin for 1 and 2 weeks, respectively. Nuclei were sedimented at 600 × *g* for 10 minutes, mitochondria I at 8,500 × *g* for 10 minutes, mitochondria II at 18,000 × *g* for 15 minutes, and microsomes at 25,000 × *g* for 2 hours. Nuclei were purified according to the method of Barnum *et al.* (4). The supernatant solution after the removal of microsomes was fractionated by careful pH adjustment to 5.2 with N acetic acid, and the sediment was homogenized in water. All operations were performed at 0–4°.

Measurement of Radioactivity—Samples were homogenized, dried to constant weight at 60–80°, and ground to a powder; 10 to 20 mg were combusted to $C^{14}O_2$ (5). An aliquot of NaOH used for absorption of expired CO_2 was precipitated as $BaCO_3$, and a known weight was digested with 35% $HClO_4$. The $C^{14}O_2$ in each case was absorbed in Hyamine and counted in a Packard liquid scintillation spectrometer. By connecting the wet combustion unit permanently to the vacuum line, the number of operations was reduced and the accuracy was increased.

Protein and Biotin Assays—Protein was determined according to the method of Lowry *et al.* (6), and total biotin was determined with *S. cerevisiae* as described by Semenza *et al.* (7). Free biotin, including biocytin, was assayed without prior hydrolysis of the sample.

RESULTS AND DISCUSSION

Biotin-C^{14}OOH Distribution in Tissues—The results of labeled biotin distribution in tissues of rats and chicks are given in Table I. The distribution pattern in biotin-deficient chicks is in general agreement with our preliminary study (1). Of the dose injected, 15 to 30% was recovered in the liver and kidney, approximately 10% was recovered in muscle and viscera, 7 to 27% remained unabsorbed in the breast muscle, and about 30% of the dose was excreted. Total dose recovered was approximately 82%. As would be expected, the activity recovered in various tissues increased with time and correlated with a decrease in the activity that remained in the injected muscle or in viscera. Spleen, heart, and lungs contained negligible activity. Similarly negligible activity was recovered in expired CO_2, which suggests that very little label was lost by direct oxidation of the terminal carboxyl of biotin. Similar distribution studies with biotin-deficient rats gave a pattern comparable to that in the chicks. Even in the case of rats, only about 4% of the dose was oxidized to CO_2, which is contrary to the results obtained when rats were given massive doses of labeled biotin and CO_2 was collected over a period of 12 hours (8). Our results support the observations of Baxter and Quastel (9) that biotin oxidase in rat liver and kidney was only one-tenth as active as in guinea pig tissues.

Biotin-C^{14}OOH Incorporation in Liver—The objective was to standardise conditions for maximal incorporation of radioactivity in the liver so that a starting material of high specific activity could be obtained for further fractionation. As will be seen from the specific activity data given in Table II, nearly maximal incorporation of radioactivity was secured in a week by use of a biotin-deficient rat or chick. This is the maximum that can be incorporated, since the normal content of biotin in liver is approximately 2 μg per g of fresh tissue. Under similar

* This investigation was supported by research grants from the National Vitamin Foundation, Hoffmann-La Roche, Inc., United States Atomic Energy Commission, and The National Institute of Arthritis and Metabolic Diseases, United States Public Health Service. Preliminary reports were presented at meetings of the Federation of American Societies for Experimental Biology (1, 2).

...nditions in a normal rat, less than one-third the activity was incorporated in the liver, as compared to the activity incorporated when a biotin-deficient rat was used.

Biotin-C^{14}OOH Distribution in Intracellular Fractions of Livers—As will be seen from Table III, the distribution of radioactivity in various cell fractions of chick and rat livers generally followed the pattern of distribution of protein in these fractions. Approximately 40% of the activity of the chick liver homogenate was found in the supernatant, another 29% in the mitochondria, 23% in the nuclei, and a very small amount in microsomes. Fractionation of the rat liver homogenate gave a similar pattern of distribution with over half of the activity being present in the cell supernatant. Extensive purification of the nuclei (4) did not increase the activity per mg of protein. On the basis of the radioactivity per mg of protein, the nuclei, mitochondria I, and the supernatant fraction were approximately as active as the unfractionated homogenate.

Total and Bound Biotin in Intracellular Fractions of Livers—Biotin content of cell fractions of normal and biotin-deficient rat and chick livers is given in Table IV. Biotin assays before and after hydrolysis showed that all fractions except microsomes and mitochondria II contained 89 to 99% of biotin in the bound

TABLE I

Biotin-C^{14}OOH distribution in chicks and rats

Chicks and rats were on the biotin-deficient raw egg white diet for 5 and 6 weeks, respectively. After deprivation of food overnight, a single dose of labeled biotin, divided into two equal parts, was administered as mentioned in the text. Chicks weighed 210 and 304 g; rats, 164 and 171 g.

Tissue	Dose recovered after hours			
	Chicks		Rats	
	1	3	1	4
	%	%	%	%
Liver	5.2	14.0	7.0	15.6
Kidney	9.0	17.0	4.6	6.4
Injected muscle	27.3	7.3	37.9	11.8
Remaining muscle	3.3	7.3	5.5	7.9
Spleen	0.1	0.3	0.2	0.1
Viscera	5.1	3.3	6.8	4.3
Lungs	0.2	0.7	0.3	0.3
Heart	0.2	0.3	0.3	0.6
Excreta	31.4	30.7	16.0	30.1
Expired CO_2	0.2	0.7	1.6	3.9
Total	82.0	81.6	80.2	81.0

TABLE II

Incorporation of biotin-C^{14}OOH in liver

Chicks and rats were on the biotin-deficient raw egg white diet for 5 and 6 weeks, respectively. The normal rat received, in addition, 100 μg of biotin intraperitoneally per week. These animals were given labeled biotin as mentioned in the text for the number of days as indicated in the table. Specific activity is expressed as micrograms per g of fresh liver.

	Days on experiment	Specific activity
...ficient rat	0.5	0.033
	1	0.062
	3	0.142
	7	0.235
	14	0.268
...rmal rat	7	0.072
...ficient chick	7	0.241

TABLE III

Biotin-C^{14}OOH distribution in intracellular fractions of chick and rat livers

Biotin-deficient chicks and rats, which received labeled biotin for 2 weeks as mentioned in the text, were used. The homogenate in each case was a pooled sample of two livers.

Fraction	Chick liver			Rat liver		
	Protein	Radioactivity		Protein	Radioactivity	
	%	%	mμc/mg protein	%	%	mμc/mg protein
Homogenate	100	100[a]	1.07	100	100[b]	1.361
Nuclei	14.7	23.0	1.57	12.2	15.1	1.68
Nuclei washed 12 times				0.1	0.1	1.71
Mitochondria I	14.5	26.3	1.76	15.0	15.3	1.10
Mitochondria II	4.8	2.6	0.59	9.6	3.0	0.42
Microsomes	7.9	2.8	0.29	11.8	1.7	0.21
Supernatant	49.1	38.6	0.84	49.1	52.6	1.45
Recovery	91.0	93.3		97.8	87.8	

[a] Equal to 1.220 μc.
[b] Equal to 0.837 μc.

TABLE IV

Biotin content of cell fractions of normal and biotin-deficient rat and chick livers

Rats and chicks were on the biotin-deficient raw egg white diet for 6 and 5 weeks, respectively. Normal animals received, in addition, biotin once a week intraperitoneally as described in the text.

Fraction	Rat			Chick	
	Normal				
	Total biotin	Bound biotin as per cent of total in each fraction	Deficient: total biotin	Normal: total biotin	Deficient: total biotin
	%	%	%	%	%
Homogenate	100[a]	92	100[b]	100[c]	100[d]
Nuclei	37	99	75	38	49
Mitochondria I	9	89	13	24	14
Mitochondria II	1	65	9	4	1
Microsomes	2	23	0	3	6
Supernatant	47	91	0	24	16
Recovery	96		97	93	86

[a] Equal to 1980 mμg of biotin per g of fresh liver.
[b] Equal to 200 mμg of biotin per g of fresh liver.
[c] Equal to 875 mμg of biotin per g of fresh liver.
[d] Equal to 291 mμg of biotin per g of fresh liver.

TABLE V

Biotin-C¹⁴OOH distribution in fractions of liver supernatant

Biotin-deficient chicks and rats, which received labeled biotin for 2 weeks as mentioned in the text, were used. Livers of three chicks and six rats were used for the preparation of the liver supernatant.

Fraction	Chicks		Rats	
	C¹⁴ in fresh liver	C¹⁴ in protein	C¹⁴ in fresh liver	C¹⁴ in protein
	mμc/g	mμc/mg	mμc/g	mμc/mg
Liver supernatant	94	0.93	147	1.46
pH 5.2 precipitate	69	1.68	142	2.82
Supernatant	23	0.40	5	0.11

form. The microsomes contained essentially free biotin, biocytin, or both. In the deficient animals, biotin stores of livers were depleted to one-third and one-tenth of the normal in chicks and rats, respectively. However, 49 to 75% of these depleted stores of the vitamin remained in the nuclei, and another 15 to 22% was found in the mitochondria, whereas the supernatant fraction contained very little biotin. In terms of actual amounts per g of fresh liver, the differences were very marked: normal supernatant contained 210 to 930 mμg compared to 0 to 47 mμg of biotin in the deficient supernatant.

Biotin-C¹⁴OOH Distribution in Fractions of Liver Supernatant Since approximately half of biotin-C¹⁴OOH in liver was found in the cell supernatant (Table III), it was further fractionated by adjusting the pH to 5.2. As shown in Table V, almost all the activity of the rat liver supernatant and two-thirds of the activity of the chick liver supernatant was recovered in the pH 5.2 precipitate, with a 2-fold increase in radioactivity per mg of protein. The close association of radioactivity with pH 5 enzymes of the supernatant and the observation that in biotin deficiency only negligible amounts of biotin were found in the cell supernatant led us to investigate the role of the vitamin in protein synthesis. The following paper deals with this study.

SUMMARY

Biotin-C¹⁴OOH distribution in tissues and liver fractions of rats and chicks after an injection of a near physiological dose of the labeled vitamin in biotin-deficient animals has been reported. Approximately 16% of the dose was incorporated in the liver after 4 hours, and nearly half of this radioactivity was found in the pH 5.2 fraction of the cell supernatant. Microsomes contained very little radioactivity. Also, very little of the labeled carboxyl was oxidized to CO_2. Most of the biotin in various cell fractions, except in microsomes, was found in the bound form. In biotin deficiency, the vitamin in liver was depleted as much as one-tenth of the normal, and only traces of biotin remained in the liver supernatant.

Acknowledgments—We are indebted to Professor O. Wiss of Hoffmann-La Roche, Basel, for the generous supply of biotin-C¹⁴OOH.

One of us (S.P.M.) wishes to express his sincere appreciation to Professor F. Leuthardt for his interest during the author's stay at the Physiologisch-chemisches Institut der Universität Zürich, Switzerland, where this study was initiated.

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Denko-'46
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Arch. Biochem. 11:109-117. 1946.

The Excretion of B-Complex Vitamins by Normal Adults on a Restricted Intake

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Received May 9, 1946

Introduction

There is much interest in the role intestinal bacteria may play in the nutrition of man. Previously we have reported that bacteria in the intestine of normal adults on a normal diet presumably synthesize relatively large quantities of B-complex vitamins which are excreted in the feces (1). In this regard, other investigators report isolated studies indicating that thiamine, riboflavin, nicotinic acid and biotin synthesized by the intestinal flora can be utilized by human subjects on a restricted dietary intake (2, 3, 4, 5).

No reports have been found in the literature regarding coexisting dietary intake and the excretion in both urine and feces of several of the lesser-known B-complex vitamins in normal adults subsisting on a diet restricted in these vitamins. Data are reported in this paper for 5 normal young men fed, for 15 weeks, a diet containing 19–60 percent as much thiamine, riboflavin, niacin, biotin, *L. casei* factor, pantothenic

acid and pyridoxine as is contained in a "normal diet." In addition, the level of protein was low (40 g., only 3 g. of which were animal protein), and corn supplied 27% of the dietary calories. Two control subjects received the same diet supplemented with animal protein and synthetic B-vitamins.

Experimental

A discussion of the subjects and details of urinary and fecal collections, as well as methods for the microbiological and chemical determinations of the B-vitamins, has been described in a previous paper (1) relating to the excretion of B-vitamins on a normal intake. Pyridoxine in the diet was determined microbiologically using *Saccharomyces carlsbergensis* according to the method of Atkin and co-workers (6).

All meals were prepared and served under the direct supervision of a trained dietitian. The menu was followed strictly and all portions were carefully weighed. Extra identical meals were prepared one day each week, carefully ground, homogenized, frozen and used for assay. The canned foods were purchased in one lot of the same pack in a quantity sufficient for the entire experiment. During an initial control period of 12 weeks a normal diet supplying liberal quantities of all nutrients was fed to all 7 subjects. The content of this diet in the B-vitamins under consideration is shown in Table I. The restricted diet provided daily about 3300 calories, 40 g. of protein, 380 g.

TABLE I

Intake of B-Vitamins

Vitamin	Control Period (all 7 subjects)	First 5 weeks restricted period (all 7 subjects)	6th–15th week restricted period	
			5 rest'd subjects	2 suppl'd subjects
Thiamine, mg.	1.44	.53	.54	1.73
Riboflavin, mg.	1.84	.36	.32	1.98
Niacin, mg.	15.6	6.8	5.7	17.6
Biotin, γ	44	24	20	80
L. casei factor, γ	64	26	22	112
Pantothenic acid, mg.	4.7	1.3	1.1	7.2
Pyridoxine, mg.	1.76	1.12	1.05	4.05

of carbohydrate, 175 g. of fat, 200 mg. of calcium, 560 mg. of phosphorus, 12 mg. of iron, 90 mg. of ascorbic acid and 16,000 I.U. of vitamin A. The determined (by analysis) average daily food and supplemental content of the B-vitamins under consideration in this report is listed in Table I. These values represent maximum intake since consumption was not always 100%. The food consisted of foods frequently consumed in certain parts of the country, such as corn meal in various forms (as fried mush, muffins and cookies), spaghetti, carrots, string beans, beets, gelatin, apples, pears, pineapple, salt pork, oleomargarine and sugar.

For 5 weeks all 7 subjects ate the restricted diet with no supplementation. At the beginning of the sixth week, and thereafter through the fifteenth week, all subjects were given daily, by capsule, 700 mg. calcium, 540 mg. phosphorus, 20 mg. iron and 666 I.U. vitamin D. In addition, the two control subjects were given daily, in tablets, the following crystalline supplements divided equally among the three meals: 1.2 mg. thiamine hydrochloride, 1.5 mg. riboflavin, 12 mg. nicotinamide, 60 γ biotin, 90 γ synthetic *L. casei* factor, 6.6 mg. *dl* calcium pantothenate, 3.3 mg. *d* calcium pantothenate, 0.3 mg. *p*-aminobenzoic acid, 3 mg. pyridoxine hydrochloride and 1 g. choline dihydrogen citrate. Corresponding placebos were given to the five restricted subjects. In addition, the controls were given in their food 45 g./day of calcium caseinate (Casec).

Results

Daily intake and excretion (in the urine and feces) of the B-vitamins are shown in Figs. 1–8. Each value represents the group 24-hour average determined on a sample collected for four consecutive days each week. On one day of each week the food was analyzed to give the values listed in Table I as dietary intake. During the period of restricted intake the fecal excretion of all vitamins remained as high as, or was higher than, during the period on the normal diet. There was no

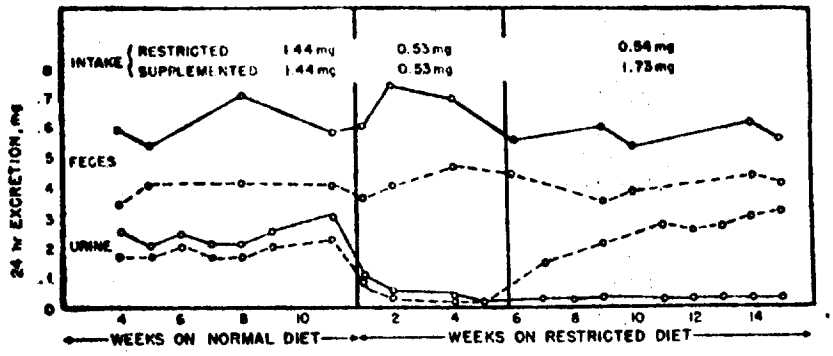

FIG. 1
Thiamine Excretion

FIGS. 1–8, inclusive, present group average urine and feces excretions of various B-vitamins. The averages for the 2 supplemented subjects are shown by broken lines, and for the 5 restricted subjects by solid lines. The first of the two vertical lines represents the end of the normal diet and the beginning of the restricted diet. The second vertical line represents the time at which supplementation of the two men was begun.

It should be noted that in Figs. 7 and 8 the abscissae for feces values are higher than for urine. This was done in order to separate the curves which would otherwise have closely coincided.

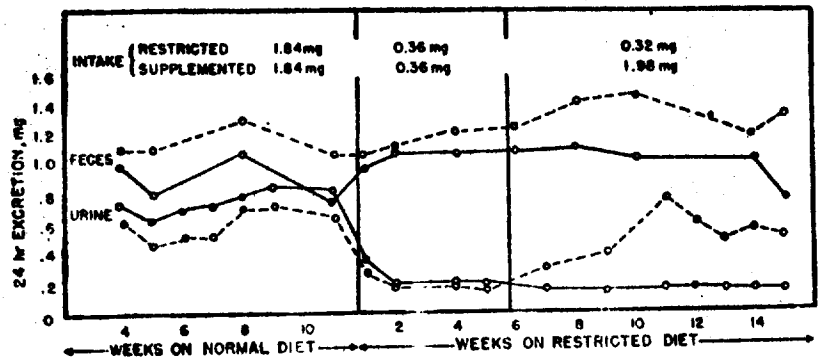

FIG. 2
Riboflavin Excretion

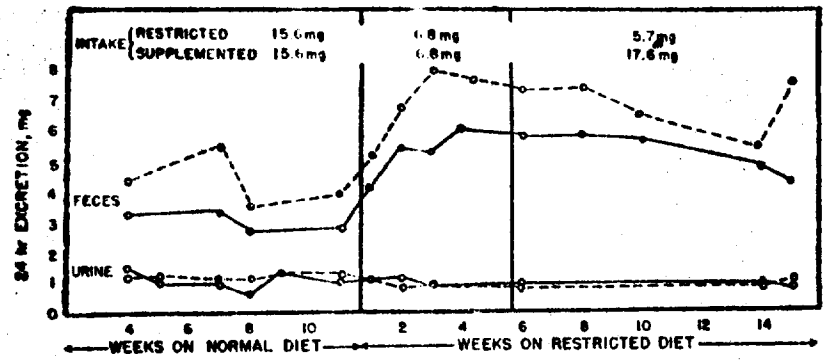

FIG. 3
Niacin Excretion

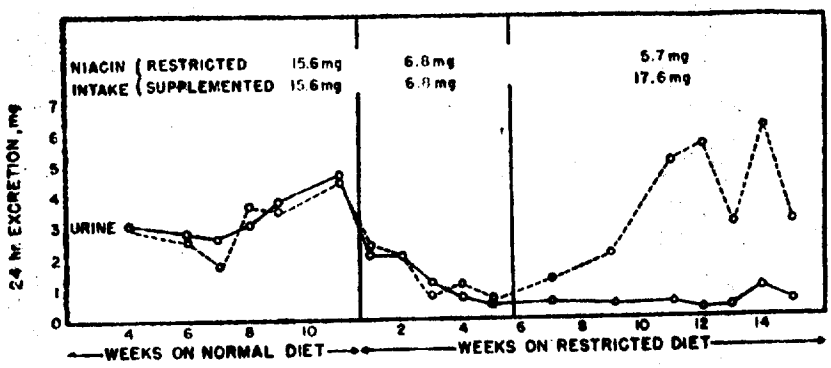

FIG. 4
N^1-Methylnicotinamide Excretion

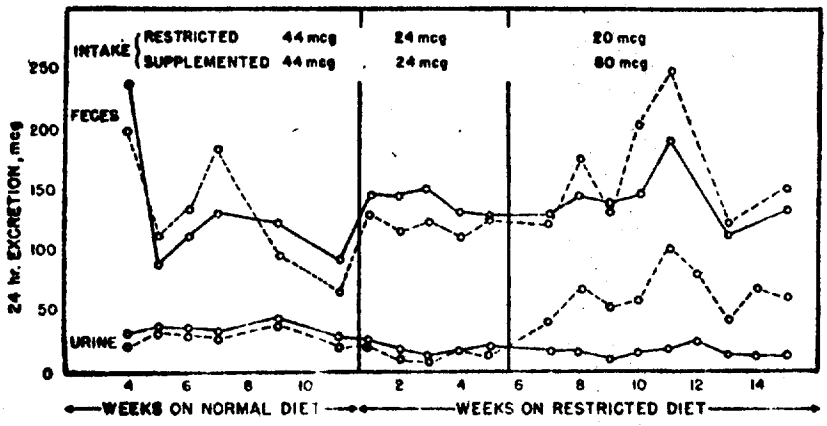

FIG. 5
Biotin Excretion

evidence, except possibly in the case of *L. casei* factor, that supplementation with crystalline vitamins increased the fecal excretion (Note Figs. 1–8).

On the other hand, the urinary excretion of thiamine, riboflavin, N¹-methylnicotinamide and pantothenic acid dropped quickly and markedly, the excretion of biotin and pyridoxine decreased to somewhat less than half their previous levels, and the excretion of niacin and *L. casei* factor decreased only slightly. When additional crystalline B-vitamins were provided to the supplemented subjects their urinary

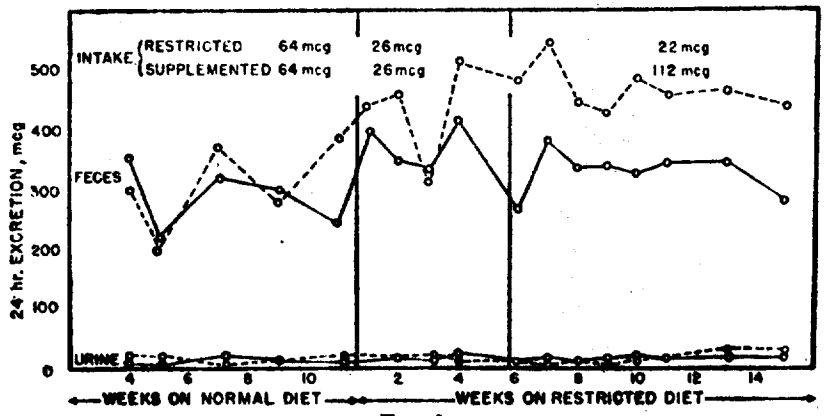

FIG. 6
L. Casei Factor Excretion

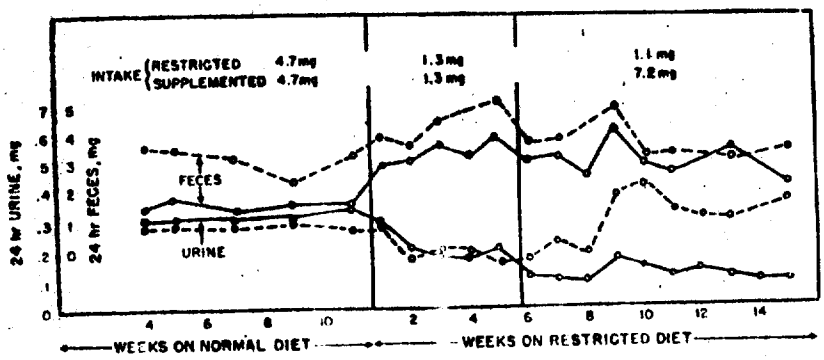

FIG. 7
Pantothenic Acid Excretion

excretion of thiamine, riboflavin, N¹-methylnicotinamide, biotin, pantothenic acid and pyridoxine gradually returned to the levels of the control period. This was not true, however, of urinary niacin and *L. casei* factor, as vitamin supplementation did not affect their excretion. As was noted above, niacin and *L. casei* factor were also the two vitamins excretion of which decreased little, if any, from levels on a normal diet.

In Table II the average excretions of the various vitamins in the urine and feces of the restricted group are compared with their intakes. The data for the 11th.–15th. weeks, inclusive, on the restricted diet are

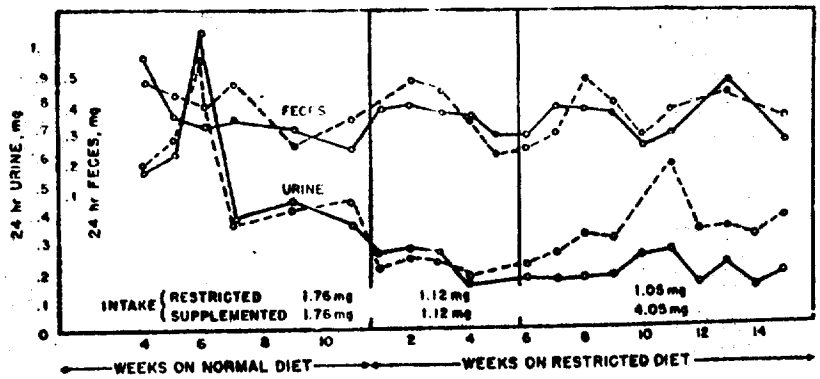

FIG. 8
Pyridoxine Excretion

TABLE II

Comparison of Urine and Feces Excretions with Intake for the 11th Week to 15th Week on a Restricted Diet

Vitamin	Means 5 men, 5 weeks			
	Intake	Urine	Feces	Urine plus feces
Thiamine, mg.	.51	.01	.56	.57
Riboflavin, mg.	.32	.16	.87	1.03
Niacin, mg.	5.7	.7	4.4	5.1
N^1-methylnicotinamide, mg.	—	.4	—	—
Biotin, γ	20	17	148	165
L. casei factor, γ	22	3	322	325
Pantothenic acid, mg.	1.1	1.0	2.8	3.8
Pyridoxine, mg.	1.05	.20	.32	.52

used for this comparison. The group average urinary excretion of thiamine, niacin, N^1-methylnicotinamide, *L. casei* factor and pyridoxine ranged from 2 to 19% of intake, while the urinary excretion of riboflavin was 50% of intake, and of biotin and pantothenic acid about equal to intake.

Fecal excretions of all vitamins except niacin and pyridoxine exceeded intake, biotin by 8-fold and *L. casei* factor by 15-fold. The combined urine and fecal excretion of all vitamins except pyridoxine approximated or greatly exceeded intake.

Discussion

Evidence is presented implying that intestinal bacteria synthesize large quantities of certain B-complex vitamins when healthy young adults subsist for 15 weeks on a restricted intake of these vitamins and of animal protein. Since the feces continued to contain relatively large amounts of these vitamins while the urinary excretion of most of them dropped markedly, it appears that only slight absorption could have occurred through the large intestine. It is possible that, in the case of certain vitamins, these quantities could be nutritionally significant on this restricted diet considering that the normal requirements may be very low. The fact that urinary excretion of pantothenic acid and biotin in the unsupplemented subjects approximated and on certain weeks exceeded dietary intake implies that the pantothenic acid and

biotin may have been absorbed through the large intestine or may have come from unidentified metabolic processes in the tissues.

Acknowledgment

The authors wish to express their appreciation to Lederle Laboratories, Pearl River, New York, for furnishing synthetic *L. casei* factor, to Merck & Company, Inc., Rahway, New Jersey, for furnishing synthetic biotin, to Abbott Laboratories, North Chicago, Illinois for preparing and furnishing the capsules and tablets used in supplementation, to Miss Jane R. Spinella and Miss Marta E. Wood of this laboratory for planning the diet, to Misses Virginia J. Penniston, Ida Shirman, Patricia K. Keegan and Velma C. Miller for their excellent technical assistance. It is a pleasure to acknowledge the cooperation of the following volunteer members of the Civilian Public Service on detached service for the project under the auspices of the Army Epidemiological Board: Delbert D. Blickenstaff, Edward L. Crill, Harvey E. Dibrell, Jr., Gareth W. Heisler, Roy W. Miller, John H. Smith and Lee Smith, Jr.

Summary

1. Average daily urinary and fecal excretions of thiamine, riboflavin, nicotinic acid, biotin, *L. casei* factor, pantothenic acid and pyridoxine and urinary excretion of N^1-methylnicotinamide are reported for five young normal adults on a restricted intake for 15 weeks. Values are also given for two subjects receiving supplements equivalent to, or in excess of, those levels found in the original normal diet.

2. Average fecal excretion of all vitamins was as high, or higher, on the restricted diet as on the normal diet.

3. Fecal excretion was unaffected by vitamin supplementation.

4. The average urinary excretion of thiamine, riboflavin, N^1-methylnicotinamide and pantothenic acid dropped markedly on the restricted diet; biotin and pyridoxine dropped moderately; and niacin and *L. casei* factor dropped only slightly, if at all.

5. Urinary excretion of thiamine, riboflavin, N^1-methylnicotinamide, biotin, pantothenic acid and pyridoxine returned to the original or to even higher levels following supplementation with crystalline vitamins. Urinary niacin and *L. casei* factor were not affected by supplementation.

6. Urinary excretion of biotin and pantothenic acid approximated intake on the restricted diet.

7. Fecal excretion of all vitamins except niacin and pyridoxine exceeded intake on the restricted diet.

8. The combined urinary and fecal excretion of all vitamins, except pyridoxine, approximated or greatly exceeded intake on the restricted diet.

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Denko-'46
Bib No. 331

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Arch. Biochem. 10(1):33-40. 1946.

The Excretion of B-Complex Vitamins in the Urine and Feces of Seven Normal Adults

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Received January 21, 1946

Introduction

Many investigators have reported unallied normal daily *urinary* excretions of some of the B-complex vitamins and their derivatives (1, 2, 3, 4, 5, 6, 7, 8, 9). Some investigators have also reported on the *fecal* output of a few of these B-vitamins (1, 3, 7, 8, 10). Balance studies of young women on a milk diet showed that the total output of biotin was slightly greater than intake, 107%, while that of pantothenic acid almost equalled the intake and that of riboflavin amounted to only 55% of the intake (1). On an unrestricted diet normal subjects excreted in the feces and urine three to six times the amount of biotin ingested in the diet (3).

Since there are, however, very few reports on the coexisting dietary intake and total excretion of the lesser known B-complex vitamins by human subjects, such values for seven healthy young men eating a

normal diet are reported in this paper. This study was undertaken preliminary to an investigation of the effects of a decreased intake of the B-vitamins (24). During a twelve-week period the urinary and fecal excretions of *p*-aminobenzoic acid, biotin, folic acid, pantothenic acid, pyridoxine, thiamine, riboflavin and nicotinic acid have been compared with the dietary intake of these vitamins. No report of similar studies of this scope has been found in the literature.

Experimental

Subjects. The subjects were seven volunteers, 23 to 28 years old, judged to be free from significant organic disease or defects. These men were housed under identical conditions in a hotel, and later a student dormitory. All ate the same diet, which remained unchanged during the entire experimental period. Strict adherence to the regimen was assured by the high character and scientific interest of the subjects, who were carefully chosen on the basis of their past records under similar experimental conditions. (As added precaution, each week's data were carefully scrutinized for signs of any possible deviation in intake of food, excretion of urine and feces, collection of samples and technical error in assay.) A constant body weight was maintained by regulating physical activity. The principal activity was provided by standardized daily tasks, *e.g.*, bicycle riding, treadmill, outdoor walks, etc. The remaining free time was spent by participating in various sports and a schedule of maintenance and assistance work.

Diet. All meals were prepared and served under the direct supervision of a trained dietitian. The menu was followed strictly and all portions were carefully weighed. Extra identical meals were prepared one day each week, carefully ground, homogenized, frozen and used for assay. The meat used was bought daily, as was the milk. The fresh vegetables were purchased three times each week at local wholesale dealers. The canned foods were purchased in one lot of the same pack in a quantity sufficient for the entire experiment. It was computed that a total of 3000 to 3300 calories daily was provided, 70 g. of protein, 860 mg. of calcium, 1.25 g. of phosphorus, 15 mg. of iron, 7400 I.U. of Vitamin A and 100 mg. of ascorbic acid. The determined (by analysis) average daily intakes of the B-complex vitamins under consideration in this report are listed in Table II.

Urinary and Fecal Collection. Urine was collected each week over a period of four consecutive days. The containers, amber-colored one-gallon jugs, were kept in a refrigerator at all times. At the end of each day the total volume and a one-tenth aliquot were measured. The aliquot of each daily sample was transferred to a sample bottle which was kept in the deep-freeze unit.

Feces were also collected over the same four-day period each week. All feces from the entire period were collected in one container, a tared one-quart jar, which was kept in a deep-freeze unit at all times. Shortly after the end of the four-day period the feces were thawed and homogenized. Daily average excretion was computed and aliquots taken for assay. In two cases fecal collection was continued beyond the four-day period until a minimum of three stools had been collected.

Methods. For the determination of thiamine the method of Hennessy and Cerecedo (11), as modified by Friedemann and Kmieciak (12), was used. For riboflavin the method of Ferrebee (13) was used with slight modification. Folic acid was determined microbiologically using the method of Teply and Elvehjem (14). Feces and food samples were prepared for folic acid, pantothenic acid and biotin assays by the procedures outlined by the University of Texas workers (15). The biotin value for food obtained by the enzyme method was checked occasionally by autoclaving a duplicate sample with HCl for ½ hour, neutralizing and assaying. Pantothenic acid was determined microbiologically by the method of Hoag *et al.* (16). In this method crystalline vitamin solutions were substituted for the yeast and Vitab mixtures. Biotin was determined microbiologically originally by the method of Shull and Peterson (17). Later the method of Teply and Elvehjem for folic acid was used for biotin assays. *p*-Aminobenzoic acid was determined with *Neurospora crassa* by using a method based on that of Thompson *et al.* (18) and that of Stokes *et al.* (19). Pyridoxine was determined by a method based on that of Stokes and co-workers (19). Nicotinic acid was determined microbiologically by the method of Krehl and coworkers (20). N ^{1}-methylnicotinamide was determined by a modification of Najjar's method (21).

Results

Daily urinary and fecal excretions of the B-vitamins are shown in Table I. These values represent the average of six to eight weekly determinations (samples collected for four consecutive days each week) made during a twelve-week period. More variation is noted in the fecal output than in the urinary excretion. Individual output varied more from week to week than did the average between individuals in any one week.

About twice as much thiamine and nicotinic acid was found in the feces as in the urine. About one and one-half times as much riboflavin and *p*-aminobenzoic acid was found in the feces as in the urine. Urinary biotin output was less than one-fourth as great as the fecal output. About seventy-five times as much folic acid was excreted in the feces as in the urine. However, the major part of the pantothenic acid and pyridoxine excreted appeared in the urine, only about 40% of the total excretion of these vitamins appearing in the feces.

The combined urinary and fecal output of thiamine, nicotinic acid and pyridoxine was considerably less than the dietary intake. The urinary plus fecal output of riboflavin was only slightly below the dietary intake. The ratio of urinary plus fecal output to dietary intake of biotin was about 3.8 to 1, pantothenic acid about 1.1 to 1, *p*-aminobenzoic acid about 2.3 to 1 and folic acid about 5.5 to 1, as shown in Table II.

TABLE I

Average Daily Urinary and Fecal Excretions*

	p-Amino-benzoic acid Low-High-Ave.	Biotin Low-High-Ave.	Folic acid Low-High-Ave.	Pantothenic acid Low-High-Ave.	Pyridoxine Low-High-Ave.	Nicotinic acid Low-High-Ave.	N¹-methyl-nicotinamide Low-High-Ave.	Thiamine Low-High-Ave.	Riboflavin Low-High-Ave.
	γ	γ	γ	mg.	mg.	mg.	mg.	γ	γ
Urine									
Subject									
1.	73–261–131	23.7–31.5–28.0	2.65–5.05–4.28	2.88–3.48–3.19	0.33–1.12–0.61	0.79–1.46–1.18	2.3–5.4–3.9	224–328–261	479–945–758
2.	85–308–148	27.4–45.5–35.5	2.66–4.39–3.91	2.88–3.90–3.37	0.34–1.01–0.60	0.95–1.37–1.13	2.0–4.3–3.1	252–392–323	498–870–694
3.	89–246–149	21.2–39.8–27.8	2.54–5.80–4.01	2.90–3.18–3.07	0.38–0.96–0.57	1.00–1.37–1.23	1.3–4.8–3.3	128–204–156	388–697–543
4.	67–260–132	19.7–39.8–29.7	2.35–5.60–3.91	2.31–2.96–2.57	0.36–0.99–0.63	0.96–1.42–1.19	2.4–4.8–3.4	184–273–227	437–747–588
5.	66–290–144	22.7–49.3–33.1	2.05–4.60–2.94	3.01–4.13–3.46	0.23–1.16–0.69	0.97–1.36–1.16	2.4–5.6–3.7	178–282–234	464–735–601
6.	89–494–198	23.6–44.4–32.5	2.04–4.99–3.86	2.40–3.19–2.68	0.38–1.17–0.67	1.15–1.55–1.39	2.0–4.1–2.7	203–312–245	475–788–651
7.	70–272–132	28.7–47.3–35.6	3.88–5.90–4.99	2.65–3.30–2.95	0.32–1.26–0.64	0.90–1.40–1.20	2.8–6.1–4.4	110–184–144	593–1120–913
Range of Averages	131–198–148	27.8–35.6–31.7	2.94–4.99–3.99	2.68–3.46–3.04	0.57–0.69–0.63	1.13–1.39–1.21	2.7–4.4–3.5	144–323–227	543–913–678
Feces									
1.	71–336–200	59–171–121	213–356–294	0.69–1.63–1.16	0.17–0.46–0.33	0.84–2.96–2.14	**	564–900–755	632–1204–869
2.	177–290–238	70–465–201	189–539–340	0.27–1.19–0.89	0.17–0.73–0.36	1.83–3.78–2.49		449–887–589	649–1580–940
3.	84–345–249	62–210–131	222–522–393	2.72–4.34–3.66	0.22–0.46–0.38	4.23–6.37–5.41		611–700–651	1158–1450–1313
4.	72–365–255	69–225–125	179–342–281	2.13–4.12–2.93	0.26–0.54–0.42	2.93–5.31–4.17		71–208–139	754–1130–972
5.	187–301–239	90–201–125	273–501–356	1.50–3.63–2.36	0.25–0.55–0.38	2.45–6.24–3.99		745–1217–895	775–1480–1117
6.	104–276–183	44–230–116	135–308–222	0.68–2.95–1.39	0.19–0.55–0.35	1.17–3.50–2.24		47–175–109	563–1034–823
7.	158–574–361	51–186–114	123–395–243	1.67–4.11–3.04	0.27–0.50–0.42	3.68–7.96–4.95		450–860–699	825–1445–1172
Range of Averages	183–361–246	114–201–133	222–393–304	0.89–3.66–2.20	0.23–0.42–0.28	2.14–5.41–3.63		109–895–548	823–1313–1089

* Each figure represents a four-day sample.
** Not determined.

TABLE II

Comparison of Daily Vitamin Intake and Urinary Plus Fecal Excretion***

	p-Aminobenzoic acid Low-High-Ave.	Biotin Low-High-Ave.	Folic Acid Low-High-Ave.	Pantothenic Acid Low-High-Ave.	Pyridoxine Low-High-Ave.	Nicotinic Acid Low-High-Ave.	Thiamine Low-High-Ave.	Riboflavin Low-High-Ave.
Urinary plus Fecal Output Subject	γ	γ	γ	mg.	mg.	mg.	mg.	mg.
1.	130–448–331	87–200–149	217–361–298	3.78–4.76–4.36	0.50–1.43–0.94	1.87–4.20–3.32	0.89–1.18–1.02	1.45–1.87–1.63
2.	283–598–386	116–493–236	193–642–344	3.44–4.98–4.27	0.62–1.31–0.96	2.44–5.11–3.48	0.79–1.26–0.93	1.37–2.23–1.64
3.	173–509–398	83–232–159	226–526–397	5.90–7.21–6.72	0.63–1.42–0.95	5.47–7.52–6.64	0.76–0.88–0.81	1.60–2.12–1.88
4.	139–517–387	92–252–155	183–440–299	4.51–6.02–5.40	0.63–1.36–1.05	4.05–6.73–5.36	0.29–0.48–0.37	1.39–1.83–1.57
5.	254–477–383	125–229–158	277–503–390	4.80–7.33–5.82	0.48–1.58–1.07	3.42–7.42–5.15	0.98–1.41–1.13	1.51–2.06–1.73
6.	209–670–365	77–256–136	139–310–226	3.34–5.75–4.07	0.57–1.69–1.03	2.42–5.17–3.62	0.25–0.49–0.36	1.15–1.69–1.48
7.	158–574–361	86–233–150	127–401–248	4.60–7.41–5.99	0.62–1.73–1.05	4.78–9.29–6.15	0.57–1.04–0.85	1.68–2.47–2.11
Range of Averages	331–398–375	136–236–163	226–397–310	4.07–6.72–5.25	0.94–1.07–1.01	3.32–6.64–4.82	0.36–1.02–0.78	1.48–2.11–1.72
Average Daily Dietary Intake	97–220–188	37–54–44	43–86–62	4.19–5.30–4.73	1.32–2.46–1.76	12.4–20.9–15.6	1.24–1.63–1.44	1.74–1.98–1.84
Per cent Excretion = $\left(\frac{\text{Urinary + Fecal Excretion}}{\text{Dietary Intake}} \times 100\right)$								
1.	131–289–209	265–460–367	477–720–539	71–110–93	43–78–57	13–27–21	67–87–80	80–97–88
2.	163–564–280	281–1040–490	297–847–613	70–116–93	44–86–36	19–27–22	58–93–72	73–112–90
3.	156–355–233	324–464–369	470–811–643	106–177–144	41–70–48	36–54–43	56–65–60	84–109–96
4.	158–313–223	259–405–331	423–681–521	92–165–122	45–81–62	32–40–35	22–35–28	75–92–83
5.	146–310–220	298–757–409	461–812–655	85–175–114	43–81–66	27–42–34	72–84–80	81–100–92
6.	103–335–226	188–508–334	322–485–380	60–137–87	42–87–57	17–30–22	20–28–23	66–85–79
7.	121–297–217	210–564–346	198–729–442	87–177–130	40–84–55	34–44–40	46–68–57	96–125–108
Range of Averages	209–280–230	331–490–378	380–655–542	87–144–112	48–66–57	21–43–31	23–80–57	79–108–91

* Each figure represents a random one-day sample.
** Each figure represents a four-day sample.

Discussion

The results of this study confirm the findings of other workers that the output of biotin in urine plus feces is greater than the dietary intake. In addition, evidence is presented suggesting that in normal young men subsisting on a normal diet, intestinal bacteria synthesize large quantities of pantothenic acid, *p*-aminobenzoic acid, biotin and folic acid. Possibly nicotinic acid is also synthesized in appreciable quantities by intestinal bacteria. N^1-methylnicotinamide, which was not included in our values for total excretion, appears in urine to the extent of thrice the quantity of niacin. Other workers (5) report over 94% of the total nicotinic acid and metabolites excreted in the urine is in the form of N^1-methylnicotinamide. If the latter substance appears in feces in quantities 2 or 3 times as great as the niacin value, total urinary and fecal excretion would equal or slightly exceed the intake.

In addition to pantothenic acid, *p*-aminobenzoic acid, biotin and folic acid, the bacteria of the intestine probably synthesize much greater quantities of pyridoxine than our data indicate. The organism we used, *Neurospora sitophila 299*, measured pyridoxine, pyridoxamine and pyridoxal (22). Another metabolite of pyridoxine, 4-pyridoxic acid, has been reported to be excreted in much greater quantities in the urine than the aforementioned three substances (9). It is quite possible feces may contain additional quantities of 4-pyridoxic acid. Thus, the figures we present do not represent the entire excretion of nicotinic acid and pyridoxine and their metabolites. The true values are probably much greater than those reported here. This is perhaps true of the other vitamins where the metabolic products are not yet known or determinable.

Dermal excretion of thiamine, riboflavin, biotin, pantothenic acid, *p*-aminobenzoic acid, pyridoxine and nicotinic acid is reported to be practically negligible as compared with the urinary excretion (6, 23). However, some folic acid is reported to be excreted in the sweat (6), but since the fecal excretion is large, this amount would be relatively negligible under the conditions of this experiment.

From these data it is apparent that on a normal diet a normal individual excretes in the urine and feces far more *p*-aminobenzoic acid, biotin, folic acid and pantothenic acid, and possibly more pyridoxine

and niacin, plus their metabolites, than is ingested in the diet. Possibly the synthesis of these vitamins in the intestine by bacteria accounts for these results. The question of whether the large quantity of B-vitamins eliminated in the feces is excreted because it is not needed or because it is not available to the body is of much interest. In these studies it was found that between 60–100% of each of the B-vitamins in feces was extractable with water.

There was no parallel in any of the individuals concerned between dietary intake and urinary plus fecal excretion. There appeared to be a familial resemblance between the urinary and fecal excretion of thiamine in subjects 4 and 6, who are brothers. In these instances, the total excretion (urinary plus fecal) as expressed in terms of percentage of the intake, was similarly low for each subject. Similar findings were obtained in these individuals for riboflavin. (See Table II.)

The authors wish to express their appreciation to Dr. Joseph J. Pfiffner of Parke, Davis and Company, Detroit, Michigan, for furnishing crystalline folic acid, and to Miss Genevieve Gales, dietitian of Passavant Memorial Hospital, Chicago, for supervising the preparation of the diet. It is a pleasure to acknowledge the cooperation of the following volunteers (members of the Civilian Public Service) on detached service for the project from the Army Epidemiological Board: Deibert D. Blickenstaff, Harvey E. Dibrell, Edward L. Crill, Lee Smith, Jr., Roy W. Miller, John H. Smith and Gareth W. Heisler.

Summary

1. Daily urinary and fecal excretion of *p*-aminobenzoic acid, biotin, folic acid, pantothenic acid, pyridoxine, thiamine, riboflavin and nicotinic acid of seven normal young men were determined.

2. Daily dietary intake of these vitamins was determined and compared with the total urinary and fecal output. Daily fecal excretion was larger than urinary excretion in all cases except pantothenic acid and pyridoxine.

3. The total daily urinary and fecal output of *p*-aminobenzoic acid, biotin, folic acid and pantothenic acid exceeded dietary intake. Dietary intake of thiamine, riboflavin, pyridoxine and nicotinic acid exceeded the combined urinary and fecal output. However, the methods employed do not measure all the metabolites of pyridoxine and nicotinic acid.

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Hosp. Management 56(1):87-90. 1943.

Elvehjem-'43
Bib No. 384

Newer Findings in Vitamin Research

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In spite of all efforts to put nutrition on a quantitative basis, optimum health is not obtainable as long as a single vitamin is missing. The presence or absence of the final necessary factor determines how efficiently the body will function. There are many newly discovered factors which may be of great significance because of their limited distribution and because they are readily inactivated.

Animals which grow almost normally on a synthetic diet of vitamins A, D, E, thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid and choline, upon the addition of a small amount of sulfa drug fail to grow and increased clotting time of the blood and fuecopenia occurs. Different species of animals require different additions, but vitamin K, biotine, inistol and paramino-benzoic acid either singly or in combination are required.

While it is not proven that these factors have an important place in human diets, it would be very surprising if a few of these unisolated factors were not necessary. Even if they are not needed directly, a more complete understanding of their chemistry and functions will undoubtedly be a great help in explaining the variations now encountered in the quantitative figures for the older vitamins.

No Long Time Plan

It is unfortunate that no definite plan has been made to maintain properly trained biochemists in the laboratory so that the fundamental studies can proceed even during the height of the war activity. Though pellagra is obviously one of our chief deficiency diseases there is no long time experiment either complete or incomplete to determine the true nicotinic acid requirement for human beings.

Theoretically the question of vitamin requirements should be disposed of by mentioning that the values of the better known vitamins have been established by the Food and Nutrition Board. These allowances which represent optimum nutrition were established before the war when there was no indication of limited food supplies. It is true that some recent studies indicate that adults may be maintained without obvious evidence of deficiency on diets somewhat low in thiamine and considerably lower in riboflavin than the figures given in the dietary allowances.

The Food and Nutrition Board intended the values to be considerably above the minimum requirements. All new evidence is now being evaluated and revisions will be recommended when the experimental results are available. There are great many individual differences and until all facts are available there can be no harm in using the rather generous values now recommended.

The vitamin values given for fo in the new tables may be accepted with confidence; in fact, the figure

for the better known vitamins may be more accurate than those for proximate analyses and for mineral elements. Optimum nutrition is more likely to result from a proper combination of all food groups than from a continual emphasis on certain selected groups. It is also important to differentiate between the foods in the same groups. For example, great emphasis is now being placed on whole grain cereals.

However, the niacin content of corn will never reach the value for wheat, nor will the thiamine content of any of the other cereals equal the value of oats. Furthermore, it is necessary to use the values for the foods as they are consumed. Example: The loss in processing has been observed in the case of flour in bread, and after a tremendous amount of effort, white bread equivalent to whole wheat bread as far as certain nutrients are concerned is now available on all markets.

The vitamin content of foods when they are about to be cooked or dehydrated should receive attention as well as the subject of the loss of vitamins during cooking dehydration. During a period of 48 hours at room temperature, the following losses of ascorbic acid occur: Spinach 70 per cent; lettuce, 70 per cent; broccoli, 65 per cent; green bananas 25 per cent. These losses are greatly reduced by proper refrigeration. Not only carotene and ascorbic acid, but thiamine disappears in practically all dehydrated foods, when they are stored at a temperature of above 80 degrees. The losses from cooking have been reduced due to improved cooking methods. Thus, with the exception of vitamin C, the actual losses in cooking are not as great as most people have assumed them to be.

Emerson-'44
Bib No. 389

Proc. Soc. Exptl. Biol. Med. 57:47-48. 1944.

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Biotin-Pantothenic Acid Interrelationship in Rats Fed Succinylsulfathiazole.

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The feeding of so-called "purified" rations containing sulfaguanidine or succinylsulfathiazole to rats has a marked depressing effect upon growth; an inhibition which can be counteracted by the feeding of liver extracts.[1,2] Daft and co-workers[3] reported that this deficiency syndrome was ascribable in part to a lack of biotin. The biotin deficiency was not progressive, due in all probability to the coexisting lack of folic acid. These findings were confirmed by Nielsen and Elvehjem[4] and by Martin.[5] The only other means of producing biotin deficiency in the rat is either by the feeding of a purified diet containing egg white or a purified ration with casein as the source of protein but supplemented with an avidin concentrate: the deficiency state thus produced progresses and leads to the ultimate death of the animal.

Wright and Welch[6,7] have reported that folic acid and biotin are necessary for the utilization of pantothenic acid by rats receiving purified diets containing succinylsulfathiazole. Animals fed this type of ration gave evidence of pantothenic acid deficiency despite the inclusion of adequate amounts of this vitamin in their diet. Even parenteral administration of pantothenic acid failed to restore the levels of this vitamin in the liver to the normal. West *et al.*[8] observed changes usually associated with a lack of pantothenic acid in rats maintained on a sulfapyridine containing low protein diet. However, the feeding of excess pantothenic acid cured these deficiency manifestations.

In the course of an investigation dealing with the influence of succinylsulfathiazole upon the utilization of pantothenic acid it was noted that signs of biotin depletion were much more marked in rats receiving the pantothenic acid deficient diet than in controls supplied with this vitamin. At a time when the rats receiving pantothenic acid showed only incipient manifestations of biotin deficiency, namely, spectacled eyes and sparse mole-like fur, the rats fed the pantothenic acid deficient diet showed a more advanced state of biotin depletion such as sore mouth, ventral alopecia and encrustations. These animals also had the porphyrin caked whiskers and pattern greying associated with a lack of pantothenic acid. Curative therapy with biotin was initiated at a time when the rats were failing rapidly from a lack of folic acid. Although some improvement was seen, weight losses were continuous and only incipient

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TABLE I.

Group	No. rats	Avg gain in wt, g 30 days	Avg gain in wt, g 42 days	No. survivors, 42 days	Deficiency signs: Biotin: First observed days on test	Deficiency signs: Biotin: Severity attained	Deficiency signs: Pantothenic acid: First observed days on test
Diet H5 (Pantothenic acid deficient)	10	60	70	All	—	—	29
Diet H5S (Pantothenic acid deficient + 0.5% succinylsulfathiazole)	10	32	42	3	16	Marked	24
Diet H5S + 5 μg biotin	8	50	53	All	—	—	30
Diet H5S + 500 μg Ca pantothenate	8	91	97	All	28	Mild	—
Diet H5S + 5 μg biotin and 500 μg Ca pantothenate	10	118	135	All	—	—	—
Diet H5 + 500 μg Ca pantothenate	10	148	202	All	—	—	—

changes were noted in the pelage during the short survival period. Accordingly a prophylactic experiment was designed in order to show the effect of biotin, pantothenic acid, and the combination of both upon the production of the two deficient states.

Methods and Results. Weanling male rats were segregated into 5 dietary groups, each being represented by 8 to 10 animals as indicated in Table I. The pantothenic acid free ration (H5) had the following composition: vitamin free casein (Labco), 24; sucrose, 60; salts (U.S.P. No. 1), 4; hydrogenated vegetable oil, 10; cod liver oil, 2. The diet was supplemented with 1 mg each of thiamin and pyridoxin, 2 mg of riboflavin, 10 mg of inositol and 100 mg of choline per 100 g. In the diet containing succinylsulfathiazole (H5S) 0.5% of the drug replaced an equivalent amount of sucrose. Groups receiving biotin and calcium pantothenate were fed these vitamins daily by stomach tube.

As can be seen from Table I the feeding of the ration deficient in pantothenic acid which contained sulfathiazole had a marked effect in depressing growth. After 6 weeks 7 of an original 10 animals had succumbed. Signs of a mild degree of biotin deficiency, namely, spectacled eyes and sparse mole-like fur, were apparent in this group after only 16 days on test. This condition progressed rapidly to involve the ventral aspects of the head and trunk. Hopping gait was observed in one animal. Signs usually associated with a lack of pantothenic acid, namely, porphyrin deposits on the whiskers and pattern greying, were first observed after 24 days on the regimen. This is at a somewhat earlier period than with the pantothenic acid deficient diet without the sulfonamide.

The daily feeding of 5 μg of biotin evoked only a slight growth response; all animals, however, survived the 42-day test period. The rats were completely free from the syndrome associated with a deficiency of biotin. Moreover, the feeding of biotin not only prevented manifestations of biotin deficiency but also tended to delay the onset of pantothenic acid deficiency. Although achromotrichia was present porphyrin caked whiskers were seen in only one rat. It is of interest to note that Unna[9] did not observe porphyrin caked whiskers in rats receiving 40 μg of calcium pantothenate although greying was not prevented.[10]

When pantothenic acid was fed, changes ascribable to a deficiency of pantothenic acid were not observed. In addition the feeding of the vitamin had a marked growth stimulating effect. This observation indicates that calcium pantothenate can be utilized to some extent, a finding in variance with that reported by Wright and Welch.[6,7] However, in the present experiments succinylsulfathiazole composed 0.5% of the diet whereas in the ration of Wright and Welch, a higher level of the sulfonamide, namely 2%, was employed.

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The feeding of calcium pantothenate alone had an attenuating effect upon biotin deficiency as reflected both in the time of onset of changes ascribable to biotin deficiency and in the severity of the lesions.

Rats receiving both biotin and calcium pantothenate were, at the termination of the experiment, completely free from the skin and pelage changes associated with biotin and pantothenic acid deficiencies. The fact that the growth did not equal that observed in the absence of the sulfonamide, could, in all probability, be ascribed to a lack of folic acid and other factors, since it has been shown that growth is restored to the normal by the feeding of folic acid concentrates.

Summary. Biotin deficiency induced in weanling rats by the feeding of a succinylsulfathiazole containing purified ration may be aggravated by superimposing a deficiency of pantothenic acid. The feeding of biotin protected against these changes and, in addition, appeared to lessen the severity of the syndrome associated with a lack of pantothenic acid. Calcium pantothenate, fed prophylactically, was completely protective against deficiency signs usually associated with the absence of pantothenic acid. Furthermore, signs of a mild degree only of biotin depletion were observed and the period required to produce such changes was extended beyond that observed in the absence of pantothenic acid. Rats receiving both biotin and calcium pantothenate were free from signs of both deficiency states.

Fraenkel-Conrat-'52
Bib No. 444

Biochem. et Biophys. Acta 8(1):66-70. 1952.

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METABOLIC FATE OF BIOTIN AND OF AVIDIN-BIOTIN COMPLEX UPON PARENTERAL ADMINISTRATION

by

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Avidin has been shown to be the protein responsible for the ability of raw egg white to bind biotin and thus render dietary biotin unavailable[1,2]. In contrast to the effectiveness of avidin to cause egg white injury upon oral administration, its injection was shown to have a curative effect on the same deficiency disease[3]. This was explained on the basis of the fact that avidin always contains some biotin (corresponding to 10–20% of its capacity) and that this bound biotin was presumably available to the mammalian organism when given parenterally. All attempts to elucidate the mode of liberation of biotin from the complex, however, were unsuccessful[4]. The complex appeared remarkably resistant to all crude or purified enzyme systems studied, as well as to wide variations of p_H; only oxidative agents seemed to liberate small amounts of biotin.

The availability to us of avidin-biotin complex containing radioactive biotin[5] appeared to supply a new tool to investigate this problem. Since a tracing of the metabolic fate of biotin could hope to be successful only if relatively large amounts of radioactivity and thus of biotin were given (at least 0.01 mg or 0.04 microcurie), pilot experiments with similar amounts of nonradioactive biotin were first conducted. It appeared that in the rat, as in man[6], administration of superphysiological doses led to marked increases in urinary excretion of biotin within the first 24 hours. The same was true for radioactive biotin, about 40% of which appeared unchanged in the first day's urine; most of the residual radioactivity was also largely excreted at that time, although not as intact biotin. In contrast, biotin administered in the form of the avidin complex appeared in the urine at a more protracted rate, a total of about 50–60% being excreted within 5 days. No evidence for protein-bound biotin was found. The liver contained approximately 7% of the administered dose at the time of autopsy, but the residual radioactivity was not concentrated to such an extent in any of the other tissues or excreta investigated that it could be determined quantitatively.

This study has supplied the first incontrovertible evidence that biotin bound to avidin is released under physiological conditions and then follows a path similar to that of free injected biotin. This finding lends independent support to the seemingly paradoxical finding of GYÖRGY AND ROSE that avidin, or rather the complex, upon parenteral

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administration may cure the symptoms of egg white injury. The present study unfortunately has not revealed any clear evidence concerning the site or mechanism of release of the biotin.

EXPERIMENTAL MATERIALS AND METHODS

Radiobiotin, labelled with C^{14} in the ureido group, was kindly supplied by Dr D. B. MELVILLE of Cornell University. The avidin-biotin complex was material which had been used for equilibrium studies[5]. It was prepared from about 90% pure avidin NA[7,8] saturated with radioactive biotin of varying specific activity and dialyzed for protracted periods of time so that it was free from excess biotin or radioactivity.

Approximately 300 g rats were injected intraperitoneally with 4–5 ml solution containing 13.5 μg radioactive biotin (equivalent to 0.063 μc) or radiobiotin-avidin complex containing about 2 mg avidin and 13.0–15.4 μg radioactive biotin (equivalent to 0.038–0.062 μc) and immediately placed in a glass respiration chamber supplied with CO_2-free air. The urine was collected under toluene, feces in a wire trap, and expired CO_2 in 2 *N* sodium hydroxide.

Radioactivity was measured in a Geiger-Müller counter with thin mica window. Aluminum discs were employed in the preparation of plates from aliquots of urine or fractions thereof, of fractions obtained from liver, or of acetone slurries of homogenized tissues or feces. Small glass cylinders with ground edges were coated inside with a thin film of Dow Corning Silicone, then were rigidly held in place over the aluminum discs while the aliquots of urine (or other samples) were dried under an infra-red heat lamp. Barium carbonate precipitates from the alkali containing the expired CO_2 were plated from alcohol suspensions according to standard technique.

Aliquots of 3–5 ml urine were used in the preparation of plates. Since much of the radiation was expected to be absorbed by the bulky residue of the urine and since the specific activity of the 3 and 5 ml aliquots did not agree, experiments were carried out to determine the amount of self-absorption incurred by 3 and 5 ml nonradioactive urine containing known amounts of added radiobiotin. The necessary correction values were thus obtained: 3.0 and 1.9 for 5 and 3 ml urine samples respectively, independent of the biotin concentration. These were so high that the corrected values listed in Table I must be considered only as approximations. A similarly high correction value (2.5) had to be used in the case of acid hydrolysates of tissue which were neutralized directly on the plate with ammonia. In order to avoid this correction, the sulfuric acid was neutralized with baryta and the barium sulfate removed by centrifugation before the aliquots were taken for determination of radioactivity. Skepticism should also be entertained in the evaluation of the plates obtained from acetone slurries of whole tissue or feces, although no attempt was made to determine the amount of self-absorption in these cases due to the wide range in weight of the samples and the scarity of radiobiotin.

Biotin assays were kindly performed by Miss N. SNELL of the Western Regional Research Laboratory, using *Saccharomyces cerevisiae* F.B., in principle by the method of HERTZ[9].

RESULTS

Excretion of radioactive material

Urine analyses (see Table I). Microbiological assays indicated that the daily excretion of biotin was increased from a control value of 2 μg to 9 μg during the first 4 hour period after administration of 16 μg free biotin and then dropped back to the control value. The excretion of radioactivity during the first 24 hours corresponded to about 13 μg of radiobiotin, although the accuracy of this value is somewhat doubtful owing to the great corrections for self-absorption that had to be applied. To ascertain how much of this excreted radioactive material was unchanged biotin, excess avidin was added to aliquots of the urine which were then dialyzed. These experiments indicated that about half of the total radioactivity contained in the urine was in the form of avidin-combinable, and thus unchanged, biotin, in agreement with the microbiological assay value*.

* The radiobiotin as available to us contained about 2% nonbiotin material[5]; this insignificant contamination could not appreciably affect the present results.

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TABLE I

FATE OF RADIOBIOTIN AND RADIOBIOTIN-AVIDIN COMPLEX UPON PARENTERAL ADMINISTRATION

		Biotin	Biotin-avidin complex	
			I	II
Dose administered	μc	0.063	0.062	0.038
	μg[1]	15.6	15.4	13.0

PERCENTAGES OF ADMINISTERED RADIOACTIVITY[2]

	Total[3]	Biotin[4]	Total[3]	Biotin[4]	Total[3]	Biotin[4]
Urine Day 1	85 (3)	39 (7)	20 (1)	23 (1)	14 2)	10 (1)
2	1 (2)		22 (2)		18 (3)	19 (4)
3	1 (1)		4 (2)		13 (2)	14 (1)
4			2 (1)		6 (1)	
5			2 (1)		7 (1)	
6			1 (1)			
	87		51		58	
	Total	Biotin	Total	Biotin	Total	Biotin
Liver	4 (1)[5]	7 (2)[7]	7 (2)[5]		10 (1)[5]	9 (1)[7]
	5 (1)[6]		6 (3)[6]		8 (2)[6]	

1. Expressed as biotin.
2. Number of determinations in parentheses.
3. Total count expressed as percent of administered dose, obtained by plating urine directly and correcting for selfabsorption of 5 and 3 ml samples.
4. Amount of radiobiotin in urine capable of combining with avidin, expressed as percent of administered dose.
5. Total count expressed as percent of administered dose, obtained by plating acetone homogenates thus only rough approximations.
6. Total count expressed as percent of administered dose, obtained by plating sulfuric acid digest
7. Amount of radiobiotin in liver liberated by sulfuric acid and capable of combining with avidin expressed as percent of administered dose.

After the administration of similar amounts of biotin complex-bound to avidin, rises in total biotin excretion above the control value of 2 μg per day were observed for 2–3 days. Three μg per day was the maximum observed. The excretion of radioactivity similarly extended over several days and amounted to 51–58% of the dose. In contrast to the results described above for the amount of true biotin compared with total radioactivity excreted by the rat receiving radiobiotin, the results obtained with the rats receiving avidin-radiobiotin complex would indicate that the total excretion of radioactive material was in the form of true biotin. Thus at least 50% of the radioactivity was dialyzable prior to the addition of avidin, but none after. The total amount of radiobiotin excreted by these rats was considerably more than the "extra" biotin excreted, as measured by microbiological assay, compared with the pre-injection control level, but was less than the total biotin appearing in the urine. Thus it would appear

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that the injection of a large dose of biotin (an eight-fold of the daily excretion value) depresses the normal mechanism of resorption from the intestine.

Preliminary studies of the effect of nonradioactive biotin (11 μg) and complex on the excretion of biotin by fasted rats indicated the same facts. Free biotin produced a markedly increased biotin excretion during the first day (the fasted control level of 1.0 μg per day was raised to 3.0 μg); whereas the complex effected an increase extending over 3 days (1.8, 2.2, and 1.4 μg per day respectively).

Expired CO_2. No count was obtained on the barium carbonate plates; it was therefore concluded that the injected biotin was not oxidized, or only to such a small extent as to be undetected in the Geiger-Müller counter.

Feces. Plates of acetone slurries of feces would indicate that approximately 7% of the total count was excreted in 3 days following the injection of radiobiotin and about 3% of the total count was excreted in the same time following the injection of avidin-radiobiotin complex.

Distribution of radioactivity in the body.

A qualitative search for radioactivity in various organs by direct plating of acetone suspensions of tissue homogenates indicated appreciable count only in the liver and considerably less in the kidney and spleen, with no radioactivity being observed in adrenal, mesentery, lung, blood, thyroid, and muscle. Acid extracts of the skin seemed to indicate a small amount of radioactivity to be present; however, the values obtained were so low and erratic that no definite conclusions could be drawn.

The nature and amounts of the radioactive material in the liver was investigated further after acid hydrolysis of samples of the tissue. The most reliable analyses were probably those in which the sulfuric acid was removed by neutralization with baryta. These indicated that 5–8% of the administered radioactivity was in the liver after 3–6 days, apparently largely as biotin, since most of it was rendered nondialyzable upon the addition of avidin (see Table I).

Liver tissue was also fractionated by the method of HOFFMANN[10], as well as by an ammonium sulfate fractionation procedure designed to reveal the presence of radiobiotin in a fraction corresponding in solubility to avidin or complex (see Table II). The former

TABLE II

FRACTIONATION OF LIVER

Experimental conditions	Free radiobiotin		Avidin-radiobiotin complex
	% of dose[1]	μg biotin per g liver[2]	% of dose[1]
Hoffmann technique			
Acid extract	0.9	1.3[3]	0.7, 1.5
Alkaline extract	3.4	2.1[4]	3.2, 2.6
Ammonium sulfate			
0.5 satd. AS			4.6
1.0 satd. AS			0
Satd. AS supernatant			0.6

1. As calculated from radioactivity.
2. By microbiological assay.
3. Active without prior hydrolysis.
4. Only 10% as active without hydrolysis.

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method yielded results concerning the fractionation of liver biotin which confirmed HOFFMANN well. The liver contained 3.4–4 μg biotin per g dry tissue, which was about 10 times its content of radiobiotin. The radioactivity appeared in both the acid and alkaline extracts, approximately in proportion to their total biotin contents.

The ammonium sulfate fractionation yielded the highest count in the globulin fraction, and no detectable amount in the fraction precipitated above 2.0 *M* ammonium sulfate, which would contain any avidin or complex. There was a small amount of non-dialyzable activity not precipitated with ammonium sulfate.

ACKNOWLEDGEMENT

The authors wish to thank Dr A. F. MORGAN for generously placing at their disposal the facilities of the laboratory.

SUMMARY

1. The parenteral administration of 0.01–0.02 mg biotin to fed or fasted rats caused a marked increase in biotin excretion for one day, accounting for up to 40% of the dose.

2. The use of labelled biotin in a similar experiment indicated that most of the residual biotin was also excreted within one day although not in microbiologically active form.

3. Of similar amounts of radiobiotin administered in complex form with avidin, about one half was excreted over a 3–5 day period, apparently largely as free biotin.

4. In each experiment, a small fraction of the administered radiobiotin was found in the liver, but no appreciable amount in the other organs tested.

RÉSUMÉ

1. L'administration parentérale de 0.01–0.02 mg de biotine à des rats alimentés ou à jeûn causa une augmentation considérable de l'excrétion de biotine pendant un jour, atteignant 40% de la dose.

2. L'emploi de biotine marquée montra, dans une expérience semblable, que la majeure partie de la biotine résiduelle était également excrétée pendant un jour, quoique non sous une forme microbiologiquement active.

3. Environ la moitié de quantités semblables de radiobiotine administrées sous forme de complexe avec l'avidine, fut excretée pendant une période de 3 à 5 jours, apparemment en majeure partie sous forme de biotine libre.

4. Dans chaque expérience une petite fraction de la radiobiotine administrée fut trouvée dans le foie; aucune quantité appréciable ne fut trouvée dans les autres organes étudiés.

ZUSAMMENFASSUNG

1. Die parenterale Verabreichung von 0.01–0.02 mg Biotin an gefütterte und nicht gefütterte Ratten verursachte eine bedeutende Zunahme der Biotinausscheidung während eines Tages, welche bis zu 40% der Dosis entsprach.

2. Die Anwendung von markiertem Biotin in einem ähnlichen Versuche zeigte, dass der grösste Teil des restlichen Biotins auch während eines Tages ausgeschieden wurde, allerdings nicht in mikrobiologisch aktiver Form.

3. Wurden ähnliche Mengen von Radiobiotin in Form eines Komplexes mit Avidin verabreicht, so wurde ungefähr die Hälfte während einer Zeitspanne von 3 bis 5 Tagen ausgeschieden, anscheinend in Form von freiem Biotin.

4. In jedem Versuch wurde eine kleine Menge des verabreichten Radiobiotins in der Leber gefunden; in den anderen untersuchten Organen wurden keine merklichen Mengen nachgewiesen.

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Received June 7, 19[illegible]

Gardner-'45
Bib No. 463

Arch. Biochem. 8:339-348. 1945.

Human Biotin Metabolism on Various Levels of Biotin Intake *

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Received August 7, 1945

Introduction

The importance of biotin in the nutrition of man and animals has been well established. The animal work on biotin and egg white injury, the isolation and characterization of biotin and avidin, and the requirement of microorganisms for biotin have been reviewed (1, 2, 3, 4, 5).

In the present study the urinary and fecal biotin elimination has been determined on diets of low, moderate and high biotin content and a moderate biotin diet with the daily administration of 50 and 100 γ of biotin as the methyl ester (70% purity).† The diurnal variation in excretion of biotin of certain subjects consuming these diets is presented. The water-soluble biotin in feces has also been measured.

Experimental

Subjects: In the two studies reported, uniform weighed diets were eaten by 10 healthy young women. Six subjects ate the three diets varying in biotin content and four were in the second study. One subject began the first period of study on the fifth day of the experiment. The weight of each subject varied little during the course of the study.

Diets: The composition of the diets is given in Table I. The first study lasted 30 days and was divided into three consecutive 10 day periods. The three diets of low, moder-

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Nutrition Foundation Inc., New York.

† The authors wish to express their great appreciation to Dr. Y. Subbarow of Lederle Laboratories, Inc., for the generous supply of biotin used during these experiments.

TABLE I

Composition of Diets

Food	Study 1		
	Low biotin, diet 1	Moderate biotin, diet 2*	High biotin, diet 3
	g.	g.	g.
Breakfast:			
orange juice, canned	150	—	200
grapefruit juice, canned	—	200	—
bread, white	65	43	—
bread, whole wheat	—	—	21
butter	20	15	10
jelly, grape	20	15	8
all bran, Kellogg's	—	—	50
egg	—	—	50
milk	—	—	300
Biotin content of breakfast	2γ	2γ	39γ
Lunch:			
baked beans, canned	150	—	—
bread, white	43	—	—
butter	15	12	8
lettuce	50	60	60
mayonnaise	15	15	10
cheese, cheddar	30	55	—
eggs, scrambled	—	—	150
bread, whole wheat	—	60	21
apple	150	150	—
ice cream	—	—	100
peanuts	—	—	20
milk	—	240	260
Biotin content of lunch	2γ	12γ	43γ
Dinner:			
cottage cheese	100	—	—
potato, baked	150	100	100
beef, ground round	—	100	—
liver, beef	—	—	100
beans, green snap	—	—	100
tomato juice, canned	—	200	200
lettuce	10	—	—
corn, canned	100	—	—
cabbage	—	30	—
bread, white	18	23	—
butter	25	23	10
ice cream	—	100	—
grapefruit sauce, canned	100	—	—
apricots, canned	—	—	100
milk	—	240	240
Biotin content of dinner	4γ	19γ	89γ

* This diet was also used in study 2.

ate and high biotin content contained by analysis 9, 33 and 171 γ of biotin daily. The water intake was not kept constant.

The diet low in biotin was deficient in several essentials. Therefore, it was supplemented daily with 1 mg. of thiamine, 1 mg. of riboflavin, 15 mg. nicotinamide, and 0.65 g. of calcium lactate. The low-biotin diet contained no milk or meat in order to keep the biotin content low.

To the diet with a moderate level of biotin, 1 mg. of thiamine was added with lunch. This diet was very similar to that described by Parsons and Collord (6). This moderate-biotin diet contained a pint of milk and a serving of ground beef.

TABLE II

Nutritive Value of Diets

Dietary essential	Low-biotin, diet 1	Moderate-biotin, diet 2	High-biotin, diet 3
Calories	1859	2080	2198
Carbohydrate, g.	207	182	164
Fat, g.	82	114	118
Protein, g.	62	75	108
Calcium, g.	0.56	1.34	1.45
Phosphorus, g.	1.37	1.45	2.54
Iron, mg.	8.1	10.2	32.2
Vitamin A,* mg.	1.5	1.3	9.6
Ascorbic acid, mg.	161	181	236
Thiamine, mg.	2.1	1.1	2.3
Riboflavin, mg.	2.2	2.4	6.9
Pantothenic acid, mg.	2.1	4.0	18.3
Nicotinic acid, mg.	18.9	12.0	50.5
Biotin, γ	8.9	32.8	171.0

* Carotene was expressed in terms of vitamin A on the basis that 0.6 γ of carotene are equivalent to 0.3 γ of vitamin A.

The high-biotin diet included four eggs, beef liver and nearly a quart of milk daily. These foods are particularly rich in biotin. The levels of the other vitamins as well as the amount and quality of protein were increased over the low and moderate-biotin diets (Table II).

The basal dietary constituents in the second study were the same as in diet 2 of the first study. During periods II and III, 50 and 100 γ of biotin as the methyl ester (70% pure) were added in two equal doses before 9 A.M. with breakfast and at 6 P.M. with dinner.

Methods: An aliquot of the foods eaten by the subjects on each diet was treated as previously (7) except that 4 *N* HCl was used instead of 2 *N* H_2SO_4 since the former was proved to yield a greater amount of biotin. Twenty-four hour urinary collections were made as in the foregoing studies. During diets 1, 2 and 3 (study 1) the first two

days' collections were discarded. The urinary and fecal collections for days 3 through 6 and 7 through 10 were composited so that a representative sample would be obtained. In the second study the biotin content of each 24-hour urinary sample was assayed.

Diurnal variation in biotin content of the urine was studied for the last day of each period (tenth day of study 1 and the sixth day of periods II and III of study 2. Aliquots of the collections for each period were assayed for biotin for subjects E. B. and J. G. on diets 1, 2 and 3 and for all four subjects during study 2 with the exception of V. S. during period II.

Fecal eliminations were collected, dried and hydrolyzed as described previously except that 4 *N* HCl was used instead of 2 *N* H_2SO_4. In diets 1, 2 and 3 (study 1) the feces were composited as were the urinary collections, days 3–6 and days 7–10 together. In study 2, period I the eliminations for days 1–4 and 5–6 were combined and thereafter days 1–3 and 4–6 were combined. In all cases 0.5 g. of carmine in a gelatine capsule was ingested to mark the beginning and end of each period.

The amounts of biotin extracted from wet and dried samples of a single fecal elimination by water and by 4 *N* HCl in 4 hours at room temperature were compared. Chloroform (1 ml.) and 50 ml. of water were added to 5 g. of wet feces. A portion of the water extract was hydrolyzed in the usual manner with 4 *N* HCl as well. A second aliquot of this fecal sample was dried and portions of it extracted with water and hydrolyzed with acid.

Results and Discussion

Metabolism of Biotin in Foods

The urinary biotin of the six subjects did not show a consistent change when the dietary biotin was increased from 9 to 33 γ *per diem*, an increase of 370% in biotin intake (Table III, diets 1 and 2). In fact, the average urinary biotin for five subjects was almost identical for these two diets (29 and 30 γ). The biotin excretion for five of the six subjects fell within a narrow range and that of the sixth was conspicuously lower than the others during all three levels of biotin intake (Table III). Subject E. W. P. was known to have eaten, previous to this study, less than three meals a day which probably entailed an abnormal selection of food. Possibly some of the biotin of the moderate and high-biotin diets was retained to elevate low body stores of the vitamin. Another possibility would be less intestinal synthesis than the other subjects. No evidence for this was afforded by the level of her fecal biotin, however, as it was very similar to the average of the other subjects. Unfortunately we were unable to study her response to the ingestion of pure biotin.

All six subjects excreted slightly less biotin on days 7–10 during the low and moderate levels of biotin intake than on days 3–6. It is possible

though not probable, that the urinary biotin would have fallen to the level of intake had the period been extended longer than the 10 days. Oppel (8) found that during an unrestricted diet or after a period of starvation similar amounts of biotin were present in human urine. These observations suggested to him that urinary biotin was based on a relatively constant level of biotin synthesis by colonic bacteria and sudden variations due to changes in biotin content of the diet.

TABLE III

Urinary and Fecal Biotin During Three Levels of Biotin Intake (Diets 1, 2 and 3, study 1)

Diet	Days	Daily biotin intake	Daily urinary biotin			Daily fecal biotin		
			5 subjects		Subject E.W.P.	5 subjects		Subject E.W.P.
			Range	Av.		Range	Av.	
		γ	γ	γ	γ	γ	γ	γ
1	3–6	9	24–38	31	25	51–84	62*	52
	7–10	9	20–36	27	18	41–75	55	63
	Av.		24–37	29	22	31–79	55	57
2	3–6	33	29–35	32	17	63–108	86	82
	7–10	33	22–30	27	12	46–99	73	77
	Av.		27–32	30	15	61–88	78	80
3	3–6	171	123–172	150	94	75–143	105	108
	7–10	171	168–200	185	108	64–147	96	103
	Av.		157–180	167	101	71–132	100	105

* Average of only four subjects.

When a high-biotin diet (171 γ daily) was consumed, the urinary biotin reflected the rise in intake (Table III). This rise is in contrast to the lack of change in urinary biotin from low to moderate intake. The dietary intake was raised 520% while the average urinary output of biotin for five subjects increased 500% (days 3–6) and 620% (days 7–10) over the average of the preceding periods. Thus, the urinary excretion of biotin was distinctly higher during the last four days of the high-biotin diet. A plateau of high biotin excretion on this high-biotin diet was not reached in 3–6 days. There was a tendency for the body

to retain quite large amounts of biotin, thus making a lag in reaching a plateau of urinary biotin excretion.

The fecal elimination of biotin on the three levels of biotin intake is shown in Table III. Possible irregularities in daily elimination of feces were somewhat minimized by pooling 4 days' samples as described previously.

The significance of differences in fecal biotin is very difficult to ascertain. Variations in fecal biotin due to dietary biotin may be masked by variations in biotin synthesized by bacteria. The food residues are an important factor in determining the growth of the intestinal bacteria and consequently their synthesis of various compounds. There is no way of distinguishing between biotin of food and microbial origin.

The fecal biotin exceeded the biotin in the diet on the low and moderate-biotin diets (Study 1), but did not equal the intake on the high-biotin diet Biotin synthesis in the feces was more obvious when biotin intake was relatively low. The ratio of fecal biotin to dietary biotin for the low, moderate and high-biotin diets was as follows (average for six subjects): 6 : 1, 2.4 : 1 and 0.6 : 1. There was, however, an absolute increase in fecal biotin from the low to moderate to high-biotin diets in a ratio of about 1 : 1.4 : 1.8 (Table III), although there was considerable individual variation. The increase in fecal biotin was nearly accounted for by the increase in weight of feces (1 : 1.2 : 1.8, for diets 1, 2, and 3). In individual cases the biotin per gram of feces did not show a direct relationship to the amount of feces eliminated. The greater weight of feces on diets 2 and 3 was not due entirely to differences in fiber content of the diet but may possibly have been due to more bacterial cells or residues.

Thus, in the three diets used, the fecal biotin increased to a limited extent when markedly different amounts of biotin in foods were ingested. Oppel (8) found no suggestion of a correlation between dietary biotin and fecal biotin on the diets he employed, but he did suggest the existence of a correlation between urinary and fecal biotin. The data from the present study do not entirely substantiate this latter suggestion in that during the moderate-biotin diet the fecal biotin was increased over the low-biotin diet while the urinary biotin was not.

The division of biotin output between urine and feces is of interest. The ratio between urinary and fecal biotin averaged 1 : 2, 1 : 2.6 and 1 : 0.6 for the low, moderate and high-biotin diets, respectively.

Metabolism with Additions of Pure Biotin (Study 2)

The daily urinary excretions of biotin by four subjects on a moderate-biotin intake and on the same diet supplemented with biotin methyl ester equivalent to 50 and 100 γ of free biotin are shown in Table IV.

TABLE IV

Urinary and Fecal Biotin During a Moderate-Biotin Diet and with Added Pure Biotin (Study 2)

Period and day		Biotin intake		Urinary biotin		Fecal biotin	
		Food	Added biotin	Range	Av.	Range	Av.
		γ	γ	γ	γ	γ	γ
I*	3	35	0	29–42	34		
	4	35	0	26–36	30	40–100	67
	5	35	0	27–46	37		
	6	36	0	25–35	32	37–148	87
II	1	35	50	30–41	36		
	2	35	50	42–54	46		
	3	35	50	38–56	43	23–83	54
	4	35	50	46–52	49		
	5	35	50	35–45	39		
	6	35	50	40–53	45	53–70	55
III	1	35	100	56–71	62		
	2	35	100	51–71	61		
	3	35	100	68–88	79	28–72	54
	4	35	100	69–86	79		
	5	35	100	58–111	91		
	6	35	100	88–117	106	40–90	70

* Samples for days 1 and 2 were not assayed as they represent a transition period from the unrestricted diet.

Two of the four subjects were the same as those on diets 1, 2, and 3 of the first study.

When 50 γ of biotin were ingested in two equal doses with breakfast and dinner, the urinary biotin showed a slight rise the first day of the addition in the case of two of four subjects (period II, Table IV). An

increased urinary biotin was observed for all subjects on the second day; a maximum occurred on the fourth day for three of the four subjects; on the fifth and sixth days there was a leveling off of biotin excretion. When an added 100 γ of biotin was ingested (period III, Table IV), the urinary biotin of all subjects rose the first day and in general continued to rise for the entire six days of the experiment. A larger percentage of added biotin was found in the urine with the higher supplement than with the lower.

In period I the urinary biotin equaled the intake. When 50 γ of biotin were added, an average of 50% of the total intake was excreted and 70% when 100 γ of biotin were added (average of days 4–6, Table IV). Increased biotin intake supplied by synthetic biotin and beef liver caused similar increases in urinary biotin, but raw or boiled bakers' yeast did not cause so marked an increase (7).

The biotin content of the feces during the second study is recorded in Table IV. As in the former studies the daily variation in biotin elimination was great for the four subjects. The daily average for the three periods was 77, 55 and 62 γ, respectively, for the moderate-diet (period I), moderate plus 50 γ of biotin and the moderate plus 100 γ (periods II and III). Thus, the ingestion of 50 or 100 γ of biotin as the methyl ester did not increase the fecal biotin over that during the first period. The added biotin unaccounted for in the urine then, must have been stored, destroyed or absorbed by the intestinal flora. The microorganisms may have synthesized less biotin when a supply of unbound biotin was present.

The total output of biotin during the moderate-biotin diet averaged 3.4, 1.2 and 1.2 times the biotin intake during the three periods. As in diets 1, 2 and 3 (study 1) the greatest excess output over intake occurred during the lowest biotin intake.

The water-soluble biotin in dried feces eliminated during days 4–6 of the second study was determined. If there were no changes in drying this water-soluble biotin would presumably represent biotin available for absorption, but not absorbed. Only 2–15% of the total fecal biotin was water-soluble (2–9 γ daily). There was slightly less water-soluble fecal biotin during the moderate-biotin diet (period I) than during the periods when 50 and 100 γ of added biotin were ingested. The average for these three periods was 4, 12 and 8% or 3, 7 and 6 γ of water-soluble biotin daily. It is entirely possible that a small part of the added biotin was excreted unchanged, which might account for the

differences in the water-soluble biotin in the feces during the three periods.

A further study of water-soluble biotin in one sample was made immediately after elimination and also after drying. The water-soluble biotin increased after drying from 3 to 9% (5 to 14γ). When the two water extracts of feces were hydrolyzed with acid, the measurable biotin was increased to 6 and 12% (10 and 20 γ) for the moist and dried feces. Acid hydrolysis of moist or dried feces resulted in nearly equal extraction of biotin (162 γ). Which fractions of biotin are available for human use are not known and the problems of form and availability need further study.

The diurnal variation in urinary biotin of certain subjects on the last days of all diets was determined. The lowest hourly excretion of biotin occurred in the hours during sleep (12 P.M. to 8 A.M.), an average of 3% per hour. The excretion during the morning (8 A.M. to 12 M.) was only slightly higher than the hours during sleep (average of 3.5% per hour). The greatest excretion came in the afternoon from 12 M.–6 P. M. (5% per hour) and in the evening from 6 P.M.–12 P. M. (5% per hour). Nearly 2/3 of the biotin was excreted in the afternoon and evening (12 M.–12 P.M.).

Biotin methyl ester when administered orally in amounts of 25 or 50 γ with breakfast and with dinner was excreted rather rapidly. A large portion of the biotin appeared in the urine in the first six hours after ingestion: the afternoon and evening excretions were high in biotin. However, the added biotin was not completely excreted within six hours after ingestion as indicated by the fact that biotin excretion for the hours during sleep was higher when the 25 or 50 γ of biotin was added than during the moderate-biotin diet with no biotin addition (period I, Table IV).

Summary

Two studies of the biotin metabolism of 10 college women who consumed a low, moderate and high-biotin diet and the moderate diet supplemented with 50 and 100 γ of biotin as the methyl ester are presented. These diets contained from 9 to 171 γ of biotin daily.

Increasing the biotin intake in food from 9 to 33 γ failed to cause a rise in urinary biotin, but the fecal biotin increased 40%. When 171 γ of dietary biotin were ingested, the urinary biotin rose markedly to

more than 5 times that excreted on the low or moderate biotin diet, while the fecal biotin increased 80% over that of the low-biotin diet.

Total biotin output averaged 9, 3 and 1.5 times the dietary intake for the low, moderate and high-biotin diets, respectively. Thus, biotin synthesis was again indicated and relatively less synthesis seemed to take place during the high-biotin diet.

When 50 γ of biotin were added to the moderate-biotin diet, a maximum of 30% of the additional biotin was accounted for in the urine. The maximum urinary output occurred on the third or fourth day after the beginning of the biotin feeding.

A larger percentage of biotin was recovered when 100 γ of biotin were ingested. A maximum of 72% was accounted for in the urine. The rise in excretion continued for the entire six days of higher intake.

Fecal biotin did not rise when either 50 or 100 γ of biotin were fed. The water-soluble biotin in dried feces varied from 2–15% of the fecal biotin. An increase in measurable biotin occurred when a water extract of feces was acid hydrolyzed.

The diurnal variation in urinary biotin was studied in diets of low, moderate and high-biotin intake. The hourly biotin excretion was greatest in the afternoon and evening and lowest during sleep and in the morning. Twenty-five or 50 γ of biotin given with breakfast and dinner were excreted promptly, a large portion but not all of the additional biotin being found in the urine within six hours after ingestion.

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Gardner-'46
Bib No. 465

Amer. Jour. Med. Sci. 211(2):198-203. 1946.

HUMAN UTILIZATION OF BIOTIN FROM VARIOUS DIETS*†

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The importance of biotin in the nutrition of man and animals has received considerable attention recently. Findlay and Stern[2] first suggested that a dermal and nervous syndrome of childhood called Swift's disease or pink disease showed a similarity to manifestations in rats fed a diet high in uncooked egg white. György mentioned several times the possible rôle in the prevention of human disease of the anti-egg white injury factor, called vitamin H by György.[4,5,6] An induced deficiency of biotin was brought about by including uncooked egg white in the diet of a group of men and was cured by a biotin concentrate (Sydenstricker, *et al.*[18]). The relation of biotin to certain pathologic states is still unsettled. A reduction in incidence of malaria in chickens and ducks when an adequate amount of biotin was present in the diet was demonstrated by Trager.[19] West and Woglom[20] reported that the biotin of tumor tissue was sometimes higher than that of normal tissues. Oppel[13] found little difference in the total urinary biotin of normal individuals and those with a variety of diseases. The urinary excretion of biotin of normal persons varied from 14 to 111 μg. per 24 hours and the daily fecal output varied from 86 to 191 μg. Biotin output in the urine and feces was 3 to 6 times as great as the intake from the diet (35 to 60 μg. per day), indicating synthesis of biotin, probably by intestinal microörganisms.

Little is known concerning the utilization by human beings of biotin from different sources. The balance between biotin intake and output during the consumption of a milk diet was recently reported from this laboratory (Gardner *et al.*[3]). The present paper reports four balance studies comparing the utilization by college women of biotin from diets containing different amounts of baker's yeast and beef liver. The human utilization of thiamine and riboflavin on two of these four diets has been reported elsewhere (Parsons and Collord[14]) and the utilization of biotin of these two diets has been presented in part (Parsons *et al.*[15]).

Experimental. *Subjects.* In the 4 metabolism studies presented, uniform weighed diets were consumed by a total of 15 healthy and reliable young women, 19-25 years of age. There were 4, 6, 2 and 3 subjects on Studies 1 through 4, respectively.

Diets. A uniform diet of moderate biotin content was supplemented daily with fresh baker's yeast (3, 4 or 7½ cakes weighing approximately 12 gm. each) or beef liver (77 or 185 gm. raw). The experimental Studies 1 through 4 as given in Table 2 lasted 13 to 15, 22, 8 and 18 days.

The original basal diet was planned for the study of the human metabolism of certain B vitamins in yeast. The diets in Studies 1 and 2 (Table 1) were those described by Parsons and Collord.[14] The various diets differed only in minor details according to the preference of the members of the squad. The amount of grape jelly, butter, and mayonnaise, as well as the amount and kind of bread was adjusted. Potato was substituted for squash in the studies following Study 1. The diet in Studies 3 and 4 contained less milk in order that a greater proportion of the biotin intake would be derived from bakers' yeast or beef

* Published with the approval of the Director of The Wisconsin Agricultural Experiment Station.
† This study was supported, in part, by a grant from The Nutrition Foundation, Inc.

ver. Pudding or bananas replaced ice cream for the same reason.

Since the basal diet was low in thiamine, 3 mg. per person was added daily during study 1 and during the basal periods when no yeast or liver was consumed during studies 2, 3 and 4.

The sequence and length of periods, the number of subjects and the supplements in the four studies are shown in Table 2.

During Studies 1 and 4, one-third of the yeast was eaten with each meal; during the second study 2 cakes were eaten with noon lunch and 1 with each of the other meals.

Methods. Twenty-four hour urinary collections were made in brown glass bottles containing 3 ml. of glacial acetic acid and 1 ml. of chloroform and were preserved with a layer of toluene. The urine was assayed for biotin after dilution. In some cases

TABLE 1.—COMPOSITION OF DIETS

		Basal diet			
		Plus raw or boiled yeast (Study 1)	Plus yeast treated 3 ways (Study 2)	Plus beef or liver (Study 3)	Plus yeast or liver (Study 4)
Breakfast:					
grapefruit juice	Gm.	200	200	150	150
bread, white	"	44	43	44	44
butter	"	10	12	10	12
grape jelly	"	20	15	10	
coffee, *ad lib.*					
Lunch:					
cheese sandwiches					
cheese, cheddar	"	55	55	50	60
bread, white	"	44	60		
bread, whole wheat	"	..	..	60	60
butter	"	10	15	20	15
lettuce	"	60	60	60	60
mayonnaise	"	..	10	15	10
apple	"	150	150	150	150
whipped cream	"	15			
sugar	"	2			
milk	"	240	240	200	200
Dinner:					
tomato juice	"	200	200		
lean beef, ground	"	150	100	180	150
cabbage	"	30	30	50	50
squash	"	100			
bread, white	"	22	22	16	16
butter	"	10	15	15	15
ice cream, vanilla	"	100	50		
ice cream, chocolate	"	..	50		
potato	"	..	100	150	125
pudding, cornstarch	"	..	..	100	
bananas	"	..	..	50	100
liver or beef	"	..	..	185*	77*
biotin content of liver	μg.	..	..	140	86
yeast, bakers', number of cakes		3†	4†	..	7.5†
biotin content of yeast	"	24	34	..	57
milk	Gm.	240			
Biotin content of basal diet	μg.	21	35	21	36

* Beef liver replaced ground beef during Period III, Study 3, and Period IV, Study 4 (Table 2).
† Baker's yeast was added to the basal diet during Periods II and IV, Study 1; Periods II, IV and VI, Study 3; and Period II, Study 4 (Table 2).

The yeast was consumed either fresh (mixed with water or fruit juice), boiled 2 minutes in water, or autolyzed 2 hours at room temperature with 2 ml. of ethyl acetate per cake of yeast. The autolysis was followed by boiling 1 hour to remove the acetate.

(Study 1) several 24 hour collections from each subject were composited to obtain a representative value for a single period.

Fecal collections were made except in Study 1. Fecal eliminations of 3 of the 6 subjects in Study 2 were collected. Charcoal and carmine were consumed on alternate

days to mark the feces. Each fecal elimination was collected directly in a 2% glacial acetic acid-alcohol mixture (Knott[8]). The samples were dried until brittle at 43° to 50° C. for 24 to 48 hours, ground and bottled. A 1 gm. aliquot was hydrolyzed with 20 ml. of 2N H_2SO_4 at 120° C. for 2 hours, neutralized with solid NaOH to about pH 3.5, filtered, and brought to a volume of 30 to 40 ml., and assayed for biotin content.

at 120° C. Six N H_2SO_4 for 4 hours at 120° C. liberated the maximum of biotin from beef liver.

Biotin was determined by a slight modification of the yeast-growth method of Snell, Eakin and Williams[17] with *Saccharomyces cerevisiæ* Strain 139. Test tubes 25 x 200 mm. in size were used instead of Erlenmeyer flasks. Three different amounts of the sample containing from 25 to 100 μμg. of biotin in a

TABLE 2.—URINARY AND FECAL BIOTIN DURING 4 STUDIES

Subjects, No.	Period No.	Period Days	Diets in various studies	Biotin intake (μg.)	Daily biotin elimination: Urine Range (μg.)	Urine Av. (μg.)	Feces Range (μg.)	Feces Av. (μg.)
			Study 1					
4	I	1-2	Basal*	21	22- 46	30		
	II	3-4	Basal + 3 boiled yeast cakes*	45	21- 29	26		
	III	2	Basal*	21	21- 31	27		
	IV	2-4	Basal + 3 fresh yeast cakes*	45	21- 30	26		
			Study 2					
6	I	3	Basal*	35	20- 36	28	57-106	83
	II	4	Basal + 4 boiled yeast cakes*	69	20- 39	30	17-114	65
	III	2	Basal*	35	21- 33	28	52- 73	61
	IV	4	Basal + 4 fresh yeast cakes*	69	16- 37	26	27-107	71
	V	2	Basal*	35	25- 37	33	39- 83	65
	VI	4	Basal + 4 autolyzed yeast cakes*	69	22- 49	35	20-109	75
	VII	3	Basal*	35	16- 39	27		
			Study 3					
2	I	1	Unrestricted		27- 35	31	105	
	II	1st	Basal with beef*	21	24- 37	31	57-202	132
	II	2nd	Basal with beef*	21	25- 40	32	65- 72	69
	II	3rd	Basal with beef*	21	25- 37	31	53- 72	63
	II	4th	Basal with beef*	21	33	33	20-123	72
	III	1st	Basal with beef liver	161	90- 95	93	48- 51	49
	III	2nd	Basal with beef liver	161	128-146	137	48- 75	61
	III	3rd	Basal with beef liver	161	114-183	148	65- 78	71
	III	4th	Basal with beef liver	161	141-165	153	36- 62	49
	IV	1	Unrestricted		84- 91	87	90	90
			Study 4					
3	I	3	Basal*	36	11- 34	23	41- 61	50
	II	4	Basal + 7½ boiled yeast cakes	93	20- 42	27	65-101	84
	III	3	Basal*	36	11- 29	22	66-118	91
	IV	4	Basal with beef liver	122	30- 83	60	72- 81	76
	V	4	Basal*	36	19- 59	33	55-100	72

* 1 mg. of thiamine hydrochloride added daily to basal diet.

The biotin content of the food was determined on aliquots of the foods consumed by the subjects. The weighed aliquots were homogenized in a Waring blender, hydrolyzed, neutralized and filtered in the same manner as the fecal samples.

The biotin contents of the beef liver and bakers' yeast were assayed separately from the rest of the foods. A maximum amount of biotin was liberated from the yeast by the methods tried with 4N H_2SO_4 for 4 hours

maximum of 2 ml. of solution were used. These levels of biotin are within the part of the standard curve obtained with pure biotin which forms a straight line. The addition of casein hydrolysate increases the range of biotin concentrations which can be measured by this method according to Hertz.[7] The curves for concentrations of pure biotin by the method of Snell *et al.*[17] and Hertz[7] coincide at the levels measured in the present studies.

The turbidity produced by the yeast growth in a sample tube after incubation at 30° C. for about 16 hours was compared with that of free acid biotin in an Evelyn photoelectric colorimeter using a 540 μ filter. If the three levels of the same sample did not agree within 10% of the lowest figure, the assays were repeated.

Results and Discussion. The urinary output is generally assumed to represent an excess of a vitamin not retained by the body and is believed to be an indirect indication of the amount used for maintenance, growth or storage. Thus, when the output of biotin exceeds the dietary intake there is either synthesis (Oppel[13]), removal from body stores or a combination of these two factors. Oppel[13] found relatively "normal" biotin excretion in fasting human subjects and those consuming egg-white for short periods. Therefore, he suggests that the level of urinary biotin is based on "biotin synthesis by colonic bacteria, and superimposed on this, sudden variations . . . due to changes in biotin content of the diet."

The urinary biotin of the 10 subjects during Studies 1 and 2 (Table 2) was not increased when the biotin intake was doubled (21 μg. increased to 45 μg. in Study 1, 35 μg. to 69 μg. in Study 2) by the inclusion daily of 3 or 4 cakes of fresh or boiled yeast. Urinary biotin during the basal periods accounted for an average of 110% of the biotin intake, while only an average of 45% of the intake appeared in the urine when fresh or boiled yeast was consumed. The absolute amounts of biotin excreted during the basal periods and the periods with fresh or boiled yeast were almost identical (average of 29 μg. for basal periods; 28 μg. for fresh and boiled yeast periods). These results are in contrast to the human utilization of thiamine and riboflavin in yeast in Study 1 (Parsons and Collord[14]). These workers found 2 to 3 times as much thiamine excreted when boiled yeast in contrast to fresh yeast was eaten. The excretion of riboflavin followed the same tendency.

When the yeast was autolyzed with ethyl acetate (Period VI, Study 2, Table 2), the urinary biotin rose only slightly for the 6 subjects, an average of 5 μg. daily over the basal periods which is equivalent to only 15% of the biotin in the ingested yeast. This slight rise in urinary biotin contrasted markedly with the sharp rise when beef liver was eaten (Study 4, Table 2).

Apparently at this level of biotin intake (69 μg.) variations in urinary excretion of biotin were nearly masked by the relatively large amounts of biotin synthesized by intestinal bacteria as indicated by the total biotin eliminated during each of the four studies (Table 2). Only at quite high levels of biotin intake such as during the periods of liver feeding of Studies 3 and 4 did the variations in urinary biotin stand out clearly from the interfering synthesis. Other factors in addition to changes influencing microbiological synthesis of biotin, which might account for the lack of consistent urinary response to ingestion of yeast biotin are: lack of utilization of biotin from yeast, perhaps due to lack of solubility; a retention of biotin by the body; or a combination of the above.

The water-soluble biotin of the fresh, boiled and autolyzed yeast amounted to 10, 10 and 21%, respectively, of the total biotin in the yeast ingested and on a weight basis equaled 3, 3 and 6 μg. per day (Study 2). If the proportion of biotin which is water-soluble is a measure of its availability to the animal, then one might expect the biotin of fresh and boiled yeast to be absorbed similarly and the ethyl acetate treated yeast to be more completely available. Some evidence of difficulty in digesting fresh yeast is indicated by the work of Montgomery *et al.*[10] who observed that over one-half of viable yeast cells passed through the stomach without being destroyed when fresh yeast was ingested. Viable yeast was found in feces of human subjects consuming fresh yeast but not for those consuming boiled yeast or an ordinary diet (Parsons, Williamson and Johnson[16]).

The fecal biotin for all four studies is

shown in Table 2. During the periods of yeast feeding (Study 2), the fecal biotin for the last 2 days averaged slightly higher (7 μg. higher daily) than during the corresponding days of the basal periods. Thus, the rise in fecal biotin accounted for only about 15% of the biotin intake from yeast.

An increase in fecal biotin could easily have been masked by changes in fecal synthesis, the yeast may have helped to make a medium less favorable for intestinal synthesis or the biotin contained in the yeast may have been utilized by the microörganisms in lieu of biotin synthesis by them. The possibility must be considered of a retention of biotin during the yeast periods, although there is no apparent reason for this inasmuch as the intake of biotin was rather liberal (Study 2).

It is possible that a longer period of adjustment to fresh yeast is necessary to reach an equilibrium in biotin excretion. There is some evidence to substantiate this need since the urinary biotin output during the basal period (V) following the ingestion of fresh yeast was noticeably higher than during the other two basal periods (III and VII, Study 2, Table 2). The first basal period is disregarded since this represents a transition from the unrestricted diet.

The purpose of Studies 3 and 4 was to determine whether or not urinary biotin could be elevated by the inclusion of moderate or large amounts of beef liver (77 and 185 gm.) and to compare the utilization of similar amounts of biotin from boiled bakers' yeast and beef liver (Study 4, Table 2). The biotin content of the diet was increased from 21 to 161 μg. daily (Study 3) and from 36 to 122 μg. (Study 4) by substituting beef liver for ground beef. The urinary biotin doubled or trebled on the first day of ingestion of liver and continued to rise for 3 or 4 days for both levels of liver feeding. A maximum of 183 μg. of urinary biotin was reached for 1 subject (Study 3). A longer period is probably necessary to reach a plateau of biotin excretion on such high levels of intake. This possibility was later substantiated (Gardner, *et al.*[36].)

When the feeding of ground beef was resumed, the fall in urinary biotin was as rapid as was the rise during liver feeding. A comparison of the utilization of somewhat similar amounts of biotin from bakers' yeast and beef liver was made in Study 4 (Table 2). The inclusion of 7½ cakes of boiled yeast daily increased the biotin intake from 36 to 93 μg. and 77 gm. of beef liver increased the intake from 36 to 122 μg. daily. Despite the large amount of boiled yeast, the urinary biotin was raised only 3 to 5 μg. daily for each of the 3 subjects (Table 2). These results corroborate those of Studies 1 and 2.

There was, however, a definite increase (average of 34 μg. daily) in fecal biotin during the ingestion of 7½ cakes of yeast (Study 4). The high fecal biotin which continued through the 2-day basal period (III, Study 4) following the yeast feeding may also have been due to the yeast. In contrast, the fecal biotin was not significantly changed by the inclusion of liver (Study 3). During Study 4 the level of fecal biotin actually dropped from 91 to 76 μg. (daily average) during the liver period. As in Study 3 the increased total biotin excretion during liver feeding was due to increased urinary output of biotin, while the reverse was true during yeast feeding.

The importance of fecal biotin is difficult to ascertain in the first place because it may be derived from several sources as follows: (1) unabsorbed food, (2) biotin absorbed from the gastro-intestinal tract and reëxcreted, (3) biotin removed from body stores, and (4) biotin originating from microörganisms. With present methods it is not possible to assign the source of biotin which is eliminated or excreted. Secondly, the question of absorption from the large intestine of biotin or other substances synthesized by microörganisms needs reëxamination since most of the intestinal flora occur below the level at which most of the absorption of nutrients other than water is believed to take place

Najjar and coworkers[11,12] have recently reported absorption of thiamine and riboflavin by man from the large intestine after large amounts of these vitamins were administered by a "retention" enema. On the other hand, Alexander and Landwehr[1] found no evidence of absorption of physiologic amounts of thiamine given in a similar manner. Mitchell and Isbell,[9] however, found that in rats the biotin and pyridoxin synthesized by intestinal bacteria apparently moved freely from the bacterial cells into the surrounding medium and were, therefore, available for absorption.

The total output of biotin during Studies 2, 3 and 4 is interesting. The range of total output varied from 54 to 261 μg. per diem which accounted for from 86 to 1073% of the dietary biotin. Each of these values is for 1 day for a single individual. The greatest excess of biotin output over intake occurred during the periods of lowest intake, *i. e.*, during the basal periods. The average output during all of the basal periods of Studies 2, 3 and 4 was 100 μg. daily or about 300% of the biotin intake.

During the periods of yeast feeding the total biotin output averaged 103 μg. daily or 137% of the intake, during the liver feeding 163μg. or 115%. Only on single days did the biotin output fall below the intake, *e. g.*, the first day of liver feeding (Study 3). Since the biotin output nearly always exceeded the intake, synthesis of biotin probably by microörganisms is indicated. It would appear that less biotin was synthesized during the ingestion of bakers' yeast and beef liver as compared with the basal periods.

Summary. Four studies of the biotin metabolism of 15 college women who consumed uniform diets and these same diets supplemented with bakers' yeast or beef liver are presented. These diets contained from 21 to 161 μg. of biotin per diem.

Doubling the biotin intake by the addition of 3 or 4 cakes of fresh or boiled yeast per diem failed to cause a consistent rise in urinary biotin and only a minor rise in fecal biotin was observed. Four cakes daily of yeast autolyzed with ethyl acetate increased the urinary output about 18%, which is nearly equivalent to the water-soluble biotin from the treated yeast.

The daily addition of 7½ cakes of boiled yeast increased the urinary biotin somewhat (14 to 33%) while a somewhat larger amount of biotin from beef liver doubled or trebled the urinary output on the first day of liver feeding. The rise continued for 3 to 4 days.

The range of biotin output in urine and feces per diem was as follows: urine 11 to 183 μg., feces 17 to 208 μg., total 54 to 261 μg.

The total output amounted to less than 1 to 11 times the intake depending to a large extent on the diet. The greatest excess of output over intake occurred on low biotin intake and the least excess occurred on a high biotin intake during liver feeding.

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Gautier-'57
Bib No. 467

Intern. Z. Vitaminforsch. 28:61-67. 1957.

Clinique Universitaire de Pediatrie de Geneve
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DERMATITIS SEBORRHOIDES (SEBORRHEIC DERMATITIS) AND BIOTIN

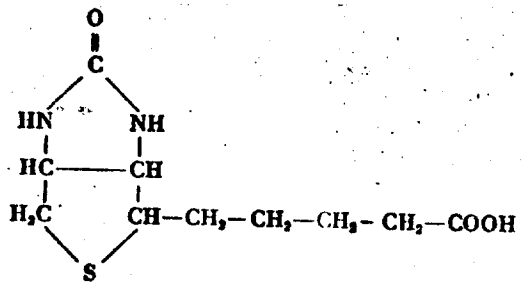
by

P. Gautier, A. Gautier and F. Thelin

We do not claim to bring in this work very new facts, but, since the role of biotin in the evolution of seborrheic dermatitis (DS) is a subject which interested M. Glanzmann, and since, in our laboratory also, we were concerned with this question (Thelin, Avrouskine), we thought that a rapid comparison of recent results with those of earlier works could be of a certain interest.

The discovery in the nature of biotin, its biological action, etc., being known facts, we will limit ourselves to a brief resume of these ideas and of the experiments carried out for the study of the deficiency in vitamin H.

In 1936, Kogl isolates, using egg yolk, a chemically pure substance, which he calls biotin. At the same time, Gyorgy concentrates vitamin H, coming from yeasts or hepatic tissue. In 1940, he recognizes the identity of vitamin H and of biotin. In 1946, Goldberg proposes a practical synthesis of d-biotin, which permits its clinical use. Its chemical formula follows:



or (+)-3,4-(2'-cetoimidazolido)-2-thiophan-n-valerianic acid.

In the organism, biotin exists in infinitesimal quantities in any living cell. It is synthesized by certain yeasts and by bacteria of the intestinal flora and it seems to have an enzymatic role in the division of the amino acids and in the metabolism of the glucides. It would also occur in certain immunization reactions.

In the animal and in human volunteers, experience has shown that biotin deficiency caused, after three or four weeks, the appearance of a non-pruriginous seborrheic dermititis, which spontaneously regresses. Consequently, if the experiment is persued, more marked phenomena are observed: atrophy of the lingual papilla, appearing of pealing dermatitis of the skin on the extremities, then neuro-vegetative symptoms: depression, anorexia, nausea, etc. All these signs disappear in four or five days under the influence of biotin in a dose of 200-300 gammas daily through injection.

Moro seborrheic dermatitis (SD) is also well known. Its description has been the subject of numerous works, among which the clinical lesson of M. Glanzmann is one of the best. Thus we will cover this subject with just a few lines.

The Moro illness begins in the first three months of life. It is characterized by the appearance of fatty scales on a more or less irritated and erythematous skin. These lesions begin in the hairy skin and the wrinkles of the body. They can extend to the gluteal region, the hind quarters, the lower part of the abdomen. The extension of the lesions to the whole of the teguments then gives the clinical picture of pealing red skin or the Leiner-Moussous illness.

The SD is not very pruriginous, and its evolution toward a complete cure, rather rapid, is the rule.

We will not take up here the question of the differential diagnostic of the SD, but we will recall however, that its reaction to egg white is in the negative rule, contrary to what is seen in the eczema of the nursing infant.

As experimentation has shown, it seems that there is a rather strict relation between biotin deficiency and the appearance in the nursing infant of the SD symptoms. Leiner had already presented the hypothysis that the pealing red skin was a deficiency illness, due to the absence in the mother's milk of a factor existing in larger quantity in cow's milk.

Is the cutaneous dystrophy of SD due to a biotin deficiency through insufficient contribution or through insufficient intestinal resorption? The question may be asked and we think with Glanzmann that the following explanation may be admitted: during the first months of life, the intestine has a great permeability to the proteins, and the intestinal flora is often insufficient to

allow the synthesis of the biotin. If the supply of biotin is weak (mother's milk) or even sufficient (cow's milk), the deficiency effect due to the incomplete disintegration of the proteins is shown, because the biotin, tied to the milk albumin is not released in sufficient quantity during this incomplete digestion.

For numerous authors, the biotin determinations which can be carried out in the blood, the milk, the urine show that the rate of it is less high in patients afflicted with SD and in infants nursed solely on the breast than in nursing young subjected to an artificial feed.

After this reminder of the elements of the problem which is the subject of our work, we must approach the very summarized study of the research devoted to the relations of SD and biotin.

Since the work of one of us, the publications concerning this subject are numerous. We will mention only the most important.

Freudenberg explains the greater frequency of SD abroad than in Switzerland in breast fed infants, by the greatest richness in biotin of the milk of Swiss women. It is to be noted that these statements were made during the post-war period, when nutrition was much more difficult abroad than in Switzerland.

Berger, out of nine children afflicted with SD and subjected to biotin and to a mixed or artificial diet, has seven cures and one improvement. The length of the treatment was much shorter than before the use of biotin.

Martin, in a series of works, reports very favorable results for the treatment of SD by biotin. The posology used was the following: six to ten injections, daily or twice daily if the case was serious, of 5 mg, up to a total of 30 to 50 mg.

Svejcar and Homolka, who during treatment controlled the rates of biotin in the patients, also obtained very favorable results, despite a lower dose than Martin: 0.1 to 0.2 mg per day, parenterally, for five to 14 days; in certain cases they give per os to the mother 4 mg per day of biotin. Finally, they recommend the addition of vitamins A, B, C and D.

We will also mention a work by Vujasin and Petrovic and those of Sara Kokil, which feel that biotin is a specific medicine of SD, in oral and parenteral doses of 5 mg per day, and that thus SD cures in two to three weeks. Kokil also observes an improvement of hyperchrome anemia which she has often seen accompany SD. Finally, the thesis of Avrouskine reports the first cases of SD treated by biotin in our clinic.

In about ten cases observed and treated by biotin, the author had drawn the conclusion that the results obtained were favorable. The question of the posology appeared, by contrast, still to be made precise, because the differences of doses and length of treatment were rather marked, as is shown in summarized table which is found in this thesis. We will only say here that the daily dose (per os or parenteral) varies, according to the authors, from 0.1 mg to 5 or 8 mg and that the length of the treatment can be from one to four weeks.

Here now are the statements that we have been able to make on a series of 30 cases, of which nine of pealing red skin of Leiner-Moussous and 21 cases of Moro dermititis, which we think we can group, given the similarity of the two affections and the results obtained.

Sex: 21 boys, 9 girls.

Feed: eight times at the breast, 20 times on cow's milk, two times mixed feed.

The beginning age is early, according to the rule: during the first month: four cases; during the second month: 16 cases; during the third month: eight cases; more than three months old: two cases.

The interval of the time elapsed between the beginning of the illness and that of the treatment is very important, because the sooner the infant is treated by biotin, the better the results obtained, as a rule. This is what our series of patients showed us, when the treatment was immediate in 16 cases, was begun less than a month after the beginning in ten cases, and more than a month after the beginning of the illness in four cases.

We can give the following precisions on the technique of treatment which we used:

The treatment per os alone was made in nine cases; on the average we administered 4 mg per day (with doses varying according to the subjects from 2 to 6 mg) and the length of treatment varied from seven to 28 days (on the average 16 days). The total dose for this series was from 20 to 140 mg (on the average 72 mg).

The i.m. treatment alone was used in 17 cases: a 5 mg syringe daily in 15 cases, for five to 17 days (on the average nine days), or a total of 25 to 85 mg (on the average 45 mg). In two other cases, three syringes per week for three and five weeks, but with less good results than when there were daily injections.

The four remaining cases received a combined parenteral and per os treatment.

These values correspond appreciably to those suggested by the authors which we mentioned above.

The results obtained through the treatment with biotin allowed us to classify the cases in the following manner:

Results on the state of the skin:

Cured	23	= 27/30 = 90%
Very improved	4	
Slightly improved	2	
Failures	1	

Results on the general state of the nursing infant: out of 11 infants in which the general state was deficient, a notable improvement was obtained nine times, or in 82% of the cases. We have not systematically controlled the action of the treatment on the blood, as Sara Kokil did, who, remember, underlined the improvement of the hemoglobin rate and the number of red corpuscles through the treatment with biotin, but it seems probable to us that the blood improvement is one of the principle factors of the improvement of the general state.

One point is still to be considered when the use of biotin is desired in the treatment of SD. We agree with Avrouskine that there must be distinguished, according to the method of feed of the nourishing young, two groups of SD which depend on partially different mechanisms: on the one hand the breast feeders, in which the biotin deficiency seems to come from an insufficient supply, on the other hand, the infants subjected to a mixed or artificial feed, with biotin supply probably sufficient, but in which the active element is eliminated without being used by the organism. In these infants, the SD is often associated with problems of the intestinal passage in the form of diarrhea. The question remains of knowing if indigestion is the cause or consequence of biotin deficiency.

We easily understand that the treatment of the infants of the second group will have to include, in addition to biotin, the medicines necessary to jugulate the intestinal problems. This is what we worked on in the nursing infants fed artificially, rather numerous in our series of observations.

And if now we attempt to draw certain conclusions from what we have learned in the literature concerning the treatment of SD through biotin, and from what we have clinically stated, we think that we can formulate them in the following way.

Conclusions

As concerns sex, SD effects boys more frequently than girls.

The results of the treatment of this disease with biotin were very favorable in 90% of the cases; the 10% of failures is not explained either by a special seriousness of the cases, or by a treatment insufficient in quantity or in length.

For the posology, the best formula seems to us to be the i.m. injection daily of 5 mg of biotin, for six to ten days (30 to 50 mg). If necessary, we can consolidate the results through a treatment per os of 15 days, using a tablet of 5 mg per day.

The interrupted treatment (three injections per week) and the oral treatment alone seem to be sufficient only for light cases.

Improvement most often begins by the third to fourth days, but the cure requires from ten to 15 days and sometimes occurs only after the halt of treatment (latent period of induction).

The effect on the general state of a patient was excellent in 82% of the cases where it was perturbed. We could not have a sufficient number of hematologic data to confirm the work of Sara Kokil on the correction of anemia through biotin.

Finally, the innocuity of this product makes it possible to recommend its use without hind-thought.

Gautier-'57
Bib No. 467

Intern. Z. Vitaminforsch. 28:61-67. 1957.

Clinique Universitaire de Pédiatrie de Genève (Dir. Prof. P. Gautier)

Dermatite séborrhoïde et Biotine

Par P. Gautier, A. Gautier et F. Thelin

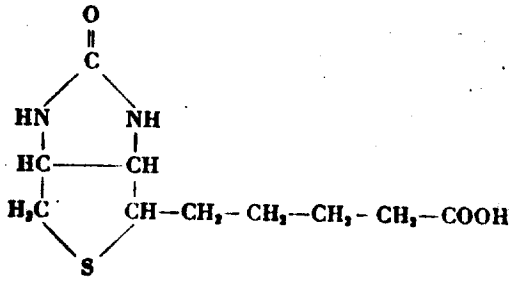
Reçu le 13 avril 1957

Nous n'avons pas la prétention d'apporter dans ce travail des faits très nouveaux, mais, comme le rôle de la Biotine dans l'évolution de la dermatite séborrhoïde (D. S.) est un sujet qui a intéressé M. Glanzmann, et comme, dans notre service aussi, on s'est occupé de cette question (Thélin, Avrouskine), nous

avons pensé qu'une rapide comparaison des résultats récents avec ceux des travaux précédents pouvait avoir un certain intérêt.

La découverte et la nature de la biotine, son action biologique, etc., étant des faits connus, nous nous bornerons à un bref rappel de ces notions et des expériences réalisées pour l'étude de la carence en vitamine H.

En 1936, Kogl isole, à partir du jaune d'œuf, une substance chimiquement pure, qu'il appelle biotine. A la même époque, Gyorgy concentre la vitamine H, provenant de levures ou du tissu hépatique. En 1940, il reconnaît l'identité de la vitamine H et de la biotine. En 1946, Goldberg propose une synthèse pratique de la d-Biotine, qui en permet l'utilisation en clinique. La formule chimique de celle-ci est:



ou acide (+)–3,4–(2′–cétoïmidazolido)–2–thiophan–n–valérianique.

Dans l'organisme, la biotine existe en quantités infinitésimales dans toute cellule vivante. Elle est synthétisée par certaines levures et par des bactéries de la flore intestinale et elle paraît avoir un rôle enzymatique dans la scission des acides aminés et dans le métabolisme des glucides. Elle interviendrait également dans certaines réactions d'immunisation.

Chez l'animal et chez des volontaires humains, l'expérience a montré que la carence en biotine provoquait, après 3 ou 4 semaines, l'apparition d'une dermatite séborrhéique non prurigineuse, qui régresse spontanément. Par la suite, si l'on poursuit l'expérience, on observe des phénomènes plus marqués: atrophie des papilles linguales, aspect de dermatite exfoliatrice de la peau des extrémités, puis des symptômes neuro-végétatifs: dépression, anorexie, nausées, etc. Tous ces signes disparaissent en 4 ou 5 jours sous l'influence de la biotine à la dose de 200—300 gammas par jour en injections.

La dermatite séborrhoïde (D. S.) Moro est, elle aussi, bien connue. Sa description a fait l'objet de nombreux travaux, parmi lesquels la leçon clinique de M. Glanzmann est d'entre les

meilleures. Aussi ne reviendrons-nous qu'en quelques lignes sur ce sujet.

La maladie de Moro débute dans les trois premiers mois de la vie. Elle est caractérisée par l'apparition de squames grasses sur une peau plus ou moins irritée et érythémateuse. Ces lésions commencent au cuir chevelu et aux plis du corps. Elles peuvent s'étendre à la région fessière, aux cuisses, à la partie inférieure de l'abdomen. L'extension des lésions à l'ensemble des téguments donne alors le tableau clinique de l'érythrodermie desquamative ou maladie de Leiner-Moussous.

La D. S. n'est pas très prurigineuse et son évolution vers la guérison complète, assez rapidement, est la règle.

Nous n'avons pas à entrer ici dans la question du dianostic différentiel de la D. S., mais nous rappellerons cependant que la réaction au blanc d'œuf y est dans la règle négative, à l'opposé de ce qui se voit dans l'eczéma du nourrisson.

Comme l'a montré l'expérimentation, il semble bien qu'il existe une relation assez étroite entre la carence en biotine et l'apparition chez le nourrisson des symptômes de la D. S. Leiner avait déjà émis l'hypothèse que l'érythrodermie desquamative était une maladie de carence, due à l'absence dans le lait maternel d'un facteur existant en plus grande quantité dans le lait de vache.

La dystrophie cutanée de la D. S. est-elle due à une carence en biotine par apport insuffisant ou par résorption intestinale insuffisante? La question peut se poser et nous pensons avec Glanzmann qu'on peut admettre l'explication suivante: pendant les premiers mois de la vie, l'intestin a une grande perméabilité aux protéines et la flore intestinale est souvent insuffisante pour permettre la synthèse de la biotine. Si l'apport de biotine est faible (lait de femme) ou même suffisant (lait de vache), l'effet de carence dû à la désintégration incomplète des protéines se manifeste, car la biotine, liée à la lactalbumine n'est pas libérée en quantité suffisante pendant cette digestion incomplète.

Pour de nombreux auteurs, les dosages de biotine, qui peuvent être pratiquées dans le sérum, le lait, les urines montrent que le taux en est bien moins élevé chez les malades atteints de D. S. et chez les enfants allaités uniquement au sein, que chez les nourrissons soumis à une alimentation artificielle.

Après ce rappel des éléments du problème qui fait le sujet de ce travail, nous devons aborder l'étude très résumée des recherches consacrées aux relations de la D. S. et de la biotine.

Depuis le travail de l'un de nous, assez nombreuses sont les publications traitant de ce sujet. Nous ne citerons que les plus importantes.

Freudenberg explique la plus grande fréquence de la D. S. à l'étranger qu'en Suisse chez les enfants nourris au sein, par la plus grande richesse en biotine du lait des femmes suisses. Il est à relever que ces constatations ont été faites pendant la période d'après-guerre, où l'alimentation était beaucoup plus difficile à l'étranger qu'en Suisse.

Berger, sur 9 enfants atteints du D. S. et soumis à la biotine et à un régime mixte ou artificiel, a 7 guérisons et 1 amélioration. La durée du traitement a été beaucoup plus courte qu'avant l'usage de la biotine.

Martin dans une série de travaux, rapporte des résultats très favorables du traitement de la D. S. par la biotine. La posologie employée a été la suivante: 6 à 10 injections, quotidiennes ou biquotidiennes si le cas était grave, de 5 mg, jusqu'à un total de 30 à 50 mg.

Svejcar et Homolka qui ont contrôlé au cours du traitement les taux de biotine chez les malades, ont aussi obtenu des résultats très favorables, malgré un dosage plus faible que Martin: 0,1 à 0,2 mg par jour, par voie parentérale, pendant 5 à 14 jours; dans certains cas ils donnent per os à la mère 4 mg par jour de biotine. Enfin, ils recommandent l'adjonction de vitamines A, B, C et D.

Citons encore un travail de Vujasin et Petrovic et ceux de Sara Kokil, qui estime que la biotine est un médicament spécifique de la D. S., aux doses orales ou parentérales de 5 mg par jour, et qu'ainsi la D. S. guérit en 2 à 3 semaines. Kokil a parallèlement observé une amélioration de l'anémie hyperchrome qu'elle a souvent vu accompagner la D. S. Enfin, la thèse d'Avrouskine rapporte les premiers cas de D. S. traités par la biotine dans notre service.

D'une dizaine de cas observés et traités par la biotine, l'auteur avait tiré la conclusion que les résultats obtenus étaient favorables. La question de la posologie lui paraissait, par contre, encore à préciser, car les différences de doses et de durée du traitement étaient assez marquées, comme le montre le tableau résumé qu'on trouvera dans sa thèse. Disons seulement ici que la dose journalière (per os ou parentérale) varie, suivant les auteurs, de 0,1 mg à 5 ou 8 mg et que la durée de la cure peut aller d'une à quatre semaines.

Voici maintenant les constatations que nous avons pu faire sur une série de 30 cas, dont 9 d'érythrodermie desquamative de Leiner-Moussous et 21 cas de dermatite de Moro, que nous pensons pouvoir grouper, vu la ressemblance des deux affections et les résultats obtenus.

Sexe: 21 garçons, 9 filles.

Alimentation: 8 fois au sein, 20 fois au lait de vache, 2 fois alimentation mixte.

L'âge de début est, suivant la règle, précoce: pendant le premier mois: 4 cas; pendant le deuxième mois: 16 cas; pendant le troisième mois: 8 cas; plus de trois mois: 2 cas.

L'intervalle du temps écoulé entre le début de la maladie et celui du traitement a une importance assez grande, car plus vite l'enfant est traité par la biotine, meilleurs sont, dans la règle, les résultats obtenus. C'est ce que nous a montré notre série de malades, où le traitement a été immédiat dans 16 cas, a été commencé moins d'un mois après le début dans 10 cas, et plus d'un mois après le début de la maladie dans 4 cas.

Sur la technique du traitement que nous avons utilisée, nous pouvons donner les précisions suivantes:

Le traitement per os seul a été fait dans 9 cas; en moyenne on a administré 4 mg par jour (avec des doses variant suivant les sujets de 2 à 6 mg) et la durée du traitement a varié de 7 à 28 jours (en moyenne 16 jours). La dose totale pour cette série a été de 20 à 140 mg (en moyenne 72 mg).

Le traitement par voie intramusculaire seule a été utilisé dans 17 cas: une ampoule de 5 mg tous les jours dans 15 cas, pendant 5 à 17 jours (en moyenne 9 jours), soit au total de 25 à 85 mg (en moyenne 45 mg). Dans deux autres cas, trois ampoules par semaine pendant 3 et 5 semaines, mais avec de moins bons résultats que lors d'injections quotidiennes.

Les 4 cas restants ont reçu un traitement combiné parentéral et per os.

Ces valeurs correspondent sensiblement à celles proposées par les auteurs que nous citons plus haut.

Les *résultats* obtenus par le traitement à la biotine nous ont permis de classer les cas de la façon suivante:

Résultats sur l'état de la peau:

guéris	23	} = 27/30 = 90%
très améliorés	4	
peu améliorés	2	
échecs	1	

Résultats sur l'état général du nourrisson: sur 11 enfants chez lesquels l'état général était déficient, une notable amélioration a été obtenue 9 fois, soit dans le 82% des cas. Nous n'avons pas systématiquement contrôlé l'action du traitement sur le sang, comme l'a fait Sara Kokil, qui, nous le rappelons, a insisté sur l'amélioration du taux d'hémoglobine et du nombre des globules rouges par le traitement à la biotine, mais il nous paraît probable que

l'amélioration sanguine est un des facteurs principaux de l'amélioration de l'état général.

Un point est encore à considérer quand ont veut employer la biotine dans le traitement de la D. S. Nous pensons avec Avrouskine qu'on doit distinguer, selon le mode d'alimentation du nourrisson, deux groupes de D. S. qui relèvent de mécanismes en parties différentes: d'une part les nourrissons au sein, chez lesquels la déficience en biotine semble provenir d'un apport insuffisant, d'autre part, les enfants soumis à une alimentation mixte ou artificielle, avec apport en biotine probablement suffisant, mais chez lesquels le principe actif est éliminé sans être utilisé par l'organisme. Chez ces enfants, la D.S. est souvent associée à des troubles du transit intestinal sous forme de diarrhées. La question reste pendante de savoir si la dyspepsie est la cause ou la conséquence de la carence en biotine.

On comprend aisément que le traitement des enfants du deuxième groupe devra comporter, à côté de la biotine, les médications nécessaires pour juguler les troubles intestinaux. C'est à quoi nous nous sommes appliqués chez les nourrissons alimentés artificiellement, qui étaient assez nombreux dans notre série d'observations.

Et si maintenant nous essayons de tirer quelques conclusions de ce que nous vous avons appris dans la littérature sur le traitement de la D. S. par la biotine, et de ce que nous avons cliniquement constaté, nous pensons pouvoir les formuler de la façon suivante.

Conclusions

En ce qui concerne le sexe, la D. S. frappe les garçons plus souvent que les filles.

Les résultats du traitement de cette maladie par la biotine ont été très favorables dans 90% des cas; le 10% d'échecs ne s'explique ni par une gravité spéciale des cas, ni par un traitement insuffisant en quantité ou en durée.

Pour la posologie, la meilleure formule nous paraît être l'injection intramusculaire du 5 mg de biotine par jour, pendant 6 à 10 jours (30 à 50 mg). On peut éventuellement consolider les résultats par un traitement per os de 15 jours, à raison d'un comprimé à 5 mg par jour.

Le traitement discontinu (3 injections par semaine) et le traitement par la seule voie buccale, ne semblent être suffisants que pour les cas légers.

L'amélioration commence le plus souvent déjà au bout de 3 à 4 jours, mais la guérison demande de 10 à 15 jours et ne survient parfois qu'après l'arrêt du traitement (temps de latence).

L'effet sur l'état général du malade a été excellent dans 82% des cas où il était perturbé. Nous n'avons pas pu avoir de données hématologiques assez nombreuses pour confirmer le travail de Sara Kokil sur la correction de l'anémie par la biotine.

Enfin, l'inocuité de ce produit permet de recommander son emploi sans arrière-pensée.

ZUSAMMENFASSUNG

Dermatitis seborrhoides und Biotin. Von P. Gautier, A. Gautier und F. Thelin.

Die Krankheit ist häufiger bei Knaben, als bei Mädchen. In 90% der Fälle reagieren die Patienten gut auf Biotin, und zwar in Dosen von 5 mg pro Tag. Die Besserung tritt schon nach 3—4 Tagen ein, die Dauer der Behandlung ist hingegen 10—15 Tage.

SUMMARY

Dermatitis seborrhoides and Biotin. By P. Gautier, A. Gautier and F. Thelin.

This disease is commoner among boys than girls. Doses of 5 mg of biotin daily produce favorable results in 90% of the cases. Improvement is already noticed by the 3rd—4th day; length of treatment is from 10 to 15 days.

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Pour la littérature antérieure à 1952, voir la thèse d'*Avrouskine.*

Gompertz-'71
Bib No. 488

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Lancet 2: 22-24. 1971.

BIOTIN-RESPONSIVE β-METHYLCROTONYLGLYCINURIA

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Summary The clinical and biochemical findings are described in a 5-month-old infant who presented with metabolic acidosis and ketosis associated with β-methylcrotonylglycinuria and β-hydroxyisovalericaciduria. The defective enzyme was presumed to be β-methylcrotonyl CoA carboxylase, and the child was treated with biotin, the coenzyme of this carboxylase. The child's clinical condition improved immediately after biotin treatment, and the abnormal metabolites disappeared from the urine. β-methylcrotonylglycinuria seems to be another inherited metabolic disease that can be vitamin-responsive.

INTRODUCTION

THE biochemical and genetic heterogeneity of inborn errors of metabolism is becoming increasingly recognised.[1] An important manifestation of this heterogeneity is "vitamin-dependent" hereditary metabolic disease. Vitamin B_{12},[2] biotin,[3] and thiamine [4] responsive metabolic errors have been described recently. In a child with thiamine-responsive maple-syrup-urine disease, the hyperaminoacidæmia responded completely to thiamine without recourse to dietary modification.[4]

Eldjarn et al.[5] have described a child with a new inborn error of the leucine-degradation pathway—β-methylcrotonylglycinuria, presumably due to an impaired β-methylcrotonyl CoA carboxylase activity [5] (fig. 1). This enzyme is a biotin-dependent carboxylase, so biotin-responsive variants are possible. In this report we describe another child with β-methylcrotonylglycinuria and his clinical and biochemical improvement associated with biotin treatment.

CASE-REPORT

This infant was born at 42 weeks' gestation of unrelated healthy parents; he weighed 3·7 kg. His sister aged 8 years is well and there is no family history of infant death.

At 5 months of age he was admitted to hospital because of persistent vomiting and an extensive skin rash. The erythematous rash had first appeared on the buttocks and

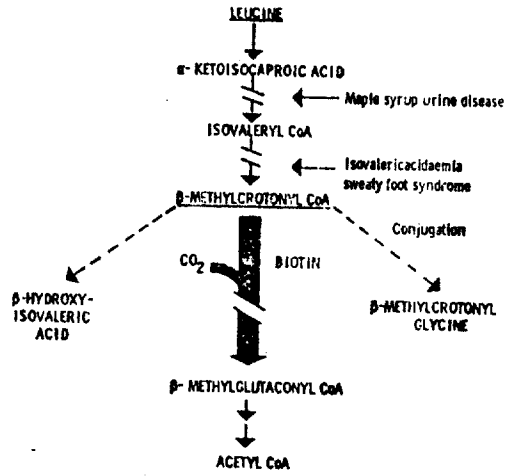

Fig. 1—Leucine-degradation pathway.

neck when he was 6 weeks old, and had gradually spread to all flexures despite topical applications including steroids and nystatin. He had tended to vomit from birth and various milk-feeding schedules had been tried without improvement. Nevertheless he had gained weight satisfactorily and was well nourished.

When he was admitted he had deep rapid respiration: this seemed to have developed slowly over the preceding few weeks. His general behaviour was abnormal, but there had been no change in consciousness and he had not had overt fits. Investigations confirmed the clinical impression of severe metabolic acidosis and ketosis. On admission he had a blood pH of 7·3, P_{CO_2} of 18 mm. Hg, and base excess of −16 meq. per litre. Routine urine analysis excluded the presence of excess protein, aminoacids, and sugars. However, a peculiar odour reminiscent of tom cats' urine was noted.

On the assumption that this metabolic acidosis and ketosis might be due to an abnormality in a pathway of protein degradation, oral feeding was stopped and the child maintained on intravenous glucose, saline, and alkalis. There was slight clinical improvement in his respiration and responsiveness, but both remained distinctly abnormal. He remained acidotic, and the rash did not improve.

BIOCHEMICAL STUDIES

Urine and plasma volatile fatty acids were analysed by steam distillation and gas chromatography.[6] β-methylcrotonylglycine and other N-acylglycine conjugates were extracted continuously with diethylether from acidified urine and were analysed as their methyl esters by gas chromatography on a diethyleneglycolsuccinate (D.E.G.S.) column at 175°. Heptadecanoic acid was used as an internal standard, and the relative detector response to β-methylcrotonylglycine was established by hydrogenation and comparison with synthetic isovaleryl glycine. β-hydroxyisovaleric acid was analysed as its methyl ester on a D.E.G.S. column at 95° using decanoic acid as an internal standard. Gas chromatography mass spectroscopy was performed on an A.E.I. M.S. 12 mass-spectrometer coupled to a 'Varian 1400' gas chromatograph, using a 'Carbowax' column at 160°.

Isovaleryl and tiglylglycine were synthesised essentially by the method of Bondi and Eissler.[7]

Results

Steam distillation and gas chromatography of the volatile fatty acids present in an initial specimen of urine from this child showed the presence of β-methylcrotonic acid. Extraction of the urine specimen with diethylether and analysis of the methylated extract on a D.E.G.S. column at 175° then revealed two large and abnormal peaks with retention-times relative to heptadecanoic acid methyl ester of 2·97 and 3·31. The second and larger peak had a retention-time identical to authenticated β-methylcrotonylglycine provided by Prof. L. Eldjarn, Institute of Clinical Biochemistry, Oslo; and its mass spectrum was also identical to the spectrum of this specimen of β-methylcrotonylglycine from the first child.[5] Catalytic hydrogenation of this second peak isolated by preparative gas chromatography gave a material with the same retention-time and mass spectrum as synthetic isovaleryl glycine.

The first and smaller peak, with a retention-time of 2·97 relative to heptadecanoic acid, had a similar but not identical mass spectrum to β-methylcrotonylglycine. Collection of this peak and hydrolysis produced free tiglic acid. The retention-time and mass spectrum of the peak was identical to that of tiglylglycine methyl ester synthesised by a modification of the method of Bondi and Eissler[7] and isolated by thin-layer chromatography. Hydrogenation of this conjugate gave a new material with a mass spectrum consistent with 2-methylbutyrylglycine methyl ester.

The further identification of the major peak as β-methylcrotonylglycine and the smaller peak as the previously undescribed compound, tiglylglycine, will be published in more detail elsewhere. The daily excretion of these conjugates is presented in fig. 2.

Measurement of the urinary excretion of β-hydroxyisovaleric acid on four occasions showed that the excretion of this metabolite was consistently less than that of β-methylcrotonylglycine. Analysis of serum samples did not reveal the presence of free β-methylcrotonic or tiglic acids.

TREATMENT AND PROGRESS

The presence of β-methylcrotonylglycine and β-hydroxyisovaleric acid in the urine of this child showed that the activity of the enzyme β-methylcrotonyl CoA carboxylase was impaired. This suggested that administration of biotin, the coenzyme of the carboxylase, might lead to clinical improvement. We planned to reintroduce protein in small amounts to his diet (0·5 g. per kg. body-weight) and, when his condition was stable, to evaluate the effect of giving biotin. Unfortunately, soon after the introduction of protein his condition rapidly deteriorated, the vomiting returned, and he became shocked and dehydrated. Staphylococcal bacteræmia was demonstrated, and gentamicin was added to the intravenous fluids. Despite initial improvement he remained acidotic and it was thought unjustified to withhold biotin until a calmer clinical state. A large and empirical dose of biotin (10 mg. per day) was given orally.[8]

The effects both clinically and biochemically were impressive. The hyperventilation and vomiting stopped and the ketosis disappeared. The blood pH and base

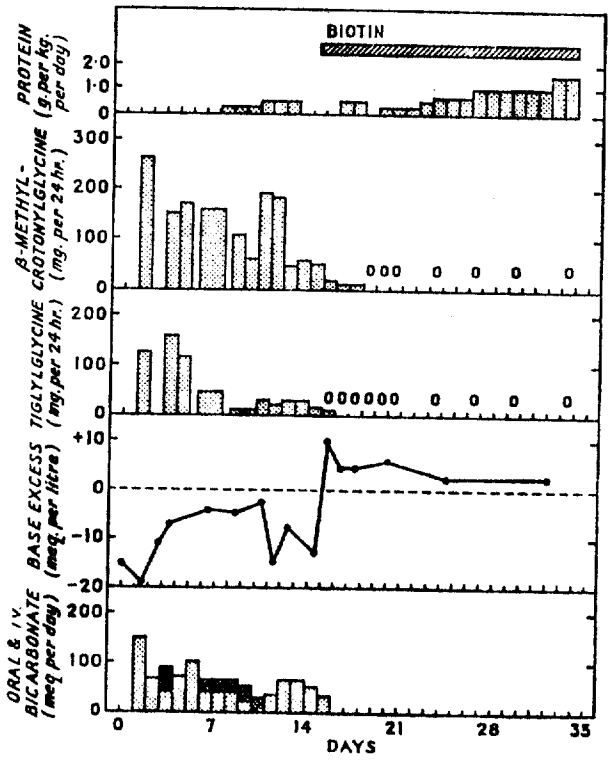

Fig. 2—Effect of biotin administration on β-methylcrotonylglycine and tiglylglycine excretion and the degree of metabolic acidosis.
Bicarbonate therapy: stippled columns, intravenous administration.

excess became normal within 24 hours, and he no longer required alkalis. The skin rash improved remarkably quickly and has not returned. He changed from being irritable and withdrawn to a happier, more responsive child. β-methylcrotonylglycine, tiglylglycine, and β-hydroxyisovaleric acid disappeared totally from the urine after starting biotin treatment, and have not reappeared (fig. 2). He had tended to be constipated, but after biotin he developed diarrhœa which threatened to be troublesome before it settled after 4 or 5 days.

DISCUSSION

We have described a child with metabolic acidosis and ketosis associated with two abnormal conjugates in the urine—β-methylcrotonylglycine and tiglylglycine. Daily excretion patterns of these conjugates (fig. 2) show that immediately after admission both were in high concentrations in the urine. When the child was receiving no protein, tiglylglycine excretion fell, leaving β-methylcrotonylglycine as the major metabolite in the urine. This suggests that the primary lesion was in the metabolism of β-methylcrotonyl coenzyme A. Tiglyl CoA, an intermediate in the degradation of isoleucine, is an isomer of β-methylcrotonyl CoA, and it is possible that increased intracellular concentrations of β-methylcrotonyl CoA block the metabolism of tiglyl CoA by enoyl hydratase, the next enzyme in the isoleucine pathway. The accumulated tiglyl CoA would, no doubt, be conjugated and excreted as the glycine conjugate in the same manner as excess β-methylcrotonyl CoA.

The metabolic pathway of leucine degradation differs from the pathways of valine and isoleucine breakdown at the β-methylcrotonyl CoA carboxylase step. The other two pathways do not include a carboxylation and so have no possibility of biotin dependency. The clinical response of this child to treatment with high doses of biotin was immediate and included a correction of the metabolic acidosis and loss of the massive ketonuria. The excretion of β-methylcrotonylglycine fell below 20 mg. per 24 hours on the day after biotin treatment was started and disappeared entirely 3 days later. It has not reappeared in the urine, although the child's daily protein intake has been increased to 4·0 g. per kg. body-weight. Eldjarn and his coworkers also concluded that their patient probably had a β-methylcrotonyl CoA carboxylase deficiency.[5] However, both the clinical and biochemical features of our patient are different from those described by Eldjarn et al. Our patient excreted over 250 mg. of β-methylcrotonylglycine per 24 hours on admission, while the previous child excreted between 60 and 90 mg. per 24 hours. The Norwegian child excreted β-hydroxyisovaleric acid as the major abnormal metabolite, this acid exceeding β-methylcrotonylglycine by a ratio 4/1. In our patient, β-methylcrotonylglycine excretion was greater than β-hydroxyisovaleric acid excretion. The relative amounts of these two metabolites of β-methylcrotonyl CoA depend on the relative activities of the conjugating enzyme, glycine-N-acylase and the hydrating enzyme, enoyl hydratase (fig. 1).[8]

In summary, this child presented with metabolic acidosis and ketosis associated with excretion of β-methylcrotonylglycine, β-hydroxyisovaleric acid; and tiglylglycine. There was an impressive and sustained clinical improvement coincident with the start of biotin administration. This clinical improvement was paralleled by the disappearance of these three metabolites from the urine. We suggest that this child has an impaired methylcrotonyl CoA carboxylase activity which responded to biotin. Early biochemical diagnosis led to appropriate coenzyme therapy. However, it is impossible to say whether vitamin-responsiveness is characteristic of this condition or whether this is just an enzyme variant.

D. G. and G. H. D. thank Prof. C. C. Booth and Prof. C. T. Dollery for their interest and encouragement. We thank the Medical Research Council and National Fund for Research into Crippling Diseases for their financial support.

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Ham-'53
Bib No. 550

J. Nutrition 51:423-433. 1953.

INTESTINAL SYNTHESIS OF BIOTIN IN THE RAT[1]

EFFECT OF DEFICIENCIES OF CERTAIN B VITAMINS AND OF SULFASUXADINE AND TERRAMYCIN

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(Received for publication June 22, 1953)

The nutritional aspects of the intestinal microflora must include the nutrition of the organisms per se. It is known that diet influences the number and type of bacteria present in the intestinal tract of animals. Porter and Rettger ('40) found that the intestinal flora in rats on high protein diets was predominately protoeolytic in nature, whereas a largely carbohydrate diet influenced a change toward fermentative bacteria.

Guerrant et al. ('34, '35) have reported lower requirements of thiamine and riboflavin when dextrinized cornstarch was used as the sole carbohydrate component in a purified diet. They suggested that this decreased requirement might be due to increased intestinal synthesis of these vitamins.

Skeggs and Wright ('46) and Couch et al. ('48) found a substantial change in biotin excretion, depending upon the carbohydrate used. Dextrin was found to promote a higher rate of synthesis than sucrose, glucose, cerelose or cornstarch. Gall et al. ('48) also found a marked change in the intestinal flora, both in type and number, when the carbohydrate component was changed.

[1] This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure 171.
Scientific Paper 1235, Washington Agricultural Experiment Stations, Pullman. Project 1041.

The addition or deletion of one vitamin can change the rate of synthesis of other vitamins. This was shown by Wegner et al. ('40). They reported that the addition of thiamine to a calf ration apparently increased the synthesis of riboflavin, pantothenic acid, pyridoxine and biotin. Neilson and Elvehjem ('42) found higher biotin synthesis in rats on stock diets than in those on a diet deficient in pantothenic acid or riboflavin. Woolley ('42) reported a greater synthesis of biotin in mice when pantothenic acid was added to the diet. He also found that inositol-deficient mice were benefitted by being fed pantothenic acid, thus indicating that the synthesis of inositol was enhanced.

Cunha et al. ('46) found that the addition of inositol and sulfathaladine to a basal purified ration for pigs alleviated, to a large extent, the deficiency symptoms which were prevented by the administration of biotin. Cunha et al. ('48) likewise reported that biotin and folic acid in combination were more effective than folic acid alone in counteracting the effect of a crude folic acid antagonist.

The biotin deficiency syndrome in rats was also demonstrated by Emerson and Keresztesy ('46) when 0.75% Marfanil (4-homosulfanilamide hydrochloride) was fed with a purified diet. They also reported an initial lowering in both the coliform and total bacterial count in the intestinal tract which returned to normal after 10 to 12 days of trial.

The use of bacteriostatic agents has been a convenient tool in the study of intestinal synthesis of the B vitamins as well as of their interrelationship. The incorporation of these drugs into the diet and the maintenance in the diet of all the factors except those under investigation make a direct balance method possible.

That the antibiotics are bacteriostatic agents and act in nutrition through this method has been shown by Reyniers et al. ('52) by feeding antibiotics to germ-free chicks and turkeys and not finding any beneficial growth response such as is found in normal animals having an established intestinal flora.

Emerson and Smith ('45) have reported biotin deficiency in rats when streptomycin was added to a purified ration. Feeding this antibiotic also produced a decrease in the total bacterial count in the intestinal tract which was of longer duration than was brought about by the administration of sulfasuxadine. A lowering of the amount of biotin synthesized by the intestinal flora was observed by them at the same time.

EXPERIMENTAL

The purified diets used were those employed by Heinemann et al. ('46). The rations were shown to sustain a good rate of growth in pigs when supplemented by B complex vitamins.

The basal diet consisted of, in per cent: vitamin-test casein [2] 26.11; sucrose 57.7; lard 11.0; and mineral mix (Osborn and Mendel; Wesson's modification) 5.2. The control ration was supplemented by the following vitamins, in milligrams per 100 gm of diet: thiamine hydrochloride, 0.3; riboflavin 0.3; niacin 2.5; Ca pantothenate 1.0; pyridoxine 0.3; choline 300.0; and menadione 0.1.

All rats received 500 U.S.P. units of vitamin A and 500 U.S.P. units of vitamin D each week. All rations were refrigerated.

Weanling female rats were used. A 25-day pre-trial conditioning period was allowed before the animals were placed in metabolism cages for the 12- to 14-day balance trials. Urine and feces were collected under 3% acetic acid, and homogenized aliquots were taken of the 12- to 14-day composite.

Two sets of trials were conducted. The rats in the first two trials were given a stock diet and were managed as previously described. This experiment was undertaken to study: (1) interrelationship of biotin and folic acid, and (2) the effect of sulfasuxadine and terramycin separately and in combination upon the intestinal synthesis of biotin. The microbiological assay for biotin of Wright and Skeggs ('44) as modified by these authors (Skeggs and Wright, '46) and using *L. arabinosus* was used.

[2] General Biochemicals, Inc.

In trial 2 (two separate trials) the basal diet was changed so that its composition was, in per cent: vitamin-free casein [3] 26; sucrose 10; starch (raw potato) 54; corn oil 5; minerals (Wesson's modification) 5; plus all the known vitamins except biotin. Added to this ration with different groups of rats were: (1) sulfasuxadine, 2%; (2) terramycin (15 mg/kg basal); and (3) 2% sulfasuxadine plus 15 mg terramycin per kilogram of feed.

In the replicate of trial 2 (designated as 2B) the starch content of the diet was reduced to 35% and the sucrose increased to 24%; otherwise the trial conditions remained the same.

RESULTS AND DISCUSSION

The data from the biotin balance trials are given in tables 1, 2 and 3. It can be seen that synthesis of this vitamin occurred in the intestinal tract of rats receiving the purified basal diet but without biotin. When sulfasuxadine was added at a 2% level, the total synthesis, as well as the quantity of the vitamin excreted per day, was diminished to about 50% that of the group not receiving the drug (based on dry weight of excreta). Synthesis of the vitamin was not completely inhibited even when the sulfa drug was included in the diet.

The addition of folic acid in the presence of sulfasuxadine produced no increase in the amount of non-dietary biotin excreted.

Thiamine deficiency produced a decrease in the total amount of excreted, non-dietary biotin but not in the quantity produced per gram of excreta eliminated, when calculated on the same basis for the control lot. This finding was interpreted as an indication that the lowering of biotin synthesis in this case was brought about because of the anorexia produced by the thiamine deficiency. Lowered food intake resulted in the excretion of a much smaller average amount of elimination products (60%). Because of the fact that there was no significant difference in the amount of biotin excreted

[3] See footnote 2, page 425.

TABLE 1

Biotin balance trials

Interrelationship with folic acid in the presence of sulfasuxadine

TREATMENT[a]	RAT NO.	NET BIOTIN EXCRETED	EXTRA-DIETARY BIOTIN/DAY [1]	VALUE COMPARED WITH CONTROLS	BIOTIN EXCRETED PER RAT PER GRAM OF EXCRETA	VALUE COMPARED WITH CONTROLS
		µg	*µg*		*µg*	
Purified basal control	2	9.13	0.76		0.71	
	3	10.42	0.86		0.92	
	5	7.48	0.63		0.71	
	10	7.97	0.67		0.76	
Purified basal + 2% sulfasuxadine	4	3.10	0.26		0.30	
	9	3.53	0.30	$t = 6.23$ [2]	0.34	$t = 6.43$ [2]
	16	4.57	0.38		0.46	
	6	4.67	0.39		0.47	
Purified basal + 2% sulfasuxadine + folic acid, 2 µg/day	1	3.17	0.26		0.33	
	7	2.95	0.28	$t = 9.39$ [2]	0.35	$t = 9.81$ [2]
	13	2.93	0.24		0.32	
	23	3.05	0.26		0.34	

[1] Twelve-day trials. Difference between second and third groups $t_s = 1.64$ (not significant).

[2] $P < 0.05$.

TABLE 2

Biotin balance trials

Effect of deficiency of certain of the B complex vitamins

TREATMENT	RAT NO.	NET BIOTIN EXCRETED	EXTRA-DIETARY BIOTIN/DAY [1]	VALUE COMPARED WITH CONTROLS	BIOTIN EXCRETED PER GRAM OF EXCRETA	
		μg	μg		μg	
Purified basal 6 B vitamins (control)	1	6.44	0.64		0.92	
	2	6.44	0.64		0.92	
	3	7.36	0.73		1.34	
	4	7.91	0.79		1.25	
Purified basal 5 B vitamins, no thiamine	5	3.44	0.34		0.82	
	6	3.48	0.35 [2]		0.83	$t = 0.32$
	7	4.28	0.43	$t = 7.87$ [3]	1.77	
	8	3.42	0.34 [2]		1.15	
Purified basal 5 B vitamins, no riboflavin	9	2.33	0.23		0.47	
	10	2.03	0.20		0.40	$t = 5.71$ [3]
	11	3.35	0.34	$t = 7.95$ [3]	0.70	
	12	3.36	0.34		0.70	
Purified basal 5 B vitamins, no niacin	13	4.38	0.44		0.59	
	14	4.62	0.46		0.62	$t = 6.84$ [3]
	15	3.31	0.33	$t = 6.12$ [3]	0.57	
	16	3.37	0.34		0.58	
Purified basal 5 B vitamins, no pantothenic acid	17	3.62	0.36		0.63	
	18	3.68	0.37		0.63	$t = 7.54$ [3]
	19	2.71	0.27	$t = 8.22$ [3]	0.46	
	20	2.61	0.26		0.46	
Purified basal 5 B vitamins, no pyridoxine	21	5.35	0.54		0.92	
	22	5.07	0.51		0.88	$t = 2.56$
	23	4.53	0.45	$t = 4.70$ [3]	1.00	
	24	4.98	0.50		1.08	

[1] Twelve-day trials.
[2] Ten-day trials.
[3] $P < 0.05$.

TABLE 3

Biotin balance trials

Effect of sulfasuxadine and terramycin on synthesis of biotin

TREATMENT	RAT NO.	TOTAL BIOTIN EXCRETED	EXTRA-DIETARY BIOTIN PER DAY [1]		BIOTIN EXCRETED PER RAT PER GRAM OF EXCRETA	
		μg	μg		μg	
Purified basal	1	88.57	7.24		1.78	
	2	87.35	7.28		1.54	
	3	77.30	6.44		1.81	
Purified basal 2% sulfasuxadine	4	32.80	2.73		0.93	
	5	35.43	2.95	$t = 14.81$ [2]	1.28	$t = 3.19$ [3]
	6	33.74	2.81		0.78	
Purified basal — terramycin (15 mg/kg ration)	7	38.07	3.17		0.78	
	8	28.07	2.34	$t = 11.35$ [2]	0.54	$t = 6.06$ [3]
	9	28.83	2.40		0.56	
Purified basal 2% sulfasuxadine — terramycin (15 mg/kg ration)	10	13.75	1.15		0.27	
	11	12.67	1.06	$t = 10.70$ [2]	0.27	$t = 3.89$ [3]
	12	12.01	1.00		0.24	

[1] Twelve-day trials.

[2] $P < 0.01$.

[3] $P < 0.05$.

per gram of excreta between the control lot and the thiamine-deficient lot, the total synthesis of the vitamin must have been limited by the indirect cause, anorexia, instead of by reduction of any of the predominant synthesizing organisms.

Deficiencies of riboflavin, niacin and pantothenic acid caused a lowering of both the total excretion of non-dietary biotin and of the quantity excreted per gram of dry excreta, indicating that the absence of these vitamins from the diet exerted a direct effect upon the synthesizing ability of the intestinal bacteria.

The absence of pyridoxine in the diet resulted in no change in the intestinal synthesis of biotin, either in terms of total excretion or on the gram basis of excreta.

The fact that the amount or bulk of fecal material present in the intestine may possibly limit the bacterial growth, and therefore synthesis, would seem to be in agreement with the theory of Guerrant et al. ('35) that the increase of synthesis which was observed with the use of dextrin was due to the production of a bulkier feces. It might likewise explain, at least partially, the increased synthesis reported to have been found with the use of natural diets containing bulk in the form of indigestible cellulose, and so forth, when compared to purified rations containing very little indigestible fiber.

In cases where synthesis of biotin was lowered both in total amount and also in the quantity per gram of excreta, it can be concluded that the effect upon synthesis of the vitamin must have been caused by failure to satisfy the nutritive requirements of the predominating synthesizing organism. Synthesis, however, was not altogether stopped under any of the conditions of the experiments, probably because of the fact that total synthesis is the summation of the production of several or many types of organisms having different nutritive requirements and even different susceptibility to the bacteriostatic action of sulfasuxadine. Obviously, the total synthesis of the vitamin is of primary significance as far as the general nutritional status of the animal is concerned. Whether the amount of synthesized vitamin is lowered because of

general lowering of the numbers of synthesizing organisms present, or due to the inhibition of the growth of specific synthesizing bacteria, the resulting effect upon the animal is the same.

The intestinal synthesis of biotin was increased in the basal group when part of the sucrose was replaced by raw potato starch, as is shown by table 3. These data confirm the work of Couch et al. ('48) and other authors previously discussed who noted variation of intestinal synthesis depending on the type of carbohydrate used. Synthesis was increased when starch was used, even in the presence of sulfasuxadine.

The bacteriostatic action of the terramycin was similar in direction and degree to that of the sulfasuxadine in inhibiting the synthesis of biotin.

A combination of terramycin and sulfasuxadine almost completely inhibited intestinal biotin synthesis. These results are similar to those reported by Monson et al. ('52), although they are not directly comparable because these workers used rations adequate in the vitamins being tested.

SUMMARY AND CONCLUSIONS

1. The microbiological synthesis of biotin in the intestinal tract of the rat, as measured by direct balance studies, was inhibited about one-half when succinylsulfathiozole was added to a purified diet at a 2% level.

2. Biotin synthesis was found to be diminished when the diet was free of riboflavin, niacin and pantothenic acid. The excretion of non-dietary biotin was lower both in total amount and when calculated in micrograms per gram (dry weight) of excreta eliminated during the trials.

3. The total amount of biotin synthesized by the intestinal flora of rats suffering a thiamine deficiency was diminished. The amount excreted per gram of excreta, however, was comparable to that of the control lot. It was concluded that, in this case, the lowering of the excretion of non-dietary biotin was caused by the anorexia of the thiamine deficiency and the resulting smaller amount of material excreted, and was

not a direct effect of the vitamin deficiency upon the synthesizing microflora.

4. A pyridoxine deficiency caused no significant change in either the total biotin excreted or in the quantity excreted per gram of excreta during the trial.

5. Terramycin added to the biotin-free diet diminished the synthesis of biotin to an extent similar to 2% sulfasuxadine; and the combination of both terramycin and sulfasuxadine lowered the synthesis to about 1 μg/day, indicating separate inhibiting action.

6. When starch replaced sucrose, there was an increased synthesis of biotin in the intestinal tract.

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Hardinge-'61
Bib No. 560

Lesser Known Vitamins in Foods[1]

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Many tables of food analyses are available for the computation of the better known food nutrients. However, none brings together, in convenient form, some of the less frequently considered vitamins, namely B_6, pantothenic acid, biotin, folic acid, choline, and inositol.

Judged by standard tables, the majority of American diets appear reasonably adequate in nutrient content. However, the possibility of a deficiency of other nutritional factors frequently arises but is not commonly investigated for lack of tables by which these factors could be readily assessed.

Since the authors found it necessary to put forth considerable effort to gather and compile this information for the study of modified diets, it was felt that its publication might be of value to others.

The data for the table were obtained from many sources (1-42). Department of Agriculture handbooks on folic acid (1) and pantothenic acid (12) provided most of the figures for these two nutrients, although data for values for foods not listed in these and also for the other four vitamins were obtained mainly from original literature. In a few instances, they were taken from other compilations or abstracts. The titles of the articles frequently indicate the specific nutrients studied. Some information was received directly from manufacturers and growers (41, 42).

Certain problems were encountered. Data had to be drawn from analyses done by different workers over a period of years (1942-1960). During this time, assay methods underwent progressive changes. Apparent inconsistencies occur when results obtained several years ago are compared with data from more recent studies. For example, older determinations of pantothenic acid, unless done by animal assay, gave results only slightly higher than the free vitamin (12). Current methods liberate bound forms of the acid before assay, and much higher values are reported. Considerable time and care has been spent to include in this compilation the most recent and representative findings available. In a few instances, where no other data for B_6 could be found, the figures of Cheldelin and Williams for pyridoxine (26) were included, even though the authors suggest that these may not be comparable to results obtained by other methods. Wherever these appear appreciably lower than other values available for similar types of food, they are enclosed in parentheses.

All six vitamins of this study are abundant in the bran and germ of whole grains, yeasts, and liver, and all are quite widely distributed in nature, yet some foods have been investigated more thoroughly than others. Those low in a certain vitamin have been but little investigated for that ingredient. For example, almost no data could be found for choline in fruits, since it is lacking or is present only in very small amounts in these foods. In general, the information on biotin and inositol content were also less complete than for folic acid, pantothenic acid, and B_6.

With these limitations in mind, this table of vitamin values is presented as a current working tool until better and more complete analyses become available.

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[1]Supported in part by a grant-in-aid from the Blalock Memorial Foundation, Walla Walla, Washington. Received for publication September 26, 1960.

TABLE 1 Vitamin B_6, pantothenic acid, biotin, folic acid, choline, and inositol content of foods, edible portion

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Cereals and Bread Products														
		gm.	mcg.		mg.		mcg.		mcg		mg.		mg.	
Cereals, cooked, whole														
Barley, whole	½ c.	30(dry)	167.1	557	0.219	0.73	9.3	31.0	15.	50.0	41.7	139.0	117.6	392.0
Corn, yellow	½ c.	20(dry)	93.4	467	0.128	0.64	4.2	21.0	5.3	26.5	12.2	61.0	10.0	50.0
Oats	½ c.	20(dry)	41.0	205	0.300	1.50	4.8	24.0	6.6	33.0	31.2	156.0	53.8	269.0
Rice, brown	½ c.	30(dry)	186.0	620	0.456	1.52	3.6	12.0	6.0	20.0	33.6	112.0	35.7	119.0
Wheat, whole	½ c.	30(dry)	158.4	528	0.411	1.37	4.8	16.0	14.7	49.0	28.2	94.0	110.0	370.0
Cereals, refined, cooked														
Cornmeal														
White	½ c.	20(dry)	(12.2)*	(61)	0.116	0.58	1.3	6.6	1.8	9.0	—†	—	10.2	51
Yellow	½ c.	20(dry)	—	—	0.138	0.69	—	—	1.8	9.0	2.0	10.0	—	—
Hominy, grits	½ c.	20(dry)	(1.2)	(6)	0.068	0.34	0.1	0.7	0.9	4.5	—	—	0.6	3
Rice														
Converted	½ c.	30(dry)	20.4	68	0.393	1.31	2.4	8.0	6.0	20.0	26.7	89.0	6.0	20
Parboiled	½ c.	30(dry)	30.0	100	0.411	1.37	3.0	10.0	5.7	19.0	29.4	98.0	7.5	25
White	½ c.	30(dry)	11.1	37	0.225	0.75	1.5	5.0	4.8	16.0	17.7	59.0	3.0	10
Cereal concentrates, raw														
Rice														
Bran	1 oz.	30(dry)	750.0	2500	0.831	2.77	18.0	60.0	43.8	146.0	51.0	170.0	138.9	463
Germ	1 oz.	30(dry)	480.0	1600	0.900	3.00	17.4	58.0	129.0	430.0	90.0	300.0	111.6	372
Polishings	1 oz.	30(dry)	600.0	2000	1.000	3.33	17.1	57.0	57.6	192.0	30.6	102.0	136.2	454
Wheat														
Bran	1 oz.	30(dry)	414.0	1380	0.900	3.00	4.2	14.0	58.5	195.0	42.9	143.0	—	—
Germ	1 oz.	30(dry)	275.4	918	0.660	2.20	—	—	91.5	305.0	121.8	406.0	231.0	770
Ready-to-eat, dark cereal														
Corn soya	1 c.	30	—	—	0.273	0.91	—	—	24.0	80.0	—	—	—	—
Oats	1 c.	25	—	—	0.230	0.92	—	—	5.6	22.4	—	—	—	—
Wheat														
Bran	1 c.	35	—	—	1.015	2.90	—	—	35.0	100.0	—	—	—	—
Flakes	1 c.	35	—	—	0.304	0.87	—	—	16.4	47.0	—	—	—	—
Shredded Wheat	1 biec.	30	—	—	0.210	0.70	—	—	16.5	55.0	—	—	—	—
Ready-to-eat, white cereal														
Corn flakes	1 c.	25	—	—	0.047	0.19	—	—	1.4	5.5	—	—	—	—
Rice	1 c.	30	(14.4)	(48)	0.114	0.38	0.4	1.3	2.3	7.6	—	—	5.7	19
Bread														
White	1 sl.	23	23.0	100	0.101	0.44	0.3	1.1	3.4	15.0	—	—	11.7	51
Whole wheat	1 sl.	23	96.0	420	0.182	0.79	0.4	1.9	6.9	30.0	—	—	15.4	67
Flour														
White	1 c.	110	363.0	330	0.594	0.54	1.1	1.0	8.8	8.0	57.2	52	51.7	47
Whole wheat	1 c.	120	1116.0	930	1.296	1.08	10.8	9.0	45.6	38.0	—	—	132.0	110
Dairy Products														
Cheese														
Cheddar	1 oz.	30	(18.7)	(66)	0.114	0.40	1.0	3.6	4.5	16.0	13.6	48	7.0	25
Cottage	1 oz.	30	—	—	0.080	0.28	—	—	8.8	31.0	—	—	—	—
Processed	1 oz.	30	—	—	0.136	0.48	1.3	4.6	3.1	11.0	—	—	—	—
Egg														
Whole	1 med.	50	126.0	252	0.795	1.59	11.2	22.5	2.5	5.1	252.0	504	16.5	33
Yolk	1 med.	17	52.4	308	0.719	4.23	8.8	52.0	2.2	12.9	253.3	1490	—	—
White	1 med.	31	67.3	217	0.043	0.14	2.2	7.0	0.2	0.6	0.6	2	—	—
Milk														
Whole	1 c.	244	87.8	36	0.756	0.31	11.5	4.7	1.5	0.6	36.6	15	31.7	13
Evaporated reconstituted	1 c.	244	53.7	22	0.805	0.33	11.0	4.5	1.7	0.7	36.6	15	—	—
Non-fat dry reconstituted	1 c.	244	80.5	33	0.830	0.34	8.3	3.4	0.5	0.2	25.4	10.4	—	—
Fats, Oils, and Oily Foods														
Butter	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Lard	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Margarine	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—
Vegetable oils	1 Tbsp.	14	—	—	—	—	—	—	—	—	0.7	5	—	—

*Data from Cheldelin and Williams (26), as only data available, even though they may not be comparable to figures obtained by other methods. Figures which are appreciably lower than other values available for similar types of foods are enclosed in parentheses.

†Dashes indicate that no representative value was found in the literature for particular constituent.

TABLE I, continued

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Fruits														
		gm.	*mcg.*		*mg.*		*mcg.*		*mcg.*		*mg.*		*mg.*	
Fruits, fresh or frozen														
Apples, medium	1	130	39.0	30	0.130	0.10	1.2	0.9	2.6	2.0	—	—	31.2	24
Apricots	3 med.	100	71.0	71	0.200	0.20	—	—	3.3	3.3	—	—	—	—
Bananas	1 med.	100	320.0	320	0.310	0.31	4.4	4.4	9.7	9.7	—	—	34.0	34
Berries														
Blackberry	⅔ c.	100	—	—	0.260	0.26	—	—	13.7	13.7	—	—	—	—
Blueberry	⅔ c.	100	91.0	91	0.120	0.12	—	—	8.0	8.0	—	—	—	—
Raspberry, sweetened	¾ c.	100	38.0	38	0.210	0.21	—	—	5.0	5.0	—	—	—	—
Strawberry, sweetened	⅔ c.	100	61.0	61	0.160	0.16	4.0	4.0	9.0	9.0	—	—	60.0	60
Cantaloupe, diced	⅔ c.	100	36.0	36	0.260	0.26	3.1	3.1	6.8	6.8	—	—	120.0	120
Cherries, sour red, pitted	⅔ c.	100	85.0	85	0.070	0.07	—	—	6.0	6.0	—	—	—	—
Figs, small	3	114	148.2	130	0.388	0.34	—	—	16.0	14.0	—	—	—	—
Grapefruit, small	½	120	25.0	21	0.288	0.24	3.6	3.0	3.4	2.8	—	—	180.0	150
Grapes, 1 bunch	3½ oz.	100	85.0	85	0.050	0.05	1.6	1.6	5.2	5.2	—	—	—	—
Oranges, small	1	100	31.0	31	0.220	0.22	1.9	1.9	5.1	5.1	—	—	210.0	210
Peaches, sweetened	½ c.	100	20.0	20	0.120	0.12	1.7	1.7	4.0	4.0	—	—	96.0	96
Pineapple	3½ oz.	100	75.0	75	0.170	0.17	—	—	6.0	6.0	—	—	—	—
Rhubarb, sweetened	3½ oz.	100	20.0	20	0.070	0.07	—	—	4.0	4.0	—	—	—	—
Watermelon, diced	1 c.	150	49.5	33	0.450	0.30	5.4	3.6	0.9	0.6	—	—	96.0	64
Fruits, canned														
Apricots	½ c.	125	67.5	54	0.125	0.10	—	—	0.6	0.5	—	—	—	—
Blackberries	½ c.	125	30.0	24	0.100	0.08	—	—	17.5	14.0	—	—	—	—
Blueberries	½ c.	125	48.7	39	0.087	0.07	—	—	5.2	4.2	—	—	—	—
Cherries, sweet	½ c.	125	196.2	157	0.150	0.12	—	—	3.7	3.0	—	—	—	—
Peaches	½ c.	125	28.8	23	0.088	0.07	0.3	0.2	0.6	0.5	—	—	—	—
Pineapple	½ c.	125	88.7	71	—	—	—	—	1.0	0.8	—	—	—	—
Plums, purple	½ c.	125	33.7	27	0.100	0.08	—	—	1.2	1.0	—	—	—	—
Fruit, dried														
Apricots, small	6 halves	30	—	—	0.225	0.75	—	—	1.4	4.7	—	—	—	—
Dates, pitted	5	45	—	—	0.351	0.78	—	—	11.1	24.7	—	—	—	—
Figs, large	2	40	128.0	320	0.208	0.52	—	—	12.8	32.0	—	—	—	—
Prunes	5 med.	35	—	—	0.126	0.36	—	—	1.9	5.4	—	—	—	—
Raisins, seedless	3 Tbsp.	30	98.0	327	0.030	0.10	1.4	4.5	3.0	10.0	—	—	36.0	120
Fruit Juices														
Canned fruit juice														
Apple	½ c.	120	42.0	35	—	—	—	—	0.2	0.2	—	—	—	—
Grapefruit	½ c.	120	15.6	13	0.204	0.17	1.0	0.8	2.3	1.9	—	—	120.0	100
Lemon	½ c.	120	61.2	51	—	—	—	—	0.2	0.2	—	—	—	—
Orange	½ c.	120	28.8	24	0.156	0.13	1.0	0.8	2.8	2.3	—	—	169.0	141
Pineapple	½ c.	120	115.2	96	0.120	0.10	—	—	1.1	0.9	—	—	—	—
Frozen or fresh														
Apple	½ c.	120	36.0	30	0.024	0.02	0.5	0.4	1.8	1.5	0.7	0.6	28.8	24
Grape	½ c.	120	25.2	21	0.048	0.04	0.4	0.3	3.6	3.0	—	—	—	—
Grapefruit	½ c.	120	16.8	14	0.192	0.16	0.8	0.7	1.6	1.3	—	—	120.0	100
Lemon, single	½ c.	120	46.8	39	0.108	0.09	—	—	1.2	1.0	—	—	—	—
Orange, fresh	½ c.	120	31.2	26	0.168	0.14	0.4	0.3	2.6	2.2	14.4	12	141.6	118
Orange, frozen	½ c.	120	26.4	22	0.168	0.14	0.4	0.3	2.6	2.2	14.4	12	110.4	92
Pineapple	½ c.	120	88.8	74	0.144	0.12	—	—	1.2	1.0	—	—	—	—
Legumes														
	cooked	*dry*												
Mung bean	½ c.	32	182.4	570	0.800	2.50	2.4	7.5	46.4	145	66.9	209	22.4	70
Cowpeas	½ c.	32	(67.2)	(210)	0.397	1.24	6.7	21.0	140.5	439	82.2	257	76.8	240
Garbanzos	½ c.	32	172.8	540	0.400	1.25	3.2	10.0	40.0	125	78.4	245	76.8	240
Lentils	½ c.	32	156.8	490	0.480	1.50	4.2	13.2	34.2	107	71.4	223	41.6	130
Lima beans	½ c.	32	176.0	550	0.416	1.30	3.1	9.8	41.0	128	—	—	54.4	170
Navy beans	½ c.	32	—	—	0.387	1.21	—	—	40.0	125	—	—	160.0	500
Kidney beans	½ c.	32	—	—	0.208	0.65	—	—	57.6	180	—	—	—	—
Split peas	½ c.	32	105.0	328	0.700	2.18	5.9	18.4	16.3	51	64.3	201	48.0	150
Soy beans	½ c.	32	204.8	640	0.538	1.68	19.5	61.0	71.7	224	108.8	340	64.0	200
Soy flour	1 c.	90	500.4	656	1.512	1.68	63.0	70.0	383.4	426	202.5	225	184.5	205
Meat and Poultry‡														
Beef														
Ground	3½ oz.	100	460.0ǂ	460	0.440ǂ	0.44	—	—	6.9	6.9	—	—	—	—
Liver	2 oz.	57	378.5	664	5.324	9.34	54.7	96.0	167.6	294.0	200.7	510	29.1	51
Rib roast, lean	3 oz.	100	480.0ǂ	480	0.600ǂ	0.60	3.4	3.4	—	—	82.0	82	—	—
Round	3 oz.	100	495.0ǂ	495	0.520ǂ	0.52	2.6	2.6	10.5	10.5	68.0	68	11.5	11.5

‡Raw values, except where indicated. ǂCooked values

TABLE I, continued

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Meat and Poultry, concluded‡														
		gm.	*mcg.*		*mg.*		*mcg.*		*mcg.*		*mg.*		*mg.*	
Lamb														
Leg	3 oz.	100	320.0/	320	0.620/	0.62	5.9	5.9	3.3	3.3	84.0	84	58.0	58
Liver	2 oz.	57	—	—	4.047	7.10	72.4	127.0	157.3	276.0	—	—	—	—
Loin chop	3 oz.	100	330.0/	330	0.590/	0.59	—	—	—	—	76.0	76	—	—
Veal														
Chop	3½ oz.	100	430.0/	430	0.550/	0.50	2.0	2.0	—	—	96.0	96	33.0	33
Leg	3½ oz.	100	200.0	200	0.914	0.914	—	—	—	—	132.0	132	—	—
Shoulder	3½ oz.	100	300.0	300	—	—	—	—	—	—	93.0	93	—	—
Stew meat	3½ oz.	100	330.0	330	0.852	0.852	—	—	4.6	4.6	96.0	96	—	—
Pork														
Bacon	2 sl.	16	88.0/	550	0.070/	0.440	1.2	7.6	—	—	12.8	80	6.9	43
Ham	3½ oz.	100	440.0/	440	0.640/	0.640	5.0	5.0	10.6	10.6	122.0	122	31.0	31
Liver	2 oz.	57	—	—	3.994	7.008	57.0	100.0	126.0	221.0	314.6	552	—	—
Loin	3½ oz.	100	480.0/	480	0.400/	0.400	5.2	5.2	2.4	2.4	77.0	77	45.0	45
Poultry														
Chicken														
Dark meat	3½ oz.	100	(25.0)	(25)	0.692	0.692	10.0	10.0	2.8	2.8	—	—	47.0	47
White meat	3½ oz.	100	(130.0)	(130)	0.804	0.804	11.3	11.3	3.0	3.0	—	—	48.0	48
Turkey	3½ oz.	100	—	—	0.748	0.748	—	—	7.5	7.5	—	—	—	—
Bologna, A.F.	2 oz.	57	—	—	—	—	—	—	—	—	34.2	60	—	—
Frankfurter, A.P.	1	50	65.0	130	0.212	0.425	—	—	—	—	28.5	57	—	—
Sausage, pork, A.P.	2 oz.	57	108.3	190	0.321	0.563	—	—	6.5	11.5	27.4	48	—	—
Fish														
Halibut, canned	3½ oz.	100	(110.0)	(110)	0.150	0.150	8.0	8.0	—	—	—	—	17.0	17
Mackerel, Pacific, canned	3½ oz.	100	270.0	270	0.470	0.470	18.0	18.0	0.6	0.6	—	—	—	—
Salmon, canned	3½ oz.	100	450.0	450	0.580	0.580	15.0	15.0	0.5	0.5	—	—	17.0	17
Tuna, canned	3½ oz.	100	670.0	670	0.420	0.420	3.0	3.0	1.8	1.8	—	—	—	—
Sardines, Pacific, canned	3½ oz.	100	280.0	280	0.600	0.600	24.0	24.0	0.5	0.5	—	—	—	—
Shellfish, canned														
Clams	3½ oz.	100	83.0	83	0.590	0.590	—	—	2.0	2.0	—	—	—	—
Crabs	3½ oz.	100	364.0	364	—	—	—	—	0.4	0.4	—	—	—	—
Oysters	3½ oz.	100	37.0	37	0.490	0.490	8.7	8.7	11.3	11.3	—	—	44.0	44
Shrimp	3½ oz.	100	111.0	111	0.210	0.210	—	—	1.8	1.8	—	—	—	—
Nuts and Oily Fruits														
Nuts, raw														
Almonds	12-15	15	15.0	100	0.087	0.578	2.7	18.0	6.7	45.0	—	—	—	—
Brazil nuts	2 med.	15	—	—	0.035	0.231	—	—	0.7	4.5	—	—	—	—
Cashews	6-8	15	—	—	0.174	1.62	—	—	—	—	—	—	—	—
Coconut, fresh	½ oz.	15	—	—	0.049	0.330	—	—	4.1	27.6	—	—	—	—
Filbert	10-12	15	—	—	0.172	1.146	—	—	10.0	66.6	—	—	—	—
Peanuts, roasted	15-17	15	45.0	300	0.320	2.137	5.1	34.0	8.5	56.5	24.3	162	27.0	180
Pecan halves	12	15	—	—	0.256	1.707	4.0	27.0	4.0	27.0	7.5	50	—	—
Walnut halves	8-15	15	144.0	960	0.146	0.970	5.5	37.0	11.5	77.0	—	—	—	—
Peanut butter	1 Tbsp.	16	48.0	300	0.400	2.500	6.2	39.0	8.5	56.5	23.2	145	28.8	180
Fruits, oily														
Avocado, Fuerte (cubed)	½ c.	75	457.5	610	0.675	0.90	4.1	5.5	22.5	30.0	—	—	—	—
Olive, ripe, mammoth	6	40	6.4	16	0.008	0.02	—	—	0.3	0.7	—	—	—	—
Vegetables														
Vegetables, fresh or frozen														
Asparagus	3½ oz.	100	136.0	136	0.620	0.62	—	—	109.0	109.0	10.0	10.0	—	—
Beans, Lima	3½ oz.	100	170.0	170	0.450	0.45	—	—	34.0	34.0	—	—	—	—
Beans, snap, green	3½ oz.	100	63.0	63	0.200	0.20	—	—	27.5	27.5	42.0	42.0	—	—
Beets (diced)	½ c.	100	(37.0)	(37)	0.170	0.17	1.9	1.9	13.5	13.5	—	—	21.0	21
Broccoli	½ c.	100	171.0	171	1.290	1.29	—	—	53.5	53.5	—	—	—	—
Brussels sprouts	3½ oz.	100	162.0	162	0.720	0.72	—	—	49.0	49.0	—	—	—	—
Cabbage (fine shreds)	1 c.	100	120.0	120	0.260	0.26	2.4	2.4	32.3	32.3	23.0	23.0	95.0	95
Carrots (grated)	½ c.	55	66.0	120	0.150	0.27	1.4	2.5	4.4	8.0	7.0	13.4	26.4	48
Cauliflower (buds)	1 c.	100	177.0	177	1.010	1.01	17.0	17.0	22.2	22.2	—	—	95.0	95
Celery (diced)	1 c.	100	—	—	0.430	0.43	—	—	7.0	7.0	—	—	—	—
Corn	3½ oz.	100	222.0	222	0.890	0.89	6.0	6.0	28.0	28.0	—	—	—	—
Lettuce	¼ hd.	100	(71.0)	(71)	0.360	0.36	3.1	3.1	21.0	21.0	—	—	55.0	55
Mixed vegetables	3½ oz.	100	122.0	122	0.310	0.31	—	—	16.0	16.0	—	—	—	—
Onion (2½ in.)	1	110	(69.3)	(63)	0.187	0.17	3.8	3.5	11.0	10.0	—	—	96.8	88
Peas, cow	3½ oz.	100	118.0	118	0.400	0.40	21.0	21.0	41.0	41.0	97.0	97.0	240.0	240
Peas, green	3½ oz.	100	150.0	150	0.820	0.82	9.4	9.4	25.0	25.0	75.0	75.0	162.0	162
Potatoes, peeled	1 med.	100	220.0	220	0.400	0.40	—	—	6.8	6.8	29.0	29.0	29.0	29
Potatoes, sweet	½ med.	100	320.0	320	0.930	0.93	4.3	4.3	12.0	12.0	11.5	11.5	66.0	66

‡Raw values, except as indicated. /Cooked.

TABLE I, concluded

FOOD	PORTION		VITAMIN B_6		PANTOTHENIC ACID		BIOTIN		FOLIC ACID		CHOLINE		INOSITOL	
	Amt.	Wt.	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent	Wt.	Per cent
Vegetables, Concluded														
		gm.	mcg.		mg.		mcg.		mcg.		mg.		mg.	
Squash, winter	3½ oz.	100	91.0	91	0.490	0.49	—	—	12.0	12.0	—	—	—	—
Squash, yellow	3½ oz.	100	63.0	63	0.390	0.39	—	—	17.0	17.0	—	—	—	—
Tomato	1 small	110	—	—	0.341	0.31	4.4	4.0	8.8	8.0	—	—	50.6	46
Mushrooms, fresh	2 oz.	60	(27.0)	(45)	1.626	2.71	9.6	16.0	14.4	24.0	—	—	10.2	17
Greens														
Beet	3½ oz.	100	—	—	0.260	0.26	2.7	2.7	60.0	60.0	—	—	21.0	21
Kale	3½ oz.	100	185.0	185	1.290	1.29	—	—	70.0	70.0	—	—	—	—
Mustard	3½ oz.	100	133.0	133	0.250	0.25	—	—	60.0	60.0	22.0	22.0	—	—
Spinach	3½ oz.	100	198.0	198	0.310	0.31	6.9	6.9	75.0	75.0	22.0	22.0	27.0	27
Turnip	3½ oz.	100	98.0	98	0.380	0.38	—	—	42.0	42.0	27.0	27.0	46.0	46
Vegetables, canned with liquid														
Asparagus, green	½ c.	120	90.0	75	0.240	0.20	2.0	1.7	32.4	27.0	—	—	—	—
Beans														
Lima	½ c.	120	97.2	81	0.132	0.11	—	—	15.6	13.0	—	—	—	—
Green string	½ c.	120	51.6	43	0.084	0.07	1.5	1.3	14.4	12.0	—	—	—	—
Beets (diced)	½ c.	125	67.5	54	0.125	0.10	—	—	3.5	2.8	—	—	—	—
Carrots (diced)	½ c.	125	51.2	41	0.137	0.11	1.9	1.5	4.1	3.3	—	—	—	—
Corn	½ c.	125	270.0	216	0.275	0.22	2.7	2.2	9.6	7.7	—	—	—	—
Mushrooms	½ c.	120	75.6	63	1.128	0.94	8.7	7.3	4.7	3.9	—	—	20.4	17
Peas														
Cow	½ c.	125	66.2	53	—	—	—	—	32.5	26.0	—	—	—	—
Green	½ c.	125	55.0	44	0.212	0.17	2.6	2.1	12.9	10.3	—	—	—	—
Spinach	½ c.	120	114.0	95	0.071	0.059	2.8	2.3	58.8	49.0	—	—	—	—
Tomatoes	½ c.	120	181.2	151	0.276	0.23	2.2	1.8	4.4	3.7	—	—	—	—
Tomato juice	½ c.	120	230.4	192	0.360	0.30	—	—	8.0	6.7	—	—	—	—
Miscellaneous														
Chocolate	1 oz.	28.4	(6.5)	(23)	0.054	0.19	9.0	32.0	28.1	99.0	—	—	24.1	85
Molasses	1 Tbsp.	20	(54.0)	(270)	0.092	0.46	1.8	9.0	1.9	9.5	17.2	86	30.0	150
Yeast, brewer's	1 Tbsp.	8	193.5	2419	0.880	11.0	16.0	200.0	161.8	2022.0	19.2	240	—	—
Food yeast, torula	1 Tbsp.	8	280.0	3500	0.800	10.0	8.0	100.0	240.0	3000.0	20.0	250	21.6	270
Honey	1 Tbsp.	20	2.0	10	0.012	0.06	—	—	0.6	3.0	—	—	—	—
Pollen, flower	—	1	9.0	900	0.027	2.70	0.3	25.0	—	—	—	—	—	—
Royal jelly	—	1	10.2	1020	0.320	32.00	4.1	410.0	0.5	50.0	—	—	0.1	10
Dairy Food Substitutes														
Soybean milk, commercial type														
Soyagen: infant, all-purpose, or malt-flavored powder	—	30	52.5	185.0	0.25	0.870	3.4	11.90	6.6	23.34	—	—	32.2	11.30
Reconstituted liquid	1 c.	240	57.5	24.0	0.27	0.112	3.7	1.54	7.2	3.00	—	—	35.3	1.47
Soyalac, infant, concentrated, liquid, reconstituted	1 c.	240	72.5	30.0	0.178	0.074	3.28	1.37	27.50	11.46	—	—	22.75	9.48
Prepared Plant Protein														
Gluten-type entrée														
Choplets	2 oz.	60	54.0	90.0	0.048	0.080	7.7	12.8	17.0	28.5	6.0	10.0	3.5	5.8
Dinner Cuts	2 oz.	60	36.0	60.0	0.032	0.054	3.0	5.0	13.2	22.0	9.5	12.5	3.0	5
Vegeburger	2 oz.	60	53.4	89.0	0.036	0.060	3.6	6.0	15.0	25.0	6.0	10.0	3.0	5
Weighted average‡	2 oz.	60	46.2	77.0	0.036	0.060	4.2	7.0	14.7	24.5	6.6	11.0	3.2	5.3
Nutmeat-type entrées														
Linketts	2 oz.	60	55.8	93.0	0.100	0.164	5.7	9.5	22.2	37.0	7.2	12.0	6.0	10
Nuteena	2 oz.	60	25.8	43.0	0.121	0.202	22.8	38.0	52.8	88.0	11.4	19.0	6.0	10
Patties	2 oz.	60	39.6	66.0	0.021	0.035	2.4	4.0	76.2	127.0	48.0	80.0	40.8	68
Proteena	2 oz.	60	43.2	72.0	0.125	0.208	13.2	22.0	19.2	32.0	6.9	11.5	6.6	11
Vegalinks	2 oz.	60	42.0	70.0	0.016	0.026	4.2	7.0	42.3	70.5	18.0	30.0	16.8	28
Weighted average‡	2 oz.	60	45.4	76.0	0.112	0.186	9.3	15.3	34.2	57.0	12.3	20.5	10.0	16.8

‡Weighted on the basis of sales per product per year.

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Breaded Shrimp

Americans consume 61,000,000 lb. breaded shrimp annually, of which, some 35 per cent is used in the institutional field, according to an estimate of the National Shrimp Breaders Association.

Producers may take advantage of the Department of Interior's voluntary grading service, a plan by which government inspectors grade the products at the plant. To belong to the National Shrimp Breaders Association, a processor must permit continuous government inspection in his plant and carry the "U.S. Grade A" seal on his package. According to this standard, the product must score not less than 85 points on a scale set up by the government, and the breading must not be more than 50 per cent of the weight.

Hertz-'46
Bib No. 576

PHYSIOLOGICAL REVIEWS
VOL. 26 OCTOBER, 1946 No. 4

BIOTIN AND THE AVIDIN-BIOTIN COMPLEX

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The present review is an attempt to bring together the available biological information regarding biotin and the avidin-biotin complex. The chemical aspect of this subject has been adequately treated in several recent reviews and will not be dealt with here (1, 2, 3).

Wildiers (4) first appreciated the requirement of certain strains of yeast for a trace factor, which he called "bios." Subsequent study indicated that the term "bios" referred to multiple factors affecting yeast growth. One of these factors was isolated by Kögl and Tonnis (5) and named "biotin."

Avidin is the substance which is considered to be responsible for the noxious effects of the ingestion of large amounts of egg white in several species of animals (6). It has also been called "anti-biotin" (7), but general usage has made avidin the more acceptable term, particularly since other substances having anti-biotin activity have been described (133), (20), (20a), (20b).

Methods of assay for biotin. In view of the quantitative requirement of numerous microorganisms for biotin, microbiological tests for this substance have been highly developed. Table 1 summarizes the essential features of the more commonly used tests. They all depend upon the same principle. The test organism is grown on a medium that will afford increasing bacterial growth as additional amounts of biotin are added. Optimal growth must be achieved upon the addition of an excess of biotin. The growth obtained may be measured turbidimetrically or acidimetrically.

The preparation of the sample for assay remains one of the most difficult steps in testing for this potent growth factor. Lampen et al. (17) have emphasized the occurrence of biotin in bound form in most natural materials. Various methods have been devised for the release of bound biotin since the vitamin is available to the test organism only in the free state. Autolysis, enzymatic digestion, and acid hydrolysis are the chief methods thus far employed, and none of them is universally applicable (8, 13, 16). Incomplete liberation by too mild a treatment and possible destruction by more drastic procedures must be carefully avoided. The shortcomings of such extraction procedures are reflected in the widely differing assay results reported for many tissues and foods (8, 11, 12, 17).

Interpretation of the microbiological results is further complicated by the existence of several biotinlike factors which support the growth of the several test organisms to varying degrees. Burk and Winzler have referred to these growth factors as "vitamers" and have indicated differences both in their heat stability and in their respective potencies for several organisms (18). Moreover, the diaminocarboxylic acid derived from biotin, the natural occurrence of which

is totally unknown, has been shown to have 10 per cent of the yeast-growth activity of biotin itself (19). A considerable series of biotin derivatives, which are discussed later, have been shown to have distinctive potencies for several different test organisms (20, 20a, 20b, 124–128a). Accordingly, a microbiological determination of biotin in a natural material is merely a resultant of numerous growth effects and represents only that proportion of the total complex of biotin and biotinlike substances which has been rendered available to the test organism employed.

Animal assays for biotin have been largely neglected because of the greater convenience and sensitivity of the microbiological methods. However, Gyorgy and his associates (21–23) successfully employed the egg-white-injured rat as a test animal in their earlier studies of the properties of vitamin H. In addition, Ansbacher and Landy (24) prepared a deficiency-producing diet for the chick

TABLE 1

Microbiological methods for biotin assay

TEST ORGANISM	RANGE IN MILLI-MICROGRAMS	METHOD OF READING	ERROR	REFERENCE
			Per cent	
Saccharomyces cervisiae	0.002–1.0	Turbidimetric		(8)
Saccharomyces cerevisiae no. 139	1.0–0.5	Turbidimetric	5	(9)
Lactobacillus arabinosus	0.25–1.5	Acidimetric		(10)
				(11)
Lactobacillus casei	40–1000	Acidimetric	10	(12)
				(14)
Lactobacillus casei	100–300	Acidimetric		(13)
Clostridium butylicum	0.01–?	Turbidimetric	20	(15
				17
Rhizobium trifolii	0.016–?	Turbidimetric		01

which could be used for assay purposes. A study of the comparative biological potency of natural material containing biotin as determined by the available animal and microbiological methods would probably afford some information regarding the various forms in which biotin may occur in nature. Similar studies in relation to pyridoxine and folic acid have led to the recognition of the several forms in which these vitamins may be found (25, 26).

Occurrence of biotin. In spite of the sensitivity of the microbiological tests, our knowledge of the actual distribution of biotin in nature is not precise. The difficulties of adequately extracting the entire biotin content from tissues and foods have led observers to accept the highest values obtained as the true measure of biotin content. For the reasons already discussed, these determinations can be regarded as only gross approximations until more is known concerning the conditions for optimal release of biotin from the bound form. Several tabulations of such determinations of biotin in a wide variety of natural materials are available (8, 11, 12, 16–18). Biotin is found in varying degree in almost all substances

tested. The high content of biotin in liver and yeast indicates its close relationship to the B-complex, an observation actually made by Boas (27) in her early studies on egg-white injury.

The presence of biotin in several highly purified natural materials warrants special consideration. Hoagland et al. (28) have shown that the elementary bodies of vaccinia contain both free and bound biotin in approximately the same amount as that found in such rich sources as egg yolk and liver. Sprince and Schoenbach (29) reported that highly purified concentrates of the tobacco mosaic virus protein contained no biotin. Miller et al. (30a) found considerable amounts of bound biotin in numerous preparations of crystalline enzymes, but the concentrations exceeded by far the expected amount of biotin, assuming an equimolar ratio of biotin to protein. This suggested that the demonstrated biotin is presumably not an integral part of the enzyme molecule. Williams et al. (30) found that biotin as well as the other B-complex factors were present in numerous preparations of presumably pure proteins, including several crystalline enzymes and viruses. The data do not determine whether biotin is an integral part of these substances or is simply present as a trace contaminant. In any event, the microbiological determination of biotin and the other B-complex factors may be utilized as a highly sensitive indicator of the purity of such biological preparations (30a).

The occurrence of biotin in numerous phyla has been reported by Woods et al. (31). This almost universal distribution of biotin further emphasizes its basic biological significance.

Biotin and avidin in nutrition and reproduction. Bond (32) reported that rats failed to grow when fed a diet containing 30 per cent dried egg white.

In 1924 Boas called further attention to the peculiar nutritive properties of dried egg white (33, 34). Her thorough description of the observable features of egg white injury in the rat includes virtually all that has been noted up to the present time.

> The rats grow well and are usually in good health for from 2 to 3 weeks. Then red scaly patches appear at the corners of the mouth, the coat becomes rough and sticky and the long hairs fall out. The fur on the abdomen shows at first a characteristic ribbed appearance, followed by the development of bald areas. Meanwhile the red patches spread to other parts of the body and the picture is one of an eczematous dermatitis. There are even skin hemorrhages in severe cases. The region round the mouth is always the most severely affected though there is often such marked blepharitis that the eyes are closed. The loss of hair is often extensive. In a few cases oedema of the feet has been seen but this does not usually occur. These rats always have a distinctive, somewhat musty smell, probably due to some constituent of the urine. The body weight remains stationary for a week or two, but falls slowly during the second stage of the disease. This is reached about 2 to 3 weeks after the development of the first signs of deficiency. To the dermatitis, symptoms of nervous upset are now added. There is pronounced spasticity of the limbs, particularly of the hind legs, and the back is arched. The rat assumes in many cases a kangaroo-like posture. . . . Some of the rats do not show marked spasticity but assume a crouching attitude and display a curious swimming movement with the front paws. Death, which occurs in the final phase, is preceded by a rapid loss of weight, and the animal shows signs of extreme cyanosis. Rigor mortis sets in rapidly. Post mortem, there is an almost complete absence of fat and the skin is infiltrated and vascularized.

The ability of certain foods to protect the rat against egg white injury was attributed by Boas to a "protective factor" (34). She concluded that the drying process rendered egg albumin toxic (34). Friedberger et al. (35, 36) claimed that in rats fed cooked eggs the toxic effects were more marked and occurred earlier than in those fed raw eggs. Contrarily, Scheunert et al. (37, 38) and Stenquist (39) reported a marked toxic effect in rats fed raw eggs and normal growth in those receiving cooked eggs. The basis for the discrepancy between these authors' findings has not been determined.

Findlay and Stern (50) also described egg white injury in the rat and suggested its similarity to "pink disease" in children.

Parsons and Kelly (40, 41) demonstrated the heat lability of the toxic factor in egg white and found that it persisted in egg white denatured by alcohol and leached in running water. The toxic effects were reversed by feeding liver or kidney and less readily by dried milk, wheat germ, dried yeast, and dried egg yolk (41). Salmon and Goodman (42) also described the toxic effects of feeding rats egg white, either fresh or dried. Lease, Parsons and Kelly described egg white injury in the chick, rabbit, monkey and guinea pig as well as in the rat (43). Further studies by Parsons and her associates extended our knowledge of the anti-egg-white-injury factor and of its distribution in various foods (44–49).

Boas (27) called attention to the similar distribution of the "protective factor X" and the factors of the B-complex. Gyorgy (51, 52) emphasized the vitaminlike character of this factor by referring to it as "Vitamin-H", a term derived from the German word "Haut" for skin. This concept guided the extended studies of Gyorgy and his associates concerning the chemical character and natural distribution of vitamin H (21–23, 56). This increasing body of descriptive and quantitative data led to the ultimate proof of the identity of vitamin H and biotin (57, 58, 59).

Nilsson et al. (60) and West and Wilson (61) meanwhile had suggested from comparative quantitative data on potent concentrates that coenzyme R (62) is identical with biotin. This was subsequently substantiated by studies reported by du Vigneaud and his associates (1).

The mechanism whereby egg white injury induces a biotin deficiency has been the subject of several investigations. Parsons et al. (63) found that the feces of rats suffering from egg-white injury proved curative of this injury only after heating. They "suggested as a tentative hypothesis that raw egg white derives its essential capacity to produce a pathological condition not from its own possible lack of the protective factor in available form nor from the presence of a hypothetical anti-enzyme but from its characteristic in the digestive tract of combining with and holding in an unabsorbable form the protective factor which originated either from the diet alone or, also from an excretion into the digestive tract." Eakin et al. (64) showed that an egg-white-injury-producing diet for chicks contained even more biotin than did a control diet containing casein, but that the tissues of chicks maintained on the former contained less biotin than the controls. They concluded that egg-white injury is attributable to the capacity of egg white to render the dietary biotin unavailable to the tissues

of the body. Eakin et al. (65) found further that a constituent of raw egg white is capable of rendering biotin unavailable to yeast cells in vitro, and that heating effected a release of the biotin for the support of yeast growth. Gyorgy et al. (66) then showed a direct correlation between the capacity of an egg-white preparation to produce egg-white injury in the rat and its effectiveness in combining with biotin in vitro. The active constituent of egg white has accordingly been called "avidin" which is a contracted form of the original "avid albumin", a term signifying its active affinity for biotin (6).

The nature of avidin and the character of the combination of avidin with biotin have been the subject of considerable study. Gyorgy and Rose (67) observed that when avidin concentrates were administered parenterally they had a decisive therapeutic effect upon egg-white injury. This effect was attributed to the bound biotin content of the avidin concentrates which presumably became available after injection. Gyorgy, Rose and Tomarelli (68) reported the sensitivity of avidin to ultraviolet light and to acidification at pH 1.8, calling particular attention to the fact that the presence of the avidin-biotin complex tended to stabilize any excess avidin present during such acidification. Gyorgy and Rose (69) further found that the avidin-biotin complex resists treatment with proteolytic enzymes including pepsin, trypsin, pancreatin and papain. In addition, incubation with preparations of liver, kidney, muscle or blood had no effect. However, about 10 to 20 per cent of the biotin could be liberated in a form which was dialyzable and would support yeast growth by treatment at pH 3 with 0.45 per cent H_2O_2. These workers therefore feel that the metabolism of biotin and avidin is more intimately related to oxidation-reduction systems than to digestive enzymes.

It should be emphasized that not all substances capable of supporting yeast growth in the absence of biotin are avidin-combinable. Thus, du Vigneaud et al. (70) prepared a diamino-carboxylic acid from biotin which supports yeast growth but is not inhibited by avidin, indicating that the urea ring is presumably essential for the formation of the avidin-biotin complex. Dittmer and du Vigneaud (20a) have shown that shortening of the side chain of desthiobiotin eliminates the avidin-combining power of the derived compounds. Oppel (71) detected the presence of an avidin-uncombinable substance in the urine of the dog, rabbit, rat and man. Burk and Winzler (18) have determined the relative proportion of yeast supporting activity of various natural materials which is not combinable with avidin. Chu and Williams have employed different testing conditions and conclude that avidin-uncombinable factors are found only in urine (18a).

Woolley and Longsworth (7) pointed out that if yeast cells are permitted exposure to a biotin-containing medium for 6 hours, the subsequent addition of avidin would be without effect upon their further growth. They interpreted this phenomenon as indicating that the absorption of the biotin into the yeast cell rendered it unavailable to the avidin. Hertz (72) has confirmed this observation and has found in addition that the acid-extractable biotin content of a 6 hour yeast culture remains avidin-combinable.

Some information concerning the chemical character of avidin has been ac-

quired. Parsons and Kelly (41) found that saturation with ammonium sulfate precipitated the active material and that it was absent from purified ovalbumen. Eakin et al. (65, 73) prepared potent concentrates by ammonium sulfate fractionation of the acetone precipitable portion of fresh egg white. Pennington, Snell and Eakin (74) prepared biologically active crystals, the chemical behavior of which indicated that the "substance may be a protein with a large carbohydrate moiety."

Woolley and Longsworth (7) derived from raw egg white an amorphous fraction which they regarded as a single substance, as evidenced by homogeneity in electrophoresis and in sedimentation determinations. The substance was 15,000 times as active as egg white and proved to be a basic protein with an isoelectric point at pH 10, and an estimated molecular weight of 70,000. Curiously, the pure substance retained activity after boiling in dilute acetic acid and was stable at room temperature in solution at a pH ranging from 1.0 to 11.0.

The distribution of avidin in nature has been studied to a limited extent. Gyorgy and Rose (75) determined that the amount of avidin present in the average hen's egg is sufficient to more than completely neutralize its biotin content. Woolley and Longsworth (7) detected traces of anti-biotin activity in certain purified basic proteins. Hertz and Sebrell (76) found relatively high avidin titres in the eggs of not only other birds such as the turkey, duck and goose, but also in the egg jelly of two species of frogs. They emphasized the fact that the egg albumen represents a secretion from the mucosal lining of the oviduct and demonstrated the presence of avidin in the oviduct tissue of the hen and frog (76).

Fraps, Hertz and Sebrell (77) found that avidin is present only in the albumen-secreting portion of the oviduct. Moreover, the oviduct of the nonlaying hen was free of avidin, indicating that avidin production may depend upon ovarian function. Hertz, Fraps and Sebrell (78, 79) further demonstrated that avidin secretion could be induced in the oviduct of the newborn chick by combined treatment with stilbestrol and either progesterone, desoxycorticosterone acetate, or testosterone propionate. Hertz (72) has tested mucosal scrapings from the oviducts of pig, cow and guinea pig for avidin with entirely negative results.

The suggested rôle of avidin and biotin in the physiology of reproduction remains unclarified, but several additional studies in this connection should be cited. Cravens et al. (80) found that the low hatchability of eggs from hens maintained on a low-biotin diet could be overcome by adding biotin-containing concentrates to the diet. Kennedy and Palmer (81) have shown that egg-white injury in the rat does not interfere with conception but leads to frequent fetal resorption and impaired lactation. Snell and Quarles (82) showed that the biotin content of the hen's egg remains almost constant throughout incubation. Singher et al. (83) found that the liver of the biotin-deficient rat retains its capacity to inactivate estradiol in vitro. These studies are as yet too diverse to permit any generalizations to be made from them.

The possible relationship between avidin and other biologically active principles found in egg white has been investigated. Laurence (53) reported a remark-

able correspondence between the lysozyme activity and avidin potency of seven egg-white concentrates. Meyer (54) reported similar results and stated further that the addition of biotin markedly enhances the lysozyme activity of egg-white preparations. However, Landy (55) had previously indicated a lack of correlation between lysozyme and avidin activity. Woolley and Longsworth (7) also noted that their pure avidin preparations lacked lytic power. Alderton et al. (84) reported that pure lysozyme not only failed to inactivate biotin but also that the addition of biotin did not enhance its potency. In addition, highly purified avidin concentrates showed no lysozyme activity. The more cogent evidence indicates, therefore, that avidin and lysozyme are distinct substances and that biotin is not involved in the lysozyme effect.

Biotin is known to be required by numerous species in addition to the rat. Norris and Ringrose (85) produced a dermatitis in chick on both a purified diet and one containing egg white. Lease and Parsons (47) also observed a marked dermatitis in chicks fed egg white. This chick dermatitis was more specifically attributed to biotin deficiency by Hegsted et al. (86), who were able to cure severe skin lesions in chicks fed a purified diet by feeding small amounts of biotin concentrates. Ansbacher and Landy (24), employing a heated ration, were able to cure the deficiency syndrome by administering crystalline biotin. Hegsted et al. (87) subsequently reported similar results with pure biotin.

Perosis has been attributed to a biotin deficiency in both chicks (88) and turkey poults (89). Richardson, Hogan and Miller (90) have indicated that perosis in chicks may be a nonspecific effect of several nutritional deficiencies in addition to biotin deficiency. Similar conclusions were reached by Patrick et al. (91) regarding perosis in turkey poults.

Among the rodents, the golden hamster has been shown to require biotin for optimal growth and reproduction (92). However, the cotton rat is able to grow on a highly purified diet without added biotin (93). Reference has already been made to the observations of Lease et al. upon egg-white injury in the rabbit and guinea pig (43).

Man and the *Macaca rhesus* monkey are the only primates whose biotin requirement has been studied. Lease, Parsons and Kelly observed a characteristic dermatitis and general debility in monkeys fed a diet containing egg white (43). Waisman and Elvejhem noted a beneficial effect of biotin upon the hair coat and skin of monkeys maintained on a purified diet (94).

Oppel (71, 95, 96) has determined the urinary and fecal excretion of biotin in man under varying conditions. The normal adult excreted in the urine from 7 to 89 micrograms of biotin daily. Ingestion of a crude biotin concentrate was promptly reflected in a marked rise in urinary output. No correlation could be made between any of the numerous disease conditions studied and the urinary output of biotin. The fecal excretion of biotin was independent of the dietary biotin and exceeded it in most instances. The occurrence in human urine of an avidin-uncombinable substance which will support yeast growth has already been mentioned (71, 18).

Gardner et al. (96a) followed the biotin excretion in 3 normal young women on

a milk diet. The urinary biotin ranged from 40 to 57 micrograms daily and the fecal biotin was in the same range. In two of the individuals the output exceeded the intake.[1] These findings are in agreement with those of Oppel (96).

Sydenstricker et al. (97, 98) fed an egg-white-containing diet to volunteers for prolonged periods of time. After 3 to 4 weeks a transient dermatitis was observed. After 5 weeks the following clinical changes were noted: lassitude, somnolence, muscle pains, hyperesthesia and anorexia. Administration of a biotin concentrate, in amounts ranging from 150 to 300 micrograms per day relieved the patients of these symptoms in 3 to 5 days. The urinary excretion level of these patients fell gradually while they were on the experimental diet and rose abruptly upon the administration of the biotin concentrate.

Williams (99) presented a detailed case report of an individual presenting a dermatitis associated with a history of having subsisted on raw eggs and wine for many years. The dermatitis cleared while the patient was on a hospital diet but could not be reproduced on a diet of raw eggs, presumably because of biotin contributed by organisms harbored in the individual's infected urinary tract. The case is an interesting clinical curiosity but is not to be considered a proved instance of human biotin deficiency.

Fraenkel and Blewett showed that the larvae of the beetle, *Tribolium confusum*, require biotin when grown on an artificial diet (100, 101, 102). Rosenthal and Reichstein reported similar observations on the same organism (103).

The microscopic pathology from egg-white injury in the rat has been described in a series of studies by Sullivan et al. (104, 105, 106). In the skin, hyperkeratosis, parakeratosis, acanthosis and edema were observed. The nervous system was found to show no change, and the hypertonicity was considered to be associated with pathological muscle changes resembling those seen in vitamin E deficiency. Shaw and Phillips (107) also reported no changes in the nervous system of egg-white-injured rats and described the muscles as grossly small and pale, with microscopic evidence of atrophy but not of true degeneration.

McHenry and Gavin (108) found that a liver fraction which was rich in biotin produced fatty livers in rats. Subsequent study (109) showed that pure biotin was also effective and that inositol and lipocaic both counteracted this type of fat deposition (110). Later, MacFarland and McHenry (110a) reported that the biotin type of fatty liver which had been previously characterized as resistant to the lypotropic action of choline was in fact only partially resistant to choline. The more complete choline-resistance induced by the administration of a liver-fraction was therefore attributed to a factor other than biotin and this special type of fatty liver was shown to be peculiarly responsive to inositol. Best et al. (110b) have concluded that the response to inositol and choline of the "biotin fatty liver" is not distinctive in character. Moreover, the apparent greater responsiveness of the liver-fraction-induced-fat to inositol is regarded by them as a synergistic lypotropic effect of inositol with the choline known to be contained

[1] Gardner, J., Parsons, H. T. and Peterson, W. H. (Arch. Biochem. 8, 339, 1945) have extended these studies in ten subjects with similar results.

in the liver fraction employed. This interpretation seems to account more adequately for the data presented.

The effects of biotin on other specific tissues have received some study. Fischer (111) noted no effect of excess biotin either upon the rate of atrophy of the denervated gastrocnemius muscle or upon the return of function following crushing of the sciatic nerve in the rat. Lazere et al. (112) similarly reported no effect of biotin deficiency in the rat upon the usual results of crushing the tibial nerve. Hamilton and Plotz (113) found that 65 per cent of explants of nerve tissue grown in vitro showed stimulating effects of biotin when added to a medium containing plasma, embryonic extract and Tyrode solution. Contrarily, Burt (114) observed no growth-stimulating effect of biotin on nerve cells from chick embryos in tissue culture.

Boas (27) and numerous other observers have noted neurological changes in the hind limbs of rats subjected to egg-white injury. Nielsen and Elvehjem (115) reported a prompt relief of the paralysis exhibited by biotin-deficient rats following biotin administration. Smith (116) has described a progressive paralysis in dogs maintained on a purified diet supplemented with eight members of the B-complex and excluding biotin. Prodromal features of the paralysis include loss of co-ordination, weakness, and a transient generalized paralysis from which the animal recovers. The final paralysis is cured in a few hours by administration of biotin in doses as low as 100 micrograms per kilogram. The syndrome is also characterized by cardiac failure which in some cases leads to sudden death.[2]

Schmidt and Landy's completely negative findings on the effect of biotin on the blood pressure, heart rate, and respiration of anesthetized cats indicate that biotin lacks marked pharmacodynamic effect in the normal animal (117).

The influence of biotin upon the susceptibility to and the course of infection has been studied to a limited extent. Trager (118, 119) found that biotin-deficient chicks and ducks exhibit an increased susceptibility to certain strains of malaria, as evidenced either by a higher peak parasite count or a prolonged persistence of high parasite counts in the peripheral blood. Moreover, the biotin level of the red blood cells and of the plasma of *P. lophurae*-infected chicks and ducks rose as the infection advanced and fell as the infection subsided. In ducks this effect preceded any increase in circulating young red cells. Control birds suffering from severe pantothenic acid deficiency showed no alteration in the character of the disease following inoculation with *P. lophurae*. These findings were interpreted as indicating a specific rôle for biotin in the resistance of birds to malarial infection. This conclusion received considerable support from confirmatory data of Seeler, Ott and Gundel (119a), who reported that chicks on an egg-white diet showed significantly higher parasite counts after inoculation with *P. lophurae* than did control chicks receiving the same diet plus biotin.

Caldwell and Gyorgy (120) reported a significant prolongation of the duration

[2] Reugamer, W. R., Elvehjem, C. A. and Hart, E. B. (Proc. Soc. Exp. Biol. & Med. 61, 234, 1946) have presented evidence indicating that this syndrome may be attributable to potassium deficiency.

of infection with *Trypanosoma lewisi* in the biotin-deficient rat. This effect could be reversed by the administration of biotin even during the course of the disease.

No effect was noted upon the susceptibility to either Lansing strain poliomyelitis or to Theiler's encephalomyelitis of mice rendered deficient in either biotin, pyridoxine or inositol (120a).

Although reference has already been made to existing reviews covering the chemistry of biotin (1, 2, 3), mention should be made of the recently accumulated data concerning the biological effects of compounds closely related to biotin. Emerson (121) employed a therapeutic test in the egg-white-injured rat and found that *dl*-biotin was half as active as biotin and that *l*-biotin was inactive when fed at 7.5 times the level of biotin. In addition, *dl*-allobiotin, the diaminocarboxylic acid, *dl*-diamino acid, *dl*-allodiamino acid, and *dl*-epiallo-diamino acid derivatives were all inactive at high levels. Ott (122) found that *l*-biotin and *dl*-allobiotin were without effect in preventing dermatitis, perosis, and growth failure in chicks fed a purified diet containing 15 per cent dried egg white. In rats rendered biotin deficient by feeding either egg white or succinylsulfathiazole, *dl*-desthiobiotin is reported to have only 0.1 to 0.01 per cent activity in comparison with *d*-biotin (123).

Duschinsky et al. (124) and Hofmann (125) independently synthesized a biotin analog in which the sulfur was replaced by oxygen. This compound, *dl*-hexhydro-2-oxo-1-furo [3,4] imidazole-4-valeric-acid, was named oxybiotin by the latter group (125) and *o*-heterobiotin by the former (124). Pilgrim et al. (126) stated that this compound in *dl*-form is half as active as *d*-biotin for *Lactobacillus arabinosus* and 40 per cent as active as *d*-biotin for *L. casei*. For *S. cerevisiae*, this compound proved 25 per cent as active as *d*-biotin at half maximum growth, but only 8 per cent as active at maximum growth. Hofmann et al. reported further that this compound possesses therapeutic potency in egg-white injury in rats and both therapeutic and prophylactic effectiveness in biotin deficiency in chicks (128). Rubin et al. (127) reported similar findings except that the activity for *Lactobacillus casei* was found to be only 25 per cent in comparison with *d*-biotin. In addition, this growth effect for *L. casei* was found to be inhibited by desthiobiotin and inferential evidence was presented indicating that the utilization of this compound by yeast involves its conversion to biotin or to a compound of similar activity for yeast growth. They also estimated this substance to be 5 per cent as active as *d*-biotin in curing egg-white injury in rats and showed that it combines with avidin in vitro (127).

Hofmann and Winnick (128a) developed a differential test for oxybiotin as opposed to biotin based on the sensitivity of the latter to treatment with dilute potassium permanganate. By means of this test they showed that *Saccharomyces cerevisiae* and *Rhizobium trifolii* utilize oxybiotin without converting it to a compound which is sensitive to permanganate treatment. Control cultures grown on biotin or on desthiobiotin contain only a permanganate-sensitive substance. The basis for the discrepancy between these findings and the data of Rubin et al. (127) indicating a conversion of oxybiotin to biotin by yeast cells remains obscure.

Additional studies concerning the effects of biotin, desthiobiotin, and related compounds in bacterial and cellular metabolism are discussed in the recent comprehensive review of Knight (133).

More recently, Tatum (134) demonstrated the synthesis by *Penicillium chrysogenum* of a substance whose biological effects are identical with those of desthiobiotin. Pimelic acid, but not cystine, enhanced the synthesis of this substance.

Numerous investigations have been devoted to the problem of synthesis of biotin and of other B-complex factors by the intestinal flora. These studies have been reviewed in detail by Daft and Sebrell (129) and by Najaar and Barrett (130). It is particularly noteworthy that in the rat a syndrome attributable in part to biotin deficiency can be produced by the inclusion in the diet of one of several sulfonamide compounds which presumably inhibit the synthesis of biotin by the intestinal bacteria (129a, 129b, 129c).

The rôle of biotin and avidin in cancer is under study in several laboratories, and this aspect of the work was recently reviewed by Burk and Winzler (131). Subsequently, Kline, Miller and Rusch (132) reported that the feeding of dried egg white at a 12 per cent level in a purified diet containing suboptimal riboflavin resulted in a reduction in occurrence of hepatomas induced with butter yellow in the rat from a control incidence of from 77 to 82 per cent to from 18 per cent to zero. Either heating the egg-white or adding biotin to the diet failed to alter the protective effect of the diet containing egg-white although the characteristic effects of biotin deficiency were prevented by both procedures. This report reopens the problem of the mechanism of the protective effect exerted by egg white.

A number of significant studies concerning the effects of biotin upon cellular metabolism have been reported. In their early observations Allison, Hoover and Burk (136) appreciated the remarkable stimulus to the respiration of rhizobium afforded by concentrates of the factor which they accordingly termed coenzyme R and which was subsequently shown to be identical with biotin (60, 61). Allison and Hoover later found that the response of rhizobium respiration to coenzyme R depended upon the presence of assimilable nitrogen (137). Burk, Winzler and du Vigneaud (138, 139) have shown that biotin deficient yeasts similarly require ammonia or some other nitrogen source in order to respond to biotin. They also found that the effect of biotin upon biotin-deficient yeast is characterized by a prompt rise in fermentation within a few minutes. This is followed after an hour by a similar increase in respiration, but the growth response is not apparent for several hours. The biotin uptake of deficient yeast was shown to be rapid even in the absence of ammonia, but ammonia was not absorbed in the absence of biotin. There was neither destruction nor synthesis of biotin accompanying yeast growth.

Burk and Winzler (18) have suggested a possible chemical relationship between the naturally occurring avidin-uncombinable form of biotin and the diaminocarboxylic acid derived from biotin which is also avidin uncombinable (19). These authors have postulated that such a substance could function as a coenzyme of CO_2 transfer by a reversible conversion to an avidin-combinable compound having a closed urea ring structure similar to biotin.

Pilgrim et al. (140) reported a marked depression of pyruvate oxidation in liver homogenates taken from both biotin-deficient as well as pantothenic-acid-deficient rats. Subsequently Summerson et al. (141) studied the effects of biotin on the in vitro metabolism of tissues from rats which had been subjected to egg-white injury. In liver slices from such animals they observed a slight rise in O_2 consumption and R.Q., and a very marked increase in the utilization of both lactate and pyruvate. The heart and brain preparations from the same animals showed no response to the addition of biotin. Assay values given for the liver slices indicated a substantial reduction in biotin content, but no biotin values are given for the heart and brain tissues studied.

General considerations. The studies leading from Boas' original description of egg-white injury (27) to our present knowledge of the subject have constituted a most remarkable research achievement. First, the recognition of the egg-white effect as a deficiency syndrome led to an appreciation of the existence of a protective dietary factor (27, 51, 52). Persistent study of the nature of this factor indicated its vitaminlike character and something of its chemical and physical properties (40–41, 56–62). The identification of vitamin H with biotin and coenzyme R is one of those rare correlations which bring together segments of information that might otherwise remain unco-ordinated for many years.

The next step forward was the demonstration that avidin exerts its characteristic effect by forming an unabsorbable complex with biotin (63–66). Such a mechanism for the production of a toxic effect had not been previously encountered. It will undoubtedly serve as a basis for many future investigations in toxicology and nutrition.

It should be emphasized that avidin is a normally occurring basic protein sustance found at least in certain specialized tissues of the genital tract in species known to require biotin (7, 74, 76–79; 87, 24). Moreover, it is hardly to be taken as coincidental that egg-yolk is a good source of biotin, while egg albumen has the peculiar property of rendering biotin ineffective (5). Thus, a distinct metabolic relationship between avidin and biotin is suggested and the affinity of this tissue protein for a highly significant metabolite is to be regarded as more than a curious toxic effect.

Although our survey has included numerous descriptions of specific biological effects of biotin and of biotin deficiency, these diverse observations give us little indication of the basic metabolic function of biotin. Yet its universal distribution in living cells, its profound effects upon bacterial and tissue growth, and its very high level of biological potency per unit weight all suggest a fundamental rôle which is yet to be elucidated.

The immediate applicability of our knowledge of biotin and avidin to clinical or technological problems is also obscure. Interesting leads have been cited regarding the possible rôle of biotin in resistance to disease (118–120). The clinical usefulness of egg white as a bacteriostatic agent has been reported (135), but such effects may be attributable to lysozyme or other anti-bacterial agents rather than to avidin. The suggested relationship between biotin and cancer

(131) is too tenuous to command early clinical consideration. Attempts are being made to relate clinical seborrheic dermatitis to the skin changes seen in egg-white injury, but no definitive data are as yet available. Synthetic biotin analogs which might function as specific anti-metabolites have far-reaching therapeutic potentialities (20, 20a, 20b, 133).

Despite the considerable body of data already at hand, the avidin-biotin problem offers a stimulating challenge for further study.

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Ivanov-'66
Bio No. 627

EFFECT OF BIOTIN ON TISSUE RESPIRATION OF CERTAIN ORGANS OF ALBINO RATS WITH EXPERIMENTAL HYPERCHOLESTEREMIA

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(UDC 616.153.922.01-085.3:577.164.18-07:616-008.922.1-074)

The effect of biotin on the tissue respiration of the organs of normal animals, and more especially in pathological conditions, has been inadequately studied. For this reason experiments were carried out to study the tissue respiration of certain organs of albino rats under the influence of biotin in normal and pathological conditions. The first experiments showed that biotin stimulates the tissue respiration of all investigated organs [1]. The pathological condition chosen was experimental hypercholesteremia, in which the intensity of the tissue respiration is depressed [2].

EXPERIMENTAL METHOD

Altogether 40 experiments were carried out on 40 albino rats weighing 162-208 g. The tissue respiration was measured in the following organs: the heart, brain, liver, kidneys, spleen, and muscle tissue. The rats were decapitated, and the tissue to be investigated was quickly extracted and miced in the cold. A sample of tissue weighing 100 mg was placed in a Warburg's flask and 2 ml of Ringer's solution (pH 7.35) were added. The tissue respiration was determined by the usual manometric method in an atmosphere of oxygen at 37.5°. The index of intensity of the tissue respiration was the volume of oxygen in cubic millimeters absorbed per hour per milligram of tissue dried to constant weight.

In the experiments of series I the effect of biotin was studied on the tissue respiration of the organs in normal conditions. The biotin solution was injected subcutaneously in a dose of 0.3 μg/100 g body weight/day for 20 days. The controls were rats kept in the same conditions but not receiving biotin. In series II experimental hypercholesteremia was caused in the albino rats by feeding them on cholesterol in a dose of 0.5 g/kg body weight with meat and fat for 120 days. Under these conditions the total cholesterol concentration in the blood serum rose on the average to 123 mg %. After the 120th day the rats began to receive subcutaneous injections of biotin in a dose of 0.3 μg/100 g body weight once daily for 20 days. The controls were rats with experimental hypercholesteremia not receiving biotin.

EXPERIMENTAL RESULTS

Initially the tissue respiration of the organs of normal rats was studied after subcutaneous administration of biotin for 20 days. Analysis of the results obtained showed that in these conditions the absorption of oxygen by the heart in the first hour of observation increased to 18.5±0.9 (compared with the normal 6.7±0.46), or by 276%, absorption by the brain—to 11.6±0.8 (normal 4.8±0.42), or by 241%, by the liver—to 28.7±1.7 (normal 5.3±0.42), or by 541% by the kidneys—to 13.2±1.1 (normal 8.4±0.5), or by 157%, and by the spleen—to 14.2±0.7 (normal 6.7±0.4), or by 211% of the normal value. Similar results were obtained during the study of the intensity of the tissue respiration at the second hour of observation. The absorption of oxygen by the heart increased to 13.4±0.9 (in the control 3.1±0.3), or by 432%, by the brain—to 8.6±0.5 (control 2.8±0.1), or by 307%, by the liver—to 24.8±1.5 (control 3.2±0.3), or by 775%, by the kidneys—to 11.0±0.8 (control 6.3±0.4), or by 174%, and by the spleen—to 11.1±0.7 (control 4.1±0.4), or by 264% (of the initial level).

In chronic experimental conditions biotin thus stimulated the tissue respiration of all the investigated organs. The increase in the absorption of oxygen by all the organs was statistically significant.

In the next series of experiments the effect of biotin was studied on the tissue respiration of the organs of rats with experimental hypercholesteremia. In these conditions biotin stimulated the absorption of oxygen by the heart to 15.8±0.4 (in the control 3.6±0.08), or by 338%, by the brain—to 7.1±0.4 (control 2.5±0.1), or by 184%, by the liver—to 19.0±0.6 (control 6.8±0.1), or by 179%, by the kidneys—to 8.9±0.6 (control 5.0±0.4), or by 78%, by the

Department of Biochemistry, Chita Medical Institute (Presented by Academician V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 11, pp. 57-58, November, 1966. Original article submitted December 28, 1964.

spleen—to 10.2± 0.8 (control 2.6± 0.1), or by 292%, and in muscle tissue—to 3.6± 0.32 (control 1.4± 0.4), or by 157%. All the differences are statistically significant.

Hence, in the conditions of experimental hypercholesteremia, biotin also stimulates the processes of biological oxidation.

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Keller-'42
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BIOTIN AND SKIN SENSITIVITY

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RECENT reports have appeared in the literature relative to hypersensitivity of given individuals to certain members of the vitamin B complex.[1] As far as is known, a skin contact reaction of the wheal and erythema type, following contact with biotin, has not as yet been recorded in the literature. Such a reaction was suspected in a laboratory worker and has subsequently been investigated.

This individual noticed red, itching patches on her arms and hands whenever mixing a dry dusty diet containing butter yellow and known members of the vitamin B complex. There were slightly raised areas in the center of the red patches which lasted about half an hour. Since this individual had been previously and repeatedly exposed to all the elements in the diet except biotin, this substance was suspected.

Accordingly, skin patch tests were employed, using the factors contacted by this individual when handling biotin. The substances tested were (1) rice and 3 per cent butter yellow in olive oil; (2) rice and the B complex including thiamine Cl, choline chloride, B_2, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, and biotin; (3) crystalline nicotinic acid; and (4) biotin concentrate.*

Since biotin gave a reaction within two hours, the above test substances were removed at the end of that time, the other substances thus serving as controls. The first test substance gave no skin reaction at all after two hours of application. The second, B complex, containing biotin, produced an area of erythema and central edema that lasted ten hours. The crystalline nicotinic acid produced an area of erythema without edema which subsided completely in an hour. The biotin concentrate, however, produced after the same period of contact a central, firm, raised, pale area with a surrounding zone of spreading erythema. This raised area was tense and shiny and persisted more than an hour after all redness in the other areas had vanished. At the end of thirty-six hours the central area was grayish tan and tender on palpation.

To rule out directly the other substances handled, as the offending agents, skin patch tests were done with thiamine chloride, riboflavin, pantothenic acid, vitamin B_6, 70 per cent alcohol, and 25 per cent hydrochloric acid (since most of the substances were tested as chlorides). All of these substances failed to produce a skin reaction after two hours of direct skin contact.

From The Lankenau Hospital Research Institute, Philadelphia.
*Concentrate No. 200, S. M. A. Corporation.

It should be noted that this individual did not get a reaction to biotin unless the skin had previously been cleansed with 70 per cent alcohol or unless nicotinic acid was present in the mixture; nor was itching noted at the site of reaction when biotin alone was applied to the cleansed skin surface. However, in conjunction with nicotinic acid, itching was present. It was further observed that biotin, in conjunction with nicotinic acid, applied to the uncleansed skin produced a reaction of the wheal and erythema type accompanied by itching. This, however, was not observed if the individual previously had applied a commercial cream to the exposed area. This latter fact was observed when measures were undertaken so that the individual could continue her work with biotin.

Skin tests were next performed with 25 μg of crystalline biotin dissolved in 1 c.c. of water. This solution was used as stock and half of it diluted serially by taking half the cubic centimeter of stock and adding 0.5 c.c. of distilled sterile water. The resulting solution was treated in a similar manner and so on until seven dilutions were obtained, including the stock solution. Three drops of each solution were applied to designated areas on the skin of the forearm previously cleansed with alcohol and allowed to remain in contact for one hour. At the end of this time, the first four dilutions produced a wheal and erythema, the erythema being least marked in the second instance. In dilutions 5 and 6 erythema alone was noted, and in the dilution 7 no reaction at all was observed.

SUMMARY

1. It has been shown that biotin can produce skin sensitivity in an individual as measured by the contact skin test.

2. Nicotinic acid apparently enhances the reaction on the uncleansed skin and exaggerates it (itching) on the cleansed and uncleansed skin surface.

3. It was further observed that the application of a commercial cream prevented a skin reaction from contact with biotin dust.

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1. Stiles, M. H.: Hypersensitivity to Thiamine Chloride, With a Note on Sensitivity to Pyridoxine Hydrochloride, J. ALLERGY 12: 507, 1941.

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Lee-'72
Bib No. 797

Jour. Nutr. 102(11):1453-1464. 1972.

[carbonyl]

Metabolism of Carbonyl-labeled ^{14}C-Biotin in the Rat[1,2]

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ABSTRACT Ureido carbonyl-labeled ^{14}C-biotin was injected intraperitoneally into young adult, male rats to study the excretion and degradation of the vitamin. After milligram quantities of the radioactive biotin were injected, the intact vitamin was found to be almost completely excreted in the urine within 24 hours. The initial excretion rate decreases with decreasing amounts of biotin administered. This initial decrease is even more marked with biotin-depleted rats. Biotin-depleted rats, treated with succinyl sulfathiazole and penicillin, were given a single injection of radioactive biotin (0.5 μg/100 g body weight). Urine was fractionated for radioactive biotin metabolites, four of which have been identified as follows: the *d*- and *l*-sulfoxides of biotin, bisnorbiotin, and a neutral ketone. No radioactive CO_2 and urea could be detected. Also, liver homogenates were incubated with carbonyl-labeled biotin at 37° for 40 or 60 minutes, with or without aeration. Under O_2-limiting conditions, the same four biotin metabolites were also found, but in different proportions. With aeration, the amount of biotin sulfoxides increased; also, trace amounts of radioactive CO_2 and urea were detected. Therefore, the rat has the ability to metabolize the vitamin via at least partial β-oxidation of the side chain and to effect some oxidation of the thioether sulfur to sulfoxides. Under physiological conditions, however, the metabolism of biotin in rats, unlike certain bacteria, does not include extensive cleavage of the ring structure of the molecule. J. Nutr. *102:* 1453–1464, 1972.

INDEXING KEY WORDS biotin · bisnorbiotin · biotin sulfoxides

Relatively little is known about the metabolic fate of *d*-biotin in mammals. The Frankel-Conrats (1) injected rats intraperitoneally with 10 to 20 μg of biotin labeled with ^{14}C in the ureido carbonyl function. They found that 85% of the administered radioactivity was excreted in the urine within 1 day. Less than half of the excreted radioactive material had biological activity with *Saccharomyces cerevisiae*. No expired $^{14}CO_2$ was detected. These results indicate that a metabolic alteration of biotin takes place in rats and that this alteration does not involve cleavage of the ureido ring of the vitamin. Baxter and Quastel (2) found formation of $^{14}CO_2$ from tissue slices of guinea pig renal cortex incubated with carboxyl-labeled ^{14}C-biotin. This finding suggested the possibility of β-oxidation of the biotin valeric acid side chain. The release of $^{14}CO_2$ from carboxyl-labeled ^{14}C-biotin in the rat was also noted by Schreier and Pfisterer (3) and by Dakshinamurti and Mistry (4). In several such early studies (1, 5, 6), biotin-like material, which did not complex well with avidin, was reported to be in human and rat urine. Burk and Winzler (7) claimed to have found in urine several biotin-related compounds, one of which was heat-labile and did not combine with avidin. Such results would indicate that metabolism of biotin in mammals involves cleavage of the ureido ring, since it is generally recognized that the formation of the avidin–biotin complex is dependent on an intact ureido carbonyl function (8). However, Wright et al. (9) found no evidence of an "avidin-uncombinable biotin" in the urine. Also, if such ring cleavage with loss of the ureido carbonyl

Received for publication April 19, 1972.

[1] Supported in part by Public Health Service Research Grants AM-08721 and AM-12224 from the National Institute of Arthritis and Metabolic Diseases and in part by funds made available through the State University of New York.

[2] Part of these data were included in a preliminary report presented at the meetings of the Federation of American Societies for Experimental Biology in Atlantic City, N. J., April, 1972.

carbon does exist, it should have led to significant $^{14}CO_2$, which was not found in the cited work of the Frankel-Conrats (1).

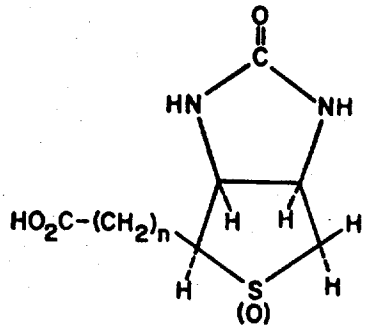
More detailed information on the degradation of biotin has come from studies using microorganisms. Christner et al.[3] and Coon et al. (10) isolated a bacterium of the genus *Pseudomonas*, which degrades 2-^{14}C-biotin to $^{14}CO_2$. From the culture filtrates of a soil pseudomonad, which grows on biotin as a sole source of carbon, nitrogen, and sulfur[4,5] (11), McCormick, Wright, and their colleagues isolated and identified several metabolites derived from biotin (12, 13), the *l*- (14) and *d*- (15) sulfoxides, and the sulfone (13). These investigators suggested that microbial oxidation of the valeric acid side chain precedes cleavage of the ureido portion of the molecule[6] (11–13, 16, 17).

This report supplies evidence for the metabolic fate of biotin in the rat, wherein β-oxidation of the side chain of the vitamin occurs, as does oxidation of the vitamin to the sulfoxides. Pertinent structures are indicated in figure 1.

EXPERIMENTAL

Experiments in vivo. Groups of young, male rats of the Sprague-Dawley strain,[7] weighing 250 to 300 g each, and fed the biotin-sufficient[8] or biotin-free[9] test diets, were given a single intraperitoneal injection of ^{14}C-biotin[10] (1 mg,[11] 4 μg, or 0.5 μg/100 g body weight) in isotonic saline adjusted to pH 7.4. Urine, feces, and expired CO_2 from each rat were collected separately in an all-glass, closed, rat metabolism cage system[12] for the first 24 hours following injection. The rats were then transferred to open metabolism cages, and collections of urine and feces continued for the succeeding experimental period. A small amount of hydrochloric acid was added to each urine collection bottle as a preservative, the pH of the urine thereby being kept below 3 throughout the collecting interval. Properly diluted urine samples and samples from the solution, originally 2 N in NaOH, which trapped the expired CO_2, were taken for assay of radioactivity. The urine samples from rats of the same experimental group were combined and fractionated for radioactive compounds. Feces were frozen, ground, digested in 0.1 N HCl, and centrifuged. The supernatant was filtered, and a sample of the filtrate was taken for determination of radioactivity.

To delay the initial excretion rate of the radioactive material, groups of rats were fed the biotin-free test diet 4 weeks prior to the single injection of radioactive biotin (0.5 μg/100 g rat). Antibacterial drugs were added to the diet to decrease possible contribution of intestinal microflora to



Biotin, n=4; Bisnorbiotin, n=2; Sulfoxides with (O)

Fig. 1 Chemical structures of biotin, bisnorbiotin, and the sulfoxides.

[3] Christner, J. E., J. L. Foote and M. J. Coon 1961 Biotin oxidation in a bacterial enzyme system. Federation Proc. 20: 271 (abstr.).

[4] Li, L.-F. 1964 The microbiological degradation of biotin. Ph.D. thesis, Cornell University.

[5] Brady, R. N., L.-F. Li, D. B. McCormick and L. D. Wright 1965 Microbial and enzymatic degradation of biotin. Federation Proc. 24: 625 (abstr.).

[6] See footnote 5.

[7] The rats were purchased from Chordata Corp., Ontario, N. Y.

[8] The composition of the biotin-sufficient diet was similar to the "normal protein" diet of Barnes et al. (18), except that the B vitamins are in cerelose instead of in sucrose, and that the amount of vitamin B_{12} contained in 2.0 g cerelose is 0.003 mg instead of 0.03 mg.

[9] The biotin-free test diet was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; for composition of this diet, see Rubin et al. (19).

[10] Radioactive *d*-biotin (58 mCi/mmole), labeled in the carbonyl carbon of the ureido function, was purchased from Amersham/Searle Corp., Arlington Heights, Ill.

[11] Specific radioactivity, 10,000 dpm/mg, was prepared by diluting the ^{14}C-biotin with nonradioactive *d*-biotin, which was purchased from Hoffmann-LaRoche Inc., Nutley, N. J.

[12] The rat metabolism cage systems were purchased from Delmar Scientific Laboratories, Chicago, Ill.

catabolism of the vitamin. In one experiment (see fig. 2), 1% succinyl sulfathiazole was added, starting on day 23 of the biotin-free diet. Rats recovered within 4 days from a diarrhea caused by the sulfa drug and were then given 0.1% penicillin,[13] in addition to 1% succinyl sulfathiazole in the biotin-free test diet. By such treatment, intestinal microorganisms in both upper and lower portions of the intestine were suppressed (20). One day after this diet, the rats were given a single intraperitoneal injection of the radioactive vitamin (0.5 μg/100 g rat), and the urine of the subsequent 5 days was collected and fractionated for radioactive biotin catabolites. In another experiment (see fig. 3), rats were treated in a similar way except that the penicillin was not added to the biotin-free diet containing succinyl sulfathiazole until the urinary excretion of the radioactivity became nearly constant (7 to 9 days after injection). One day after this, the rats were transferred to a biotin-sufficient diet, which contained 0.1% penicillin and 1% succinyl sulfathiazole; urine collected over the next 4 days was pooled for isolation of radioactive metabolites.

Experiments in vitro. A 20% (w/v) homogenate of normal rat liver was incubated with ^{14}C-biotin (1 μg/1 g liver) at 37° for 40 minutes. The reaction mixture was buffered with a solution of 0.1 M potassium phosphate containing 10^{-3} M $MgCl_2$ and 10^{-4} M $MnCl_2$ (final pH 7.4). As a control, a heat-inactivated homogenate was incubated in the same way. For the detection of $^{14}CO_2$ production during incubation of small samples, the use of septum-stoppered flasks with appended center wells, containing base, followed the procedure described by Yang and McCormick (21). In the large-scale experiments, 50 ml of homogenate was added in a stoppered 500-ml baffled flask, which connected in series with two gas wash bottles, containing 50 ml of 1 N NaOH, and connected, in turn, to a vacuum pump. The CO_2 released during incubation was trapped by the NaOH solution. The liver homogenate was analyzed for radioactive compounds after incubation.

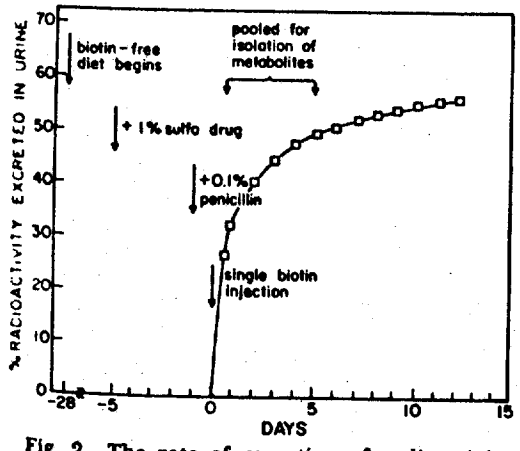


Fig. 2 The rate of excretion of radioactivity in the urine from 12 rats, treated as indicated, before and after injection with 0.5 μg/100 g body weight of carbonyl-labeled ^{14}C-biotin (523,000 dpm/μg).

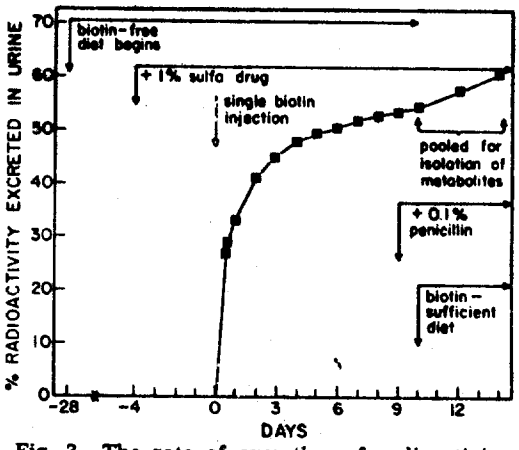


Fig. 3 The rate of excretion of radioactivity in the urine from 12 rats, treated as indicated, before and after injection with 0.5 μg/100 g body weight of carbonyl-labeled ^{14}C-biotin (523,000 dpm/μg).

Assay of radioactivity. All measurements were made in a liquid scintillation spectrometer [14] with Bray's solution (22) as the liquid scintillator. Aliquots of 0.1 to 0.4 ml of aqueous solution in this scintillation liquid gave satisfactory counting results. In measuring the radioactivity of $^{14}CO_2$, which was absorbed into 1 N or 2 N NaOH solution, Bray's solution, containing 4% thixotropic gel, was used to prevent precipitation of the sample in the

[13] Penicillin "G" procaine was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.
[14] The Model 3375 TriCarb Liquid Scintillation Spectrometer used was manufactured by and purchased from Packard Instrument Co., Downers Grove, Ill.

counting vial. The data were expressed as disintegrations per minute by the external standard method (23). Radioactivity on the developed paper chromatogram was localized and quantified with a radiochromatogram scanner.[18]

Isolation procedures. The methods used were similar to those of Yang et al. (24) for isolation of metabolites from culture of an *Endomycopsis* sp. grown on biotin. The neutralized urine or liver homogenate was filtered, the filtrate was concentrated at reduced pressure, then adjusted to pH 2 with HCl. The radioactive materials in the acidic filtrate were adsorbed onto active charcoal and subsequently eluted with a 9:1 mixture of 50% aqueous ethanol and 28% ammonium hydroxide. To the eluate, 10 volumes of 95% EtOH was added and the mixture centrifuged. The supernatant was concentrated and poured onto a 100–200 mesh Dowex 50-X8 (H^+) column (2.2 by 60 cm). Radioactive substances were eluted from the column with H_2O. The eluate was concentrated and then poured over a second column (1.6 by 120 cm; 100–200 mesh) of Dowex 1-X2 (formate). The radioactive materials were stripped from the column by successive elution with water and two gradients of formic acid: 0 to 0.05 M and 0.05 to 0.2 M. Fractions of 15 ml were collected and 0.1- or 0.2-ml aliquots taken for determination of radioactivity. The elution patterns from chromatography of ^{14}C-compounds from urine or liver homogenates on such a column are shown in figures 4 and 5, respectively. These fractions, which contain most of the radioactivity, were separately concentrated to a small volume. Concentrated fraction A was neutralized and poured over a column (1.5 by 50 cm) of Dowex 1-X2 (formate). The radioactive substances were eluted with water. The radioactive eluate was concentrated (fraction A), poured over a column of Dowex 50-X8 (1.6 by 50 cm), and eluted with water. A substance, which washed through quickly (A-1), was thereby separated from one (A-2) weakly retained by the cation-exchange resin (fig. 6). Both fractions were separately concentrated to a small volume under reduced pressure. Crystals formed in the concentrated fraction, A-2, were recrystallized from methanol.

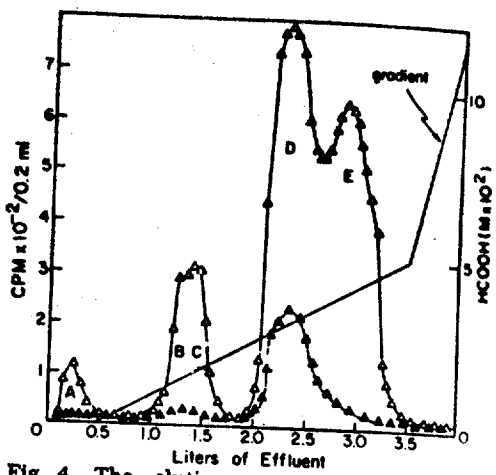


Fig. 4 The elution patterns from column chromatography of carbonyl-labeled ^{14}C-biotin and urinary ^{14}C-metabolites on Dowex 1-X2 (formate). The ^{14}C-material from pooled rat urine, as described in figure 2, was poured over a column (1.6 by 120 cm) for successive elution with water and 0 to 0.05 M and 0.05 to 0.2 M formic acid gradients. The rats had been treated as described in figure 2 (△) or figure 3 (▲) to obtain "early" or "late" urine.

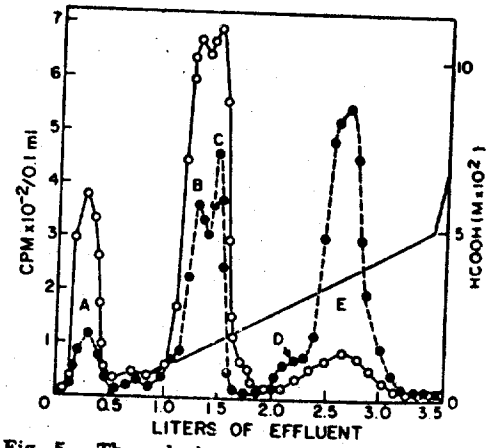


Fig. 5 The elution patterns from column chromatography of carbonyl-labeled ^{14}C-biotin and radioactive liver homogenate metabolites on Dowex 1-X2 (formate). The ^{14}C-material, enriched from 50 ml of incubated homogenate of rat liver (as figure 2), was poured over a column (1.6 by 120 cm) for successive elution with water and 0 to 0.05 M and 0.05 to 0.2 M formic acid gradients. Radioactive biotin (523,000 dpm/μg; 1 μg/gram liver) was incubated with (○) or without (●) aeration.

[18] The Actigraph III Radiochromatogram Scanner used was manufactured by and purchased from Nuclear-Chicago Corp., Arlington Heights, Ill.

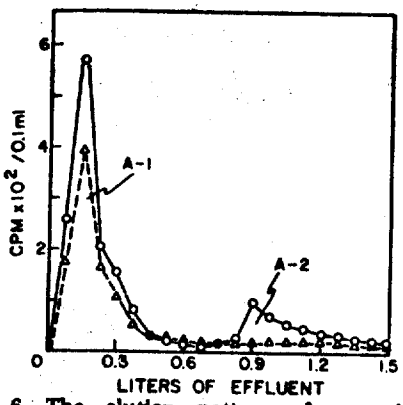


Fig. 6 The elution patterns from column chromatography of radioactive fraction A (from Dowex 1-X2), which derived from liver homogenate (○) or urine (△) of the rats, on Dowex 50-X8 (H^+). Material was eluted with water.

Analytical techniques. Appropriate aliquots of the concentrated fractions, together with internal and external standard biotin catabolites,[16] were applied to sheets of Whatman no. 1 chromatography paper; the sheets were developed ascendingly with acidic, neutral, and weakly alkaline solvent systems including: *n*-butyl alcohol–acetic acid– water (2:1:1, v/v/v); *n*-butyl alcohol–formic acid–water (4:1:1, v/v/v); *n*-butyl alcohol–1.5 N ammonium hydroxide (1:1, v/v); water-saturated *n*-butyl alcohol; methanol–pyridine–water (85:4:15, v/v/v). In an experiment to test for the presence of a neutral ketone, a sample of fraction A-1 was reacted with dansyl hydrazine, according to the method of Chayen et al. (26), prior to application on the paper chromatogram. In general, the chromatograms were sprayed with H_2SO_4–*p*-dimethylaminocinnamaldehyde reagent (27), and the intensity of color developed was measured with a recording densitometer.[17] A keto compound on the developed chromatogram was detected by 2,4-dinitrophenylhydrazine spray reagent (13). Ultraviolet absorbing compounds were detected under a Mineralight lamp.[18] In the isotope-dilution method, commercial urea or chemically-made biotin sulfoxides was (were) used as carrier. The melting point of crystals from fraction A-2 was determined on a Fisher-Johns melting point apparatus. Infrared spectra were run in KBr pellets with an infrared spectrophotometer.[19] The urease reaction was performed by incubating 1 mg of standard ^{14}C-urea or ^{14}C-unknown with 2.16 units of urease[20] in 5 ml of 0.5 M potassium phosphate buffer, containing 10^{-4} M EDTA and 10^{-6} M glutathione, pH 7.0, at 37° for 115 minutes. The reaction mixture was incubated in a 50-ml Erlenmeyer flask stoppered with a rubber septum. The reaction was terminated by an injection of 20% trichloroacetic acid, and the $^{14}CO_2$ was collected into a solution of hydroxide of Hyamine, which was adsorbed on a filter paper wick in a polypropylene center well appended to the rubber septum. The filter paper and the washings from the polypropylene center well, with Bray's solution, were counted for radioactivity.

RESULTS

Excretion of radioactivity after administration of carbonyl-labeled ^{14}C-biotin. No significant $^{14}CO_2$ was trapped by the glass metabolism cage system, and only a trace amount of radioactivity was excreted through the feces. The major portion of the radioactivity injected into biotin-sufficient rats is excreted through the urine. When milligram quantities of radioactive biotin were administered, more than 90% of the given dose was excreted through urine within 12 hours. The initial excretion rate of the radioactivity was decreased with decreasing dosage of the radioactive biotin. As shown in figure 7, the radioactivity excreted in the urine from young male rats was 83 ± 4% during the initial 8 hours for animals that had received 4 μg of radioactive biotin per 100 g of body weight, while 47 ± 4.5% was excreted during the initial 12 hours for the animals that had received 0.5 μg per 100 g body weight. When rats were fed the biotin-free

[16] The standard bisnorbiotin and biotin sulfoxides were previously isolated in this laboratory from culture filtrates of a pseudomonad (12). Chemically-synthesized biotin *d*- and *l*-sulfoxides were made by the method of Melville (25). A sample of bisnorbiotin was a gift from Dr. K. Ogata, Kyoto University, Japan.

[17] The Recording Electrophoresis Densitometer used was manufactured by and purchased from Photovolt Corp., New York.

[18] Mineralight is a registered brand name; the lamp used was manufactured by and purchased from Ultra-Violet Products, Inc., San Gabriel, Calif.

[19] The Model 137 Infracord Spectrophotometer used was manufactured by and purchased from Perkin-Elmer Corp., Norwalk, Conn.

[20] The crystalline urease used in these experiments was a gift from Dr. W. J. Visek, Department of Animal Science, Cornell University.

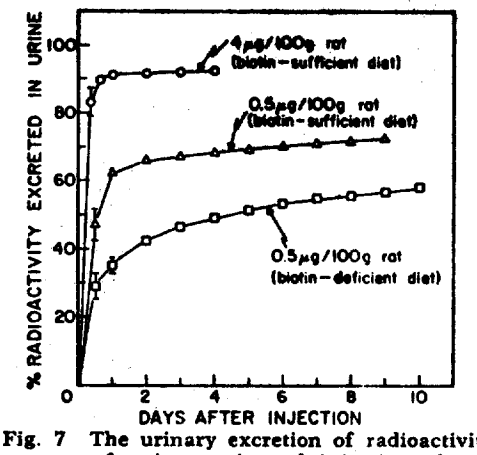


Fig. 7 The urinary excretion of radioactivity from rats after intraperitoneal injection of carbonyl-labeled ^{14}C-biotin (523,000 dpm/μg) to normal rats or those fed the biotin-free test diet for 4 weeks. Each point on each curve represents the mean value from six rats.

test diet for 4 weeks prior to the injection of 0.5 μg/100 g body weight, the initial excretion rate was further decreased to 29 ± 4% per 12 hours.

Isolation and characterization of compounds. The elution patterns from Dowex 1-X2 (formate) column chromatograms of carbonyl-labeled ^{14}C-biotin metabolites, which had been extracted from urine or from liver homogenate, are shown in figures 4 and 5, respectively. From such columns, radioactive fractions (A–E) were isolated. Mobilities of these radioactive fractions and standards, which were applied externally or internally with the unknowns, were compared on paper chromatograms. In over five different solvent systems, the R_f values of fractions B, C, D, and E were found to be the same as those of biotin *l*-sulfoxide, biotin *d*-sulfoxide, bisnorbiotin, and biotin, respectively. Most of the R_f values for these compounds are listed in table 1. A mixture of radioactive fraction B and chemically synthesized, nonradioactive biotin *l*-sulfoxide gave constant specific radioactivity after repeated recrystallization from absolute ethanol. This further confirms that the compound in fraction B and biotin *l*-sulfoxide are identical. In the same way, the isotope dilution technique was used to confirm the identity of fraction C with biotin *d*-sulfoxide. In addition, dried material from fraction B or C, when treated with excess H_2O_2 in glacial acetic acid, resulted in a new compound, which gave the same R_f value as biotin sulfone on paper chromatograms. Oxidation of the dried fraction D with H_2O_2 led to the sulfoxides and sulfone corresponding to the oxidation products of known bisnorbiotin. The R_f values of these derived compounds on paper chromatograms are also given in table 1. This further established that the material from fraction D is identical to bisnorbiotin.

The radioactive compounds in fraction A, which washed directly through Dowex 1-X2 columns with water, are clearly not anionic. The fraction A isolated from incubation of ^{14}C-biotin with liver homogenates was further fractionated by chromatography on a Dowex 50-X8 column where elution with water resulted in fraction A-1, which washed through quickly, and fraction A-2, which was weakly retained by the cation exchanger, as shown in figure 6. However, the neutral fraction A isolated from the urine could not be subfractionated by water elution from the Dowex 50-X8 column. The position of elution is identical to that of A-1 from liver homogenates. The mobilities of fraction A-1 and the weakly basic (cationic) A-2 are similar on paper chromatograms developed with butanol–acetic acid–water (2:1:1, v/v/v) or butanol–formic acid–water (4:1:1:, v/v/v). However, the mobilities of A-1 and A-2 are different on paper chromatograms developed with pyridine–methanol–water (4:85:15, v/v/v); the R_f value of A-1 is 0.58 to 0.59, whereas the R_f value of A-2 is 0.43 to 0.44, the same as for urea. When fraction A-2 was concentrated, white, needle-like radioactive crystals were formed. The melting point of these crystals was 128 to 130°, which is close to that of pure urea (132.7°). The infrared spectra of A-2 and commercial urea are identical. The specific radioactivity of the crystals became constant (30! dpm/mg) upon repeated recrystallizations from methanol. After incubation of A-2 (or ^{14}C-urea in the control) with purified urease at 37° for 15 minutes, the radioactivity was liberated as $^{14}CO_2$, which was trapped and counted. When fraction A-1 was reacted with urease, only a trace amount of $^{14}CO_2$ was trapped. Pure urea (based on an infrared spectrum) was also

TABLE 1

Mobilities of biotin analogues on paper chromatograms

Biotin catabolites	Butanol: acetic acid: water (2:1:1, v/v/v)	Butanol: formic acid: water (4:1:1, v/v/v)	Butanol: ammonium hydroxide (1.5 N) (1:1, v/v)	Water-saturated butanol	Pyridine: methanol: water (4:85:15, v/v/v)
Biotin	0.81–0.83	0.82–0.83	0.77–0.78	0.62–0.63	0.37–0.38
Biotin *d*-sulfoxide	0.63–0.64	0.62–0.64	0.76–0.77	0.31–0.33	
Biotin *l*-sulfoxide	0.61–0.62	0.58–0.60	0.75–0.76	0.20–0.22	
Biotin sulfone	0.67–0.68	0.68–0.69		0.19–0.21	
Bisnorbiotin	0.71–0.73	0.72–0.73	0.67–0.69	0.40–0.41	0.34–0.35
Bisnorbiotin *d*-sulfoxide	0.59–0.60	0.45–0.47			
Bisnorbiotin *l*-sulfoxide	0.52–0.53	0.40–0.42			
Bisnorbiotin sulfone	0.64–0.65	0.53–0.55			

isolated from the fraction A from rat urine; however, no significant radioactivity could be detected in this urea. The radioactive spot on the developed paper chromatogram of either fraction A-1 from the liver homogenate or fraction A from the rat urine exhibits the specific pink color reaction with the acidic *p*-dimethylaminocinnamaldehyde and an orange-yellow color reaction with 2,4-dinitrophenylhydrazine. Thus, the radioactive material contained in these truly neutral fractions possesses both a ureido ring (30) and a neutral keto group (13). The presence of a neutral keto group in the radioactive compound in these fractions was further confirmed by its ability to react with dansyl hydrazine (26). On paper chromatograms developed with pyridine–methanol–water (4:85:15, v/v/v), the R_f value is 0.59 for the radioactive ketone and 0.70 for the fluorescent dansyl hydrazine. When the reaction product of dansyl hydrazine is chromatographed with the radioactive unknown, the radioactive and fluorescent spot shifts to a new position with an R_f value of 0.50. This indicates that the radioactive compound from fraction A-1 of the liver homogenate and from fraction A of the urine are identical and can combine with dansyl hydrazine, which is known to react with neutral ketones to form hydrazones (26). Ketones are formed as a result of the decarboxylation of β-keto acids, known to be intermediates in the β-oxidation of biotin (13).

Differences between the metabolism of biotin in vivo and in vitro. No detectable radioactive CO_2 was respired from the rats injected with various amounts of carbonyl-labeled ^{14}C-biotin, while a significantly detectable amount of $^{14}CO_2$, which is equivalent to the breakdown of 2.6 μg/gram liver of radioactive biotin (130 dpm/μg) was detected when an excessive amount of radioactive biotin (834 μg/gram liver) was incubated with rat liver homogenate at 37° with aeration for 1 hour. The significance of the $^{14}CO_2$ production is also supported by the observation that the radioactivity trapped by the NaOH solution was linearly increased with incubating time. Since the amount of biotin added was excessive, the percentage of the added radioactivity metabolized to $^{14}CO_2$ was low (0.16%).

There is no detectable radioactive urea in the experiment in vivo, while urea with low specific radioactivity (301 dpm/mg) was isolated in the experiment in vitro. As previously pointed out, in the neutral fraction isolated from the experiment in vitro, both radioactive urea and a neutral ketone were detected, whereas in that from the experiment in vivo, only a neutral ketone was found.

The relative amounts of other ^{14}C-biotin metabolites isolated in vitro under different situations are different from one another. For each experimental condition, one representative set of data measured from the fractions that were best resolved by the Dowex 1-X2 column is shown in table 2. In an experiment in vivo, the amount of metabolites isolated from the initial urine of a group of drug-fed, biotin-depleted rats, treated as described under the conditions relating to figure 2, is higher in bisnorbiotin, lower in biotin sulfoxides, and even lower in the neutral metabolites, compared to the biotin isolated. The amount of metabolites isolated from the urine of rats under similar conditions, but at a later time after treatment as described for figure 3, is dominant in bisnorbiotin, and nonsignificant in sulfoxides and neutral metabolites, as compared to the biotin isolated. In the experiment in vitro, where radioactive biotin was incubated with liver homogenates (1 μg/gram liver) in a stoppered flask as described for figure 5, the amount of biotin sulfoxides isolated is higher than that of bisnorbiotin, although lower than that of biotin, while the amount of neutral metabolite isolated was similar to that of bisnorbiotin. When the incubation was carried under aeration, the amounts of sulfoxides and neutral metabolites isolated were higher than that of biotin, while only a trace amount of bisnorbiotin was detected. In all these cases the amount of biotin *d*- to *l*-sulfoxide isolated appeared in a ratio between 2 to 3

DISCUSSION

Sydenstricker et al. (28) found that the excretion of biotin in urine from normal human volunteers decreased from the range of 29 to 52 μg per day when a normal diet was eaten, to the range of 3.5 to 7.3 μg per day when a biotin-deficient regimen was followed for 8 weeks; administration of 75 to 300 μg of biotin per day increased immediately the urinary biotin excretion by these deficient subjects to 55 μg per day. The daily requirement of biotin for a healthy man can be expected, from this and other considerations, to be lower than 75 to 300 μg or 0.17 to 0.43 μg per 100 g body weight. At least the same magnitude per body weight appears reasonable for the rat. In the present study, where 0.5 μg of biotin per 100 g of body weight was injected into the rat, the amount may be considered a reasonable physiological dose. The very rapid clearance of the injected radioactive biotin in normal rats

TABLE 2

Comparison in vivo and in vitro of the metabolism of biotin in rats

Radioactive compounds (column fractions)	Radioactivity recovered (%) [1]			
	in vivo (urine)		in vitro (liver homogenate)	
	Early [2]	Late [3]	With aeration [4]	Without aeration [5]
Neutral metabolite (A)	3.4 [6]	trace [6]	14.0 [7]	4.0 [7]
Biotin *l*-sulfoxide (B)	2.5	trace	15.0	6.5
Biotin *d*-sulfoxide (C)	6.6	trace	27.0	16.5
Bisnorbiotin (D)	37.5	84.0	trace	4.0
Biotin (E)	25.5	4.0	4.0	42.0
Total	75.5	88.0	60.0	73.0

[1] Percentage of total radioactivity prior to isolation.
[2] Pooled urine samples collected during the initial 5 experimental days, as shown in figure 4.
[3] Pooled urine samples collected during days 11 to 14, as shown in figure 5.
[4] Experimental conditions as given for figure 7, top curve (○).
[5] Experimental conditions as given for figure 7, bottom curve (□).
[6] Major radioactive component is a neutral ketone.
[7] Includes urea, as well as a neutral ketone.

indicates that the rat possesses a very low renal threshold for reabsorption of biotin.

About 75% of the total radioactivity contained in the liver homogenate incubated without aeration or in the initial urine sample was recovered in the final isolation fractions (cf. table 2). The major loss of radioactivity occurred during the processes of adsorption and elution from charcoal and anion-exchange chromatography. The total recovered radioactivity from the homogenate incubated with aeration is lower (60%); the decreased recovery is due to increased loss during both processes mentioned. While the total recovered radioactivity from the "late" urine sample is higher, the increased recovery of the radioactivity is mainly due to decreased loss during adsorption from charcoal. These observations may indicate that the "late" urine contains the least amount of relatively more water-soluble radioactive metabolites of small molecular weight. The liver homogenate incubated under aeration probably (and expectedly) contains most such water-soluble, less nonpolar radioactive metabolites. However, since biotin, bisnorbiotin, and biotin sulfoxides are generally close to each other in both their molecular size and their ionic charge in the solutions used during isolation, assuming the loss of radioactivity from all these fractions during the isolation processes is roughly proportional to their amounts contained in the original samples, the metabolic fate of radioactive biotin can be interpreted on the basis of the amounts of radioactive metabolites isolated. No significant radioactive biotin metabolites are detected in the urine of the rat after injection of biotin of low specific radioactivity (10,000 dpm/mg) in high (mg) amounts, but, as seen, various radioactive metabolites can be isolated from rat urine after injecting the animals with biotin of high specific radioactivity (523,000 dpm/μg) in the physiological range. These results indicate that the capacity for the metabolism of biotin in the living rat is limited. The metabolism of radioactive biotin to the sulfoxides in the liver in vitro is, of course, much higher than that in the living rat, where excretion of the vitamin occurs rapidly. The major site for formation of biotin sulfoxides in the rat, though, is the liver. The ratio of sulfoxides to biotin in the incubated liver homogenate or in the urine decreases with decreasing amounts of biotin administered. The mechanism for oxidizing biotin to sulfoxides is nonspecific. Since the sulfoxides produced in the liver homogenate are very significantly higher when incubated with aeration than incubated without, the formation of biotin sulfoxides from biotin is molecular-oxygen dependent. Probably, the oxidation is catalyzed by the NADPH- and oxygen-dependent system in the liver microsomes, which are known to oxidize various organic sulfur-containing compounds to their sulfoxides (29–32). When the administered dose is in a physiological range, bisnorbiotin is the most apparent biotin metabolite in vivo, whereas the quantity of biotin sulfoxides is dominant in vitro. This finding indicates that the capacity of the liver for forming biotin sulfoxides is much higher than that for forming bisnorbiotin and that the formation of bisnorbiotin is more generalized among the organs in the living rats. In the biotin-depleted rats treated with both sulfa drug and penicillin, the urinary excretion of radioactivity is prolonged after the single injection of radioactive biotin. When the excretion rate of radioactivity becomes constant, replacement of the biotin-free test diet by the biotin-sufficient diet results in a constant excretion of urinary radioactivity, which is twice the original one (cf. fig. 3). Radioactive bisnorbiotin accounts for 84% of the radioactivity contained in the urine of the succeeding 4 days. From this finding, together with the observation that the ratio of bisnorbiotin to biotin isolated in the urine increases with decreasing amounts of biotin injected, it is reasonable to suggest that the formation of bisnorbiotin is related to the turnover mechanism of biotin from its storage pool and/or from biotin enzymes.

The isolation of radioactive bisnorbiotin indicates that β-oxidation of the valeric acid side chain occurs, undoubtedly as a result of mitochondrial action. However, no radioactive tetranorbiotin has so far been detected, either in vivo or in vitro. Also, no significant radioactivity has been found in exhaled CO_2 or in the urea isolated from urine. Only a trace amount of

radioactivity was found in the CO_2 and urea produced in the liver homogenate in vitro. These latter results are different from the findings concerning the metabolic fate of biotin in the pseudomonad grown on biotin as sole organic substrate. This bacterium readily cleaves an additional two-carbon fragment from the side chain of bisnorbiotin to yield tetranorbiotin and further degrades the ring system of the molecule to yield such terminal products as urea (12). It seems that all but the most primitive microorganisms may have lost the ability to utilize the sulfur and nitrogen within biotin. Hence, the small amount of the vitamin, which is required by nearly all organisms, rats and man being no exception, is rather well conserved metabolically.

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Lee-'73
Bib No. 796

Proc. Soc. Exp. Biol. Med. 142:642-644. 1973.

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Urinary Excretion of Biotin and Metabolites in the Rat[1] (37085)

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The metabolism of the vitamin, *d*-biotin, in the rat has recently been investigated by Lee *et al.* (1). When carbonyl-labeled biotin is injected intraperitoneally (ip) at the mg level, the radioactivity is almost completely excreted as intact biotin within one day, though the rate of excretion is less when the amount of biotin given is decreased to the μg level, especially in animals previously depleted of the vitamin. However, not all of the injected radioactivity was recovered within a few days.

In addition to the recovered vitamin, small amounts of several biotin metabolites, namely, the *d*- and *l*-sulfoxides, bisnorbiotin (two-carbon shorter side chain), and a neutral ketone were isolated from rat urine (1), but tetranorbiotin (four-carbon shorter side chain), which also accumulates in the culture medium from a biotin-degrading bacterium (2), was not detected.

The present study was made to determine the excretion rates in the rat of biotin and some of the more commonly occurring metabolites.

Materials and Methods. *d*-Biotin was purchased from Hoffmann-La Roche, Inc. The radioactive compounds used were exclusively ureido carbonyl[^{14}C]-labeled, with specific radioactivities, in dpm/mg, as follows: biotin, 20,240; bisnorbiotin, 20,100; tetranorbiotin, 20,440; biotin *d*-sulfoxide, 20,700; biotin *l*-sulfoxide, 20,500; and biotin sulfone, 20,450. The [^{14}C]biotin was supplied by Amersham/Searle Corp. The [^{14}C]catabolites formed by β-oxidation of the valeric acid side chain of [^{14}C]biotin were isolated from culture filtrates of a pseudomonad grown on the carbonyl-labeled biotin as described by Iwahara *et al.* (2). The sulfoxides and sulfone were prepared from carbonyl-labeled biotin by oxidizing with peroxide according to Melville (3). All radioactive compounds were dissolved in 0.1 *M* sodium phosphate buffer, final pH 7.4.

Young, male, Sprague-Dawley rats (250–310 g), which were obtained from Chordata Corp., were fed, for six weeks, a biotin-free, avidin-containing test diet[4] (4), which additionally contained 1% succinyl sulfathiazole. These animals were then divided randomly into six groups of five rats each. Each rat was given a single ip injection of 0.5 mg/100 g body weight of one of the six radioactive compounds. A control group of rats, maintained on a biotin-sufficient diet,[5] was given an injection of 0.5 mg/100 g body weight of radioactive biotin. Urine, feces, and expired CO_2 from each rat were collected separately in an all-glass, closed, rat metabolism cage system.[6] Assay of radioactivity from

[1] This work was supported in part by Research Grants AM-08721 and AM-12224 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service, and in part by funds made available through the State University of New York.

[2] Supported by Section of Biochemistry and Molecular Biology Undergraduate Research Participation Program Grant GY-8914 from the National Science Foundation, summer, 1971.

[3] Holder of Career Award K6-AM-16,612 from the National Institutes of Health, U. S. Public Health Service.

[4] Purchased from Nutritional Biochemicals Corp., St. Louis, Mo.; for composition of this diet, see Rubin *et al.* (4).

[5] Rat-Mouse Pelleted Diet from Agway, Inc., Specialties Division, Syracuse, N.Y.

[6] Purchased from Delmar Scientific Laboratories, Chicago, Ill.

samples was done according to Yang and McCormick (5). Isolation of radioactive compounds from samples of urine followed the method of Yang *et al.* (6). The identification of recovered radioactive compounds was accomplished with paper chromatography as described in previous work (1).

Results. No significant radioactivity was excreted through feces or expired CO_2. The major excretion occurs in the urine. The urinary excretion rates of biotin from both biotin-sufficient and biotin-deficient rats, which have been injected ip with milligram amounts of the vitamin, are similar to each other. However, there are notable differences if one compares the initial excretion rates of biotin with certain of the metabolites, although all six compounds tested were almost completely excreted within one day.

The differences among the urinary excretion rates of biotin and analogues from rats are presented in Table I. The initial excretion rates of tetranorbiotin, biotin sulfoxides, and biotin sulfone are similar and significantly faster than those of biotin and bisnorbiotin, which latter two behave similar to each other. In each case, the radioactive compounds recovered from urine evidenced but one major radioactive peak upon column (Dowex 1-X2, formate) and one spot upon paper chromatograms. Positions for elution and mobilities were identical to the original injected compounds. Thus, none of these compounds, when injected at the milligram level, appeared to be metabolized very extensively, even though some small fraction is. This relatively small amount becomes quite apparent when only microgram amounts of the vitamin are given (1).

TABLE I. Urinary Excretion Rates of Biotin and Biotin Metabolites from the Rat.*

Compound	% Urinary radioactivity recovered after injection		
	0–3 hr	3–6 hr	6–24 hr
	Biotin-sufficient diet		
Biotin	84.3 ± 5.1	9.5 ± 2.2	2.7 ± 0.4
	Biotin-deficient diet		
Biotin	83.8 ± 6.3	9.3 ± 2.7	2.6 ± 0.4
Bisnorbiotin	85.8 ± 2.2	10.4 ± 0.6	2.2 ± 0.0
Tetranorbiotin	96.4 ± 0.2	2.9 ± 1.2	
Biotin *d*-sulfoxide	97.4 ± 1.7	1.4 ± 0.4	
Biotin *l*-sulfoxide	97.4 ± 1.1	1.5 ± 0.2	
Biotin sulfone	97.8 ± 1.2	1.4 ± 0.2	

* Values shown are means ± SD.

Discussion. The urinary excretion rates of biotin, bisnorbiotin, tetranorbiotin, biotin sulfoxides, and biotin sulfone from the biotin-deficient rats are all of the same magnitude, but clear differences do exist. The urinary excretion rate of biotin appears to be the same from the biotin-deficient as from the control rat. Hence, the lowered initial excretion rate of such relatively large amounts (milligram level) of biotin, which is less than that of most of the metabolites, is not likely due to greater need (microgram level) of the vitamin in the biotin-deficient rat. The similar excretion rate of bisnorbiotin, which presumably cannot be incorporated into biotin-dependent enzymes, to biotin alone argues against a special need for retaining the larger amounts of the vitamin. The fact that each of the present metabolites is excreted at least as quickly and completely as biotin minimizes the possibility that the major part of the urinary radioactivity not recovered within a few days after microgram amounts of biotin are injected, as in the previous work (1), might be accounted for by any single one of these five biotin metabolites, rather than by several of them. The relatively fast and complete excretion of biotin and its metabolites at the milligram level may suggest that an active transport mechanism for reabsorbing these compounds in the renal tubule of the rat may not be so important as a simple passive absorption and/or that excretion is facilitated. This is further supported by examining the differential excretion rates of these compounds: biotin sulfone $\geqq$ biotin sulfoxides $>$ biotin, and tetranorbiotin $>$ bisnorbiotin $\geqq$ biotin. This behavior at least generally parallels the water solubilities.

Oxidation of the thioether sulfur in biotin expectedly leads to increased polarities and greater water solubilities in the sulfoxides and sulfone, just as does shortening the hydrophobic side chain in the bisnor- and tetranor-compounds.

Finally, the urinary excretion rates of biotin and its metabolites from the rat are also consistent with the rates of uptake of these compounds by the bacterial cell, where the sulfone > sulfoxides > biotin (7, 8).

Summary. Approximately 95% of an ip dose of 0.5 mg/100 g body weight of [^{14}C] biotin is excreted by the young, male rat within 24 hr; 84% is excreted within the first 3 hr. The rate and extent of excretion of bisnorbiotin is essentially the same. The more water-soluble tetranorbiotin, both *d*- and *l*-sulfoxides of biotin, and biotin sulfone are excreted even more rapidly, with 96–98% of the injected dose appearing in the urine within the first few hours.

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Received Sept. 1, 1972. P.S.E.B.M., 1973, Vol. 142.

NAS /NRC Question.
BIB NO. 954

BIOTIN

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Dr. B. Fust's Report of March 28, 1951

Acute Toxicity Tests

Abstract

Acute toxicity determinationsin mice are reported for 42 experimental substances. Results with GRAS substances are as follows:

Biotin	$LD_{10} > 10.0$ g/kg p.o.
DL-a-Tocopherol (oily solution)	$LD_{10} > 10.0$ g/kg p.o.

In addition, DL-a-tocopherylquinone was tested and found to be atoxic (no symptoms) at 10.0 g/kg per os.

Translated and abstracted by W. R. Sullivan, 8/31/71

/k

Translation of Author's Summary

From the results reported on pages 4-10 as well as the publications which have appeared in our area on biotin, it appears that the substance is suitable for the treatment of seborrheic dermatitis and a very severe form of it, Leiner's disease. Biotin has also proved useful in allied conditions. Seborrheic eczema does not respond to vitamin H; subacute and chronic arthritides also show little effect. To some extent, chronic colitides responded surprisingly well to biotin. In cases of pernicious anemia and iron-deficiency anemias, it can also be cautiously tried with some expectation of success.

Intolerance has never been observed even at the very high dose of 100 mg/day per os or parenterally. Side effects, in the sense of allergic reactions, have never occurred. It has to be stressed that in normal times with adequate nutrition, biotin deficiency states are hardly ever seen. Widespread dermatitis seborrhoides in babies and children is only to be expected if mothers are undernourished during pregnancy. It must also be mentioned that biotin deficiency symptoms can be avoided by giving raw liver and kidney to the mothers.

A significant demand for biotin is thus only to be expected in times of need, which naturally is accompanied by worsening of nutritional status.

It is recommended that 5 mg tablets and ampuls be added to the price list but not promoted as a specialty.

Abstract of clinical findings (pp. 4-10)

Trials, supervised by Prof. Opitz (University of Heidelberg) in 5 infants with Leiner's disease who were given DL-biotin per os or i.m. for 8, 9, 18, 23 and 26 days showed no discernible effect and were stopped in June 1948 as hopeless. Prof. Hilber (University of Munich) also had negative results (details are not available) in Leiner's disease.

After optically active D-biotin became available, rather substantial amounts (table on page 7) were made available and evidence mounted (details are not included in this report) that biotin was indeed useful in vitamin H deficiency states such as Leiner's disease and other seborrheic dermatitides. However, since the investigators always added other vitamins (BVK) when biotin alone did not give positive results, criteria for selecting patients for biotin therapy were not developed.

Seborrheic eczema and true allergic eczemas were not affected by biotin administration. Yet vitamin H seemed to have a supportive action in a number of cases of acne vulgaris (Kiessling). According to Schmid's publication, a trial of biotin seems indicated in cases of iron-deficiency anemia and also in pernicious anemia. He also indicated a good effect in toxically conditioned panmyelopathies, in which erythropoiesis was definitely stimulated. Somewhat surprising results were obtained in chronic colitides.

On the basis of Thiers' observations (in which 12 arthrosis patients were benefited by 50 mg/day of biotin), therapeutic trials with massive doses were undertaken at 4 different clinics, but all gave negative results. Dr. Schettler (Marburg) gave two patients 2 x 50 mg/day for 3 weeks with no benefit and Dr. Scheiffarth (Erlangen) had no success in 2 polyarthritis patients given 50 mg/day for 14 days.

Dr. Zickgraf tried high doses in 22 arthritics. He gave i.m. injections of 2 x 50 mg/day and oral doses of 3 x 20 mg/day and in a few cases used both routes simultaneously. No benefits were observed. Dr. Stoeber (Garmisch-Partenkirchen) tried it in 3 rheumatic children, following the same regimen as Thiers, without success.

(pg. 11) No indications of hypervitaminosis have been seen even with massive doses. At Heidelberg University's skin clinic daily doses up to 30 mg and total doses up to 770 mg have been given. In trying to repeat the work of Thiers, daily doses of 100 mg. i.m. and 60 mg p.o. for more than 8 days and up to 3 weeks have been given. Intolerance has never been reported, nor any side effects.

Translated and abstracted by W. R. Sullivan, 8/31/71

/jk

Report by Dr. Dübler

Department W 2

Biotin Ro 1-5492

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Summary

On the basis of the experiments described on pages 4-10 and the publications concerning Biotin that have appeared, it seems that this preparation is suited to the treatment of seborrhoid dermatitis and its most serious form, Leiner's disease. Biotin has also been used successfully on diseases that are similar to the above. Seborrhoic eczema does not respond to vitamin H, and subacute and chronic polyarthritis types are likewise unaffected by this vitamin. To a certain extent, chronic colitis types reacted surprisingly well to Biotin. Where there are cases of pernicious and iron-deficiency anemia that do not respond to customary therapy, the preparation may be tried with good chances of success.

Even when the highest does of 100 mg Biotin daily are administered, either orally or parenterally, intolerance has never been observed. Likewise, side effects in the form of allergic reactions have never occurred. However, it must be emphasized and remembered, that in normal times, with sufficient nutrition, diseases due to Biotin deficiency hardly ever are observed. Only when pregnant mothers eat poorly -- thus when there is qualitative of quantitative undernourishment-- can we expect widespread seborrhoid dermatitis during infancy and early childhood. It must further be pointed out that Biotin deficiency phenomena can be avoided by administering doses of raw liver and kidney to the mothers at the appropriate time.

Thus, a greater requirement of biotin can be expected in times of emergency and scarcity, which are naturally always associated with a worsening of nutritional conditions.

For these reasons, in our opinion, the introduction of Biotin as an express specialty is unjustified. However, in order to be able to meet the demands for Biotin, we recommend that the preparation be added to the price lists in its therapeutic forms of tablets and pills containing 5 mg each, without any propagandistic proclamations.

Biotin Ro 1-5498

It seems wise and purposeful to us to come to a decision as soon as possible regarding the further fate of our Vitamin H preparation, Biotin.

There has been no systematic and thorough examination of Biotin, and the preparation has only been included in the clinical testing of other experimental preparations, because there was little promise of success in its use on ailments other than Leiner's disease and related diseases. However, it seemed to us to be time to examine more closely its therapeutic usefulness, so that the questions before us -- "Where do we stand with our Biotin experiments?" and "What should be done to assure the preparation of successful use?" -- could be answered clearly and uniformly.

Of course, within the framework of this report it is impossible to discuss all the cases treated with Biotin, because -- as already briefly mentioned -- tests could not be performed systematically and taking into consideration all the details necessary for a critical analysis. In the years 1947 to 1950-51, test charts with precisely defined questions, the reponses to which could be used in forming a judgment, were not published. Beyond that, the data available for analysis, except for a few cases, did

not contain sufficiently precise details (concerning number of cases, diagnoses, effects, dosage, tolerance and side effects, as well as additional therapy, etc.), and were loose and too general. Thus, the attempt to report on Biotin at least relatively exactly and completely, is doomed to failure a priori. Therefore, this report can offer only an approximate overview which, however, permits us to obtain a satisfactory answer to the questions raised.

General Clinical Observations and Results

Racemic DL-Biotin Ro 1-5125/13 and /14 was first supplied to us in the middle of November, 1947, in a quantity of 30 x 20 tablets of 5 mg each, and 10 x 6 pills with lcem to likewise 5 mg working material each. It was supplied by Basel VI. In the article pertaining to this matter (No. 48, November 13, 1947), mention was made of a communication between Basel and Grenzach. The object of the discussion was Biotin, especially the contents of the exposés by B VI entitled "On the Significance of Biotin in Dermatology", and probably a manuscript by Thélin already available at that time.[1]

Since, for good reasons, treatment experiments are performed preferably not only in one testing place, but rather at various distant clinics, it was necessary to divide the rather modest sample supply obtained among three examiners. Professor Opitz of the University's Children's Clinic at Heidelberg received 15 x 20 tablets and 5 x 6 pills, Professor Hilber of the University's Children's Clinic at Munich received 10 x 20 tablets and 5 x 6 pills, and the physician for children's diseases, Dr. Folberth of Lörrach, received the remaining 5 x 20 tablets.

The fact that 3 pediatricians were chosen for this Biotin testing, and that they were given the first ever available test amounts in order to perform therapeutic examinations and research, leads to the conclusion that the erythematic-scaling dermatoses that pediatricians observe most frequently in infancy and young childhood were probably the object of greatest interest.

Biotin indications originated for the most part in the works of Gyögy, the experiments of Sydenstricker and an observation of Williams.

According to experiments performed largely in Czechoslovakia, Biotin has been shown to be most effective chiefly in the cases of Erythrodermia desquamativa Leiner, and especially the ailment related to this disease that affects the infant: seborrheid Dermatitis, which can be viewed as a non-generalized form of Leiner. Biotin is also effective against two particular forms of dermatitis — seborrhea and milk scaling. It was our task to verify these treatment results in our work, by confirming the findings with similar or equally favorable results, and possibly strengthening them by examining a wider spectrum of ailments, or in the case that the tests were negative, it was our task to search for other viable indications for Biotin.

Experiences with Biotin from our own Work with Seborrheid Dermatitis and Leiner's Disease

The first treatment experiments, conducted under the supervision of Professor Opitz, involved 5 cases of Leiner's disease. The infants daily

1 Ann. Pediatr. 172, 193 (1949)

received 4 x 1/2 tablets of DL-Biotin Ro 1-5125/13 perorally or 2 x 1 ccm DL-Biotin Ro 1-5125/14 intramuscularly. The duration of treatment for the individual patients was 8, 9, 18, 23 and 26 days.

Since no detectable effect could be determined after either oral or parenteral application, the experiments were terminated as purposeless in June of 1948. On the basis of cases of seborrhoid dermatitis described in the Thelin article and preserved in photographs, where the ailment cleared up after 2 weeks of treatment with 5 mg Biotin daily intramuscularly, the experiments were supposed to be resumed, but they never were, because the patients who were most suited to treatment with Biotin were far more frequently admitted to the University Skin Clinic rather than the University Pediatric Clinic. To this circumstance we attribute the fact that physicians who were active in the Heidelberg Dermatological Clinic at the time, such as Professor Kimmig and Dr. Kiessling, also undertook treatment experiments with Biotin, the results of which were printed in the publication by the same dermatologists, entitled "Clinical Experiments with Biotin Doses Administered Orally and Intramuscularly" (Dermatolog. Wochenschrift, volume 51, p. 1246 ff, 1951). As concerns the orally treated cases in the pediatric clinic, it could undoubtedly be shown, in light of the disorders of the digestive tract that often accompanied the Erythrodermia desquamativa Leiner, that only insufficient amounts of Biotin were absorbed. As regards the remaining intramuscularly treated cases, the lack of success could be attributed to the fact that either the amount of material administered was still too small, or that Biotin, as a single responsible deficiency factor, is not always sufficient to cure Leiner's disease.

Professor Hilber of the Munich University Pediatric Clinic also obtained a similar unsatisfactory result in Leiner's diseases with these first test amounts of Biotin.

There are no available data on the number of cases he treated therapeutically with Biotin. However, in a visiting lecture of Dr. Borkowsky, we find the mention that Biotin had remained ineffective in all cases of patients with Leiner's disease, whether administered orally or parenterally. As a result, the tests were considered terminated.

We could expect no report on experiences with Biotin from Dr. Folberth, who had, by chance, received only 5 x 20 tablets, and who very probably observes genuine Leiner only in exceptional cases in his pediatric practice.

The unfavorable results obtained at the Heidelberg and Munich University Pediatric Clinics with the first available test amounts of Biotin did not offer much hope for testing with the preparation and represented for us a beginning that was not exactly inspiring. However, in our efforts with Biotin, two facts seemed to stand out.

1. We succeeded in splitting the racemic DL-Biotin into its two optically active components, so that we could replace the Biotin Ro 1-5125 with d-Biotin Ro 1-5492, which is optically active and identical to the natural material.

2. Through a series of publications, d-Biotin won more and more significance, so that our vitamin H test preparation became the object of ever greater interest, and the yearly rising demand for samples could be filled completely.

Test Amounts Released

The following are the total test amounts of Biotin released to over 130 doctors from the end of 1947 until September 30, 1954.

DL-Biotin	Ro 1-5125/13	600 tablets of 5 mg each and
"	Ro 1-5125/14	60 pills of 1 ccm each with 5 mg working material
d-Biotin	Ro 1-5492/1	6400 tablets of 2 mg each
"	Ro 1-5492/2	14.860 pills of 1ccm with 5 mg
"	Ro 1-5492/3	37.140 tablets of 5 mg each
"	Ro 1-5492/4	70 pills of 2 ccm with 25 mg/ccm each
"	Ro 1-5492/5	3100 tablets with a break line to every 20 mg working material

Very interesting and impressive conclusions can be drawn from the comparison of Biotin use in individual years. Here, the samples provided for microbiological purposes are not taken into consideration.

a. Jahrgang	b. Ampullen à 5 mg	c. Tabletten à 5 mg	d. Tabletten à 2 mg	e. Gesamt-Wirkstoffmenge
1947	60 = 300 mg	600 = 3.000 mg	-	3,3 g
1948	687 = 3.435 mg)	-	1.400 = 2.800 mg	6,235 g
1949	636 = 3.180 mg	-	4.000 = 8.000 mg	11,18 g
1950	2.266 =11.330 mg	2.000 = 10.000 mg	1.000 = 2.000 mg	23,33 g
1951	1.578 = 7.890 mg	9.640 = 48.200 mg	-	56,09 g
1952	2.742 =13.710 mg	13.880 = 69.400 mg	-	83,11 g
1953	2.556 + 70 à 50 mg =16.280 mg	6.720 + 1.800 à 20 mg = 69.600 mg	-	84,38 g : 12 =7,03 g f. monatlich
1954	1.614 = 8.070 mg	5.020 + 700 à 20 mg = 26.500 mg	-	47,17 g : 9 =5,24 g f. monatlich.

Key -
a. year
b. pills of 5 mg
c. tablets of 5 mg
d. tablets of 2 mg
e. total working amount
f. monthly

To the same degree that the use of test amounts increased from one year to the next, the sphere of users grew, though we will refrain here from naming the various researchers individually. It will be sufficient to point out here that, from the oral and written reports we have received, and from the lecture manuscripts available to us and the works that have in the meantime been published, it is clear that Biotin is suitable for the treatment of diseases caused by vitamin H deficiency, such as Leiner's disease and other forms of seborrhoid dermatitis. Very often, it is capable alone of producing good effects. If Biotin causes no response in the case of the indications named, then, almost without exception, a positive result can be achieved with supplementary BVK doses. There are no clues for deciding whether or not a patient is favorably inclined to therapy with Biotin. It is always necessary to experiment. With the recession of the dermatologic al phenomena, there is almost always an improvement in the general condition and an increase in weight. Biotin administration causes a noticeable retardation of the healing process, but the liver treatment of the nursing mother, who is not to ingest any egg albumin during this period, leads to a significantly more rapid healing, as comparative treatments in the infant clinic in Chemnitz have shown. In the case of infants, medication with tablets was seen to be more favorable than injections.

Biotin and other Ailments

Seborrhoic eczema and genuine allergic eczema were not affected by supportive Biotin doses. On the other hand, vitamin H seemed to have a supportive effect in a number of cases of Acne vulgaris (Kiessling). According to Schmid ("Therapy with Biotin", Wiener Zeitschrift f. innere Medizin, v. 32, no. 1, 1951) an experiment with Biotin seems indicated in cases of iron-deficiency anemia and perniciosa that do not respond, or respond only insufficiently, to usual therapy. In his opinion, toxically caused pan-myelopathies are also favorably affected, whereby the erythropoesis is above all clearly stimulated. Partially surprising success could be obtained with Biotin in cases of chronic colitides, especially in the cases of patients whose feces were similar to those found in putrefaction dyspepsia.

High-dose Biotin in cases of rheumatic ailments

On the basis of observations by Thiers ("Therapeutic value of Biotin in Rheumatology" "Interet therapeutique de la biotine en rheumatologie", Rv. Rhum. No. 10, 543, 1951), who successfully treated 12 patients with arthrosis -- of whom 11 had bilateral rheumatic Cox arthrosis -- with 50 mg Biotin daily, corresponding therapy experiments with massive Biotin doses were undertaken at four different clinics. This after-check of Thiers' work was negative in all cases. In 2 patients with arthrosis who received 2 x 50 mg Biotin daily for 3 weeks, Dr. Schettler (Med. Univ. Clinic Marburg) was unable to induce any improvement. Biotin failed likewise in 2 cases of chronic polyarthritis, given in doses of 50 mg daily for 14 days (Dr. Scheiffarth, Head Physician at the Med. Univ. Clinic in Erlangen).

Dr. Zickgraf reports on a total of 22 cases treated with high doses of Biotin (3 of acute polyarthritis, 8 of primary-chronic and 11 of chronic or subacute polyarthritis forms). In cases of acute and primary-chronic polyarthritis, he injected 2 x 50 mg intramuscularly daily. The chronic and subacute cases received 2 x 20 mg orally each day, and in single cases 2 x 50 mg intramuscularly as well. Dr. Zickgraf was unable to determine any truly convincing effect in any of his patients, and came to the conclusion

that subacute or chronic polyarthritis is not a promising indication for Biotin therapy.

The treatment experiments undertaken by the head physicians at the rheumatism division of the pediatric clinic of the Inner Mission in Garmisch-Partenkirchen (Dr. Stoeber) likewise led to no positive results. Dr. Stoeber used the dosage prescribed by Thiers, and reported that in 3 cases of primary-chronic arthritis, she was unable to determine the effect of the Biotin.

Effect and Dosage

Biotin-deficiency dermatoses such as Leiner's disease and related ailments respond rapidly and certainly to Biotin administration, while so-called Biotin-insensitive dermatoses do not respond to vitamin H.

In the case of ailments with a questionable relation to Biotin (Akne vulgaris), vitamin H appears to produce a supportive effect. Likewise in the case of iron-deficiency anemia that does not respond to normally therapy, in the case of toxically caused panmyelopathies, chronic forms of colitis and also Perniciosa, a favorably effect of Biotin can more frequently be expected. In general, in the indications named, a daily dose of 5-10 mg orally is sufficient. The duration of treatment is usually 1-3 weeks. Occasionally it is possible to achieve a surprising effect after fewer days.

Tolerability -- Side Effects

Since no signs of hypervitaminosis were seen after large amounts of Biotin, either in animal experiments or clinical tests, we knew from the start that we need not reckon with any sort of side effects due to massive dosage. In the Heidelberg University Dermatological Clinic, single doses of up to 30 mg were administered daily and total doses of up to 770 mg were given for a cure. Also, in the Thiers after-check, up to 100 mg intramuscularly and 60 mg orally were given daily for 8 days to 3 weeks. Intolerance was never observed, whether the administration was oral or parenteral, and side effects such as increase in pruritus stimulation etc. were never reported.

Publications

The following articles concerning Biotin and our testing realm have already appeared -- 319

Lemke — "On the Ätiology and Therapy of Seborrhoid Dermatitis" ("Zur Ätiologie und Therapie der Dermatitis seborrhoides") Monatsschrift für Kinderheilkunde, V. 98, no. 8, pp. 350-353

Kiessling — "Clinical Experiences with Biotin Doses administered Orally and Intramuscularly" ("Klinische Erfahrungen mit innerlichen und intramuskulären Biotingaben") Dermatol. Wochenschr. 1951, no. 51, p. 1246

Liebe — "On the Aspect of Erythrodermia desquamative - Leiner" ("Zum Krankheitsbild der Erythrodermia desquamative - Leiner") Kinderärztl. Praxis, 20: 5, 216-23 (1952)

Koch — "Effect of Pyridoxine (Vit. B_6) and Biotin on the Glycogen Content of the Rat Liver" ("Einfluss der Pyridoxin (Vit. B_6) und Biotin auf den Gykogengehalt der Rattenleber") Internationale Zeitschr. f. Vitaminforschung, V. XXII, No. 4, 1951.

Guttmann — "The Treatment of Chronic Ailments, especially Multiple Sclerosis, with a Polyvitamintherapy" ("Die Behandlung chronischer Erkrankungen, insbesondere der multiplen Sklerose mit einer Polyvitamintherapie") Psychiatrie, Neurologie und medizinische Psychologie, V. 5, no. 10, pp. 362-369.

The following publications are yet to appear --

Vogtherr — "On the Effect of Biotin on the Water and Mineral Retention" ("Über die Beeinflussung des Wasser- und Mineralhaushaltes durch Biotin")

Geiner — "Biotin (vitamin H) and its Intracutaneous Administration in cases of Dermatosis" ("Das Biotin (Vitamin H) und seine intracutane Applikation bei einigen Dermatosen")

Noack — "Experiences with Biotin in the Pediatric Clinic of Stralsund" (Über Erfahrungen mit Biotin in der Kinderklinik Stralsund")

Final Remarks

From the information on pages 4-10 we see that Biotin is suitable for the treatment of seborrhoid dermatitis and its most acute form, Erythrodermia desquamative Leiner. Biotin has also proved useful in ailments related to the above. However, seborrhoic eczema does not respond to vitamin H. Sub-acute and chronic forms of polyarthritis likewise do not react to Biotin. In the cases of Pernicious and iron-deficiency anemias that do not respond to normal treatment, biotin can be tried with a promise of some success. Chronic forms of colitis reacted in part surprisingly well.

Intolerability phenomena have never been observed, even in the case of the highest dosage of 100 mg Biotin daily, administered orally or parenterally.

Thus the first question we posed in the beginning -- "Where do we stand with our Biotin experiments?" -- can be answered quite satisfactorily.

However, in order to be able to provide an answer to thesecond question that will take care of all the pros and cons, we must be clear on the point that in normal times, with a regulated and sufficient supply of nourishment, ailments due to Biotin-deficiency are hardly ever observed. Only when pregnant mothers eat incorrectly, thus when there is qualitative and quantitative undernourishment, need we expect an increase in seborrhoid dermatitis during infancy and the early years of childhood. Further, it must be considered that Biotin deficiency phenomena can also always be slightly reduced by administering raw liver and kidney to the mother.

Thus, a greater need for Biotin is only to be expected during times of emergency and scarcity, which are naturally accompanied by a rapid and com-

siderable worsening of nutritional conditions.

For these reasons, in our opinion, introduction of Biotin as an express specialty medicine is not justified. The supply and cost of such are certainly not at all proportionate to the need.

Whether the demand for our vitamin H preparation had been as great if the material had had to be paid for, cannot be judged objectively.

However, in order to be able to meet the demands for Biotin, we recommend the inclusion of the therapeutic forms of the preparation, tablets and pills of 5 mg each, in the price lists, without adding any kind of propaganda for the preparation.

Grenzach, October 13, 1954

[redacted]

Rapport von Dr. Dübler

Abteilung W 2

0030

4

Biotin Ro 1-5492

Inch-Nr 1

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Sowohl aus den auf den Seiten 4-10 geschilderten Erfahrungen als auch aus den in unserem Arbeitsbereich erschienenen Publikationen über Biotin geht hervor, daß sich das Präparat zur Behandlung von Dermatitis seborrhoides und seiner schwersten Form: dem Morbus Leiner eignet. Biotin hat sich auch bei diesem Formenkreis nahestehenden Affektionen bewährt. Seborrhoisches Ekzem spricht auf Vitamin H nicht an; ebenso wenig lassen sich subakute und chronische Polyarthritiden damit beeinflussen. Chronische Colitiden reagierten auf Biotin teilweise überraschend gut. Bei auf übliche Therapie nicht ansprechenden Fällen von Perniciosa und Eisenmangel-Anämien kann das Präparat mit Aussicht auf Erfolg versucht werden.

Unverträglichkeit ist auch bei höchster Dosierung von täglich 100 mg Biotin, gleichgültig ob oral oder parenteral verabreicht, nie beobachtet worden. Auch Nebenerscheinungen im Sinne allergischer Reaktionen sind niemals aufgetreten. Es muß jedoch betont und festgehalten werden, daß in normalen Zeiten mit ausreichender Ernährungslage durch Biotin-Mangel bedingte Erkrankungen kaum zur Beobachtung kommen. Nur wenn schwangere Mütter sich falsch ernähren, wenn also qualitative oder quantitative Unterernährung vorliegt, ist mit einer stärkeren Verbreitung von Dermatitis seborrhoides im Kleinkind- und Säuglingsalter zu rechnen. Es muß ferner darauf hingewiesen werden, daß Biotin-Mangelerscheinungen auch durch jeweils an die Mütter zu verabfolgende Gaben von roher Leber und Niere beseitigt werden können.

Ein größerer Bedarf an Biotin wird daher nur dann zu erwarten sein, wenn Notzeiten, die naturgemäß stets mit Verschlechterung der Ernährungsverhältnisse einherzugehen pflegen, hereinbrechen.

Aus diesen Gründen läßt sich unseres Erachtens die Einführung des Biotin als ausgesprochene Spezialität nicht rechtfertigen. Um dennoch den jeweiligen Anforderungen nach Biotin nachkommen zu können, wird empfohlen, die therapeutischen Anwendungsformen des Präparates, Tabletten und Ampullen zu je 5 mg Wirkstoff, in die Preisliste aufzunehmen, ohne daß irgendein propagandistischer Aufwand für das Präparat erfolgt.

B i o t i n Ro 1-5492

Es will uns scheinen, daß es sinnvoll und zweckmäßig ist, sobald als möglich eine Entscheidung über das weitere Schicksal unseres Vitamin H-Präparates B i o t i n herbeizuführen.

Wenn auch eine systematische und eingehende Prüfung des Biotin nicht erfolgte, das Präparat neben der klinischen Prüfung anderer Versuchs-Präparate gewissermaßen nur "mitgelaufen" ist, weil außer der Leiner'schen Erkrankung und diesem Krankheitsbild verwandten Affektionen weitere Indikationen für seine Anwendung nur wenig Aussicht auf Erfolg boten, so dürfte es dennoch an der Zeit sein, uns nunmehr Rechenschaft über seine therapeutische Brauchbarkeit abzulegen, damit die Fragen, vor die wir uns gestellt sehen "Wo stehen wir mit unseren Biotin-Erfahrungen?" und "Was sollte geschehen, dem Präparat einen gebührenden Umsatz zu sichern?" klar und eindeutig beantwortet werden können.

Es ist im Rahmen dieses Rapportes natürlich nicht möglich, über alle mit Biotin behandelten Fälle zu berichten, weil - wie schon kurz erwähnt - die Prüfung nicht systematisch und unter Berücksichtigung aller zu einer kritischen Beurteilung erforderlichen Einzelheiten durchgeführt werden konnte. Da in den Jahren 1947 bis 1950/51 Prüfungsbogen mit genau definierten Fragen, deren Beantwortung zur Urteilsbildung herangezogen werden könnte, nicht verausgabt worden sind, und weil die zur Auswertung vorliegenden Unterlagen bis auf

spärliche Ausnahmen zu wenig präzise Angaben (über Fallzahl, Diagnose, Wirksamkeit, Dosierung, Verträglichkeit und Nebenerscheinungen sowie Zusatztherapie u.a.m.) enthalten, also unzulänglich und zu allgemein formuliert sind, so ist der Versuch, auch nur einigermaßen exakt und vollständig zu referieren a priori zum Scheitern verurteilt. Daher kann hier lediglich eine lückenhafte Schau gegeben, nur ein ungefährer Überblick vermittelt werden, der aber gestattet, eine befriedigende Antwort auf die aufgeworfenen Fragen zu erhalten.

Allgemein klinische Beobachtungen und Ergebnisse

Racemisches DL-Biotin Ro 1-5125/13 und /14 wurde uns Mitte November 1947 erstmals in einer Menge von 30 x 20 Tabletten zu je 5 mg und 10 x 6 Ampullen mit 1 ccm zu gleichfalls je 5 mg Wirkstoff von Basel VI avisiert. In dem betreffenden Schreiben (Nr.48 vom 13.11.47) wurde Bezug genommen auf eine zwischen Basel und Grenzach erfolgte Rücksprache. Bei dieser dürfte das Thema Biotin: im besonderen der Inhalt des von B VI verfaßten Exposés "Über die Bedeutung von Biotin in der Dermatologie" und wahrscheinlich ein damals schon vorhandenes Manuskript der Arbeit Thélin +) Gegenstand der Unterredung gewesen sein.

Da man Behandlungsversuche aus guten Gründen nicht nur an einer Prüfstelle allein sondern nach Möglichkeit an verschiedenen örtlich voneinander getrennten Kliniken anstellen

+) Ann.Paediatr. 172, 193 (1949)

läßt, war es notwendig, die erhaltene überaus bescheidene Mustermenge noch unter 3 Prüfer aufzuteilen. Es erhielten Prof.Opitz von der Univ.Kinderklinik Heidelberg 15 x 20 Tabletten + 5 x 6 Ampullen, Prof.Hilber von der Univ.Kinderklinik München 10 x 20 Tabletten + 5 x 6 Ampullen und der Facharzt für Kinderkrankheiten, Dr.Folberth, Lörrach, den Rest von 5 x 20 Tabletten.

Wenn insbesondere 3 Pädiater für diese Biotin-Prüfung ausgewählt und ihnen die ersten überhaupt verfügbaren Versuchsmengen zur Durchführung therapeutischer Untersuchungen angeboten worden sind, so läßt diese Tatsache unschwer darauf schließen, daß wohl vornehmlich die im Säuglings- und Kleinkindalter Kinderärzten häufiger zur Beobachtung kommenden erythematös-schuppenden Dermatosen im Vordergrund des Interesses gestanden haben mögen.

Die Indikationen von Biotin gehen im wesentlichen auf Arbeiten von György, auf Versuche von Sydenstricker und auf eine Beobachtung von Williams zurück.

Nach vor allem in der Tschechoslowakei gesammelten Erfahrungen hatte sich Biotin hauptsächlich bei Erythrodermia desquamativa Leiner, der diesem Krankheitsbild nahestehenden Affektion des Säuglings: der Dermatitis seborrhoides, die als ein nicht generalisierter Leiner aufgefaßt werden kann, sowie bei zwei besonderen Formen der Dermatitis, dem Gneiss und dem Milchschorf wirksam erwiesen. Es galt nun, diese Behandlungsergebnisse in unserem Arbeitsgebiet nachzuprüfen, um die Befunde durch ähnliche oder gleich günstige Resultate

zu bestätigen und evtl. durch umfangreicheres Krankengut zu erhärten, oder bei negativem Ausfall der Untersuchungen nach anderen tragbaren Indikationen für Biotin zu fahnden.

Erfahrungen mit Biotin aus unserem eigenen Arbeitsgebiet bei Dermatitis seborrhoides und Morbus Leiner

Die unter Aufsicht von Prof.Opitz durchgeführten ersten Behandlungsversuche erstreckten sich auf 5 Fälle von Leinerscher Erkrankung. Die Säuglinge erhielten täglich 4 x 1/2 Tablette DL-Biotin Ro 1-5125/13 peroral oder täglich 2 x 1 ccm DL-Biotin Ro 1-5125/14 intramuskulär. Die Behandlungsdauer der einzelnen Patienten betrug 8, 9, 18, 23 und 26 Tage.
Da weder nach peroraler noch parenteraler Applikation eine nachweisbare Wirkung festgestellt werden konnte, sind die Versuche als aussichtslos im Juni 1948 abgebrochen worden. Sie sollten auf Grund des in der Arbeit Thélin beschriebenen und in Bildern festgehaltenen Falles von Dermatitis seborrhoides, der bei täglich 5 mg Biotin intramuskulär in 2 Wochen abheilte, zwar wieder aufgenommen werden, doch blieb es dabei, weil die zur Behandlung mit Biotin geeigneten Patienten weit mehr in die Univ.Hautklinik als die Univ.Kinderklinik eingewiesen wurden. Auf diesen Umstand war es zurückzuführen, daß damals an der Heidelberger Hautklinik tätige Ärzte wie beispielsweise Prof.Kimmig und Dr.Kießling ebenfalls Behandlungsversuche mit Biotin aufnahmen, deren Ergebnisse in der Publikation des zuletzt genannten Dermatologen "Klinische Erfahrungen mit innerlichen und intramuskulären Biotingaben" (Dermatolog.Wochenschrift, Heft 51, S.1246 ff, 1951) ihren Niederschlag

gefunden haben. Bei den in der Kinderklinik peroral behandelten Fällen wäre in Anbetracht der mit der Erythrodermia desquamativa Leiner nicht selten einhergehenden Störungen des Verdauungstraktes zwanglos vorstellbar, daß nur ungenügende Biotinmengen zur Resorption gelangt sind; bei den restlichen intramuskulär behandelten Fällen läßt sich der Mißerfolg nur so deuten, daß entweder die verabfolgte Wirkstoffmenge noch zu gering war, oder daß Biotin als allein verantwortlicher Mangelfaktor zu einer Heilung des Leiner nicht immer ausreicht.

Zu einem ebenso unbefriedigenden vorläufigen Behandlungsergebnis Leinerscher Erkrankungen mit ersten Biotinversuchsmengen kam auch Prof.Hilber von der Univ.Kinderklinik in München.
Über die Anzahl der von ihm in die Therapie mit Biotin einbezogenen Fälle liegen Angaben nicht vor. Doch findet sich in einem Besuchsbericht Dr.Borkowsky's der Hinweis, daß Biotin bei allen an Leiner erkrankten Patienten sowohl bei peroraler als auch bei parenteraler Darreichung versagt habe, weshalb die Prüfung zunächst als abgeschlossen betrachtet werden müsse.

Von Dr.Folberth, der anscheinend bloß gefälligkeitshalber 5 x 20 Tabletten erhalten hatte und der sehr wahrscheinlich echte Leiner in seiner Kinderpraxis nur ganz ausnahmsweise zu Gesicht bekommt, konnte ein Bericht über Erfahrungen mit Biotin nicht erwartet werden.

Diese an den Univ.Kinderkliniken in Heidelberg und München gewonnenen ungünstigen Erfahrungen mit den ersten verfügbaren Biotinversuchsmengen waren für die Prüfung des Präparates wenig ermutigend und bedeuteten für uns einen nicht gerade verheißungsvollen Anfang. Doch kamen uns zwei Tatsachen in unseren Bemühungen um Biotin außerordentlich entgegen.

1. war es gelungen, das racemische DL-Biotin in seine beiden optisch aktiven Komponenten zu spalten, so daß wir Biotin Ro 1-5125 durch das mit dem natürlichen Wirkstoff identische, optisch aktive d-Biotin Ro 1-5492 ersetzen konnten; und

2. gewann, hervorgerufen durch eine Reihe von Publikationen, d-Biotin mehr und mehr an Bedeutung, so daß unser Vitamin H-Versuchs-Präparat zunehmend wachsendem Interesse begegnete und der sich von Jahr zu Jahr steigernde Bedarf an Mustern auch voll gedeckt werden konnte.

Verausgabte Versuchsmengen

Die seit Ende 1947 bis 30.9.1954 an über 130 Ärzte und Ärztinnen insgesamt verausgabten Versuchsmengen belaufen sich für

DL-Biotin	Ro 1-5125/13	auf 600	Tabletten zu je 5 mg und
"	Ro 1-5125/14	" 60	Ampullen zu je 1 ccm mit 5 mg Wirkstoff
d-Biotin	Ro 1-5492/1	" 6400	Tabletten zu je 2 mg
"	Ro 1-5492/2	" 14.860	Ampullen zu je 1 ccm mit 5 mg
"	Ro 1-5492/3	" 37.140	Tabletten zu je 5 mg
"	Ro 1-5492/4	" 70	Ampullen zu 2 ccm mit je 25 mg/ccm
"	Ro 1-5492/5	" 3100	Tabletten mit Bruchrille zu je 20 mg Wirkstoff.

Recht interessante und eindrucksvolle Aufschlüsse lassen sich aus der Gegenüberstellung des Biotin-Verbrauchs in den einzelnen Jahren gewinnen, wobei die für mikrobiologische Zwecke zur Verfügung gestellten Muster im Ansatz unberücksichtigt geblieben sind:

Jahrgang	Ampullen à 5 mg	Tabletten à 5 mg	Tabletten à 2 mg	Gesamt-Wirkstoffmenge
1947	60 = 300 mg	600 = 3.000 mg	-	3,3 g
1948	687 = 3.435 mg)	-	1.400 = 2.800 mg	6,235 g
1949	636 = 3.180 mg	-	4.000 = 8.000 mg	11,18 g
1950	2.266 =11.330 mg	2.000 = 10.000 mg	1.000 = 2.000 mg	23,33 g
1951	1.578 = 7.890 mg	9.640 = 48.200 mg	-	56,09 g
1952	2.742 =13.710 mg	13.880 = 69.400 mg	-	83,11 g
1953	2.556 + 70 à 50 mg =16.280 mg	6.720 + 1.800 à 20 mg = 69.600 mg	-	84,38 g : 12 =7,03 g monatlich
1954	1.614 = 8.070 mg	5.020 + 700 à 20 mg = 26.500 mg	-	47,17 g : 9 =5,24 g monatlich.

Im selben Maße wie der Verbrauch an Versuchsmengen von einem Jahr zum anderen sich vergrößerte, wuchs der Abnehmerkreis,

doch soll verzichtet werden, die verschiedenen Prüfer hier alle einzeln aufzuführen. Es mag an dieser Stelle genügen, darauf hinzuweisen, daß aus den mündlich und schriftlich erhaltenen Erfahrungsberichten, den bei uns vorliegenden Vortragsmanuskripten und aus den inzwischen zur Veröffentlichung gelangten Arbeiten eindeutig hervorgeht, daß Biotin zur Behandlung von durch Mangel an Vitamin H ausgelösten Krankheiten, wie z.B. des Leiner und anderer seborrhoischer Dermatitiden geeignet ist. Recht häufig vermag es schon allein gute Wirksamkeit zu entfalten. Spricht Biotin bei den genannten Indikationen nicht an, so läßt sich fast ohne Ausnahme durch zusätzliche BVK-Gaben ein positives Behandlungsergebnis erzielen. Anhaltspunkte, ob ein Patient einer Biotin-Therapie zugänglich ist oder nicht, gibt es nicht; stets ist man auf den Versuch angewiesen. Mit dem Rückgang der Hauterscheinungen kommt es nahezu regelmäßig zur Besserung des Allgemeinbefindens und zum Gewichtsanstieg. Durch Biotin-Verabfolgung wird eine auffällige Beschleunigung des Heilverlaufs erreicht, jedoch führt die Leberbehandlung der stillenden Mutter, die während dieser Zeit kein Eieralbumin zu sich nehmen soll, einen wesentlich schnelleren Eintritt der Heilung herbei, wie in der Säuglingsklinik in Chemnitz angestellte Vergleichsbehandlungen ergeben haben. Bei Säuglingen hat sich die Medikation von Tabletten besser bewährt als die von Injektionen.

Biotin bei anderen Affektionen

Seborrhoisches Ekzem und echte allergische Ekzeme konnten durch unterstützende Biotin-Gaben nicht beeinflußt werden. Indessen schien Vitamin H bei einer Anzahl von Akne vulgaris-Fällen eine unterstützende Wirkung zu haben (Kießling).

Nach Schmid ("Therapie mit Biotin", Wiener Zeitschr.f.innere Medizin, 32.Jahrg., H.1, 1951) erscheint in Fällen von Eisenmangelanämien und auch bei Perniciosa, die auf die übliche Therapie nicht oder nur unzureichend ansprechen, ein Versuch mit Biotin angezeigt. Auch toxisch bedingte Panmyelopathien werden nach seiner Meinung günstig beeinflußt, wobei vor allem die Erythropoese deutlich stimuliert werde. Teilweise überraschende Erfolge konnten mit Biotin bei chronischen Colitiden erzielt werden, besonders bei Patienten, deren Stühle denen der Fäulnisdyspepsie ähnlich waren.

Biotin hochdosiert bei rheumatischen Affektionen

Auf Grund von Beobachtungen von Thiers ("Intéret thérapeutique de la biotine en rhumatologie", Rv.Rhum.No.10, 543, 1951), der 12 Arthrosekranke - davon 11 mit bilateraler rheumatischer Coxarthrose - erfolgreich mit 50 mg Biotin täglich behandelte, wurden an 4 verschiedenen Kliniken entsprechende Therapieversuche mit massiven Biotin-Dosen veranlaßt. Diese Nachkontrolle der Arbeit Thiers ist an allen Prüfstellen völlig negativ verlaufen. Doz.Schettler (Med.Univ.Klinik Marburg) vermochte bei 2 Arthrotikern, die täglich 2 x 50 mg Biotin über 3 Wochen bekamen, keine Besserung zu erzwingen. Ebenso versagte Biotin bei 2 Fällen von chronischer Polyarthritis unter Gaben von 50 mg täglich über 14 Tage (Doz.Dr.Scheiffarth, Oberarzt a.d.Med.Univ.Klinik Erlangen).

Über Erfahrungen mit Biotin hochdosiert an insgesamt 22 Fällen (3 akute Polyarthritis, 8 primär-chronische und 11 chronische bzw. subakute Polyarthritiden) berichtet

Doz.Dr.Zickgraf. Er injizierte bei akuter und primär-chronischer Polyarthritis täglich 2 x 50 mg intramuskulär, die chronischen und subakuten Fälle erhielten täglich 3 x 20 mg per os und in einzelnen Fällen gleichfalls 2 x 50 mg intramuskulär.Dr.Z. konnte bei keinem einzigen Patienten einen wirklich überzeugenden Behandlungseffekt feststellen und kommt zu dem Resultat, daß die subakute oder chronische Polyarthritis wohl kaum eine aussichtsreiche Indikation für die Biotin-Therapie darstellt.

Auch die an der Rheuma-Abteilung der Kinderklinik der Inneren Mission in Garmisch-Partenkirchen angestellten Behandlungsversuche durch die Chefärztin, Priv.Doz. Dr.Stoeber mit hoher Biotin-Dosierung führten zu keinem positiven Behandlungsergebnis. Dr.St. hielt sich an die von Thiers angegebene Dosierung und teilte uns mit, daß sie bei 3 Fällen von primär-chronischer Arthritis keine Wirkung des Biotin feststellen konnte.

Wirkung und Dosierung

Biotin-Mangel-Dermatosen wie Leiner und ihm nahestehende Affektionen sprachen rasch und sicher auf Biotin-Zufuhr an, während sog. Biotin-unempfindliche Dermatosen auf Vitamin H-Verabreichung nicht reagierten.
Bei Krankheiten mit fraglicher Beziehung zum Biotin (Akne vulgaris) scheint Vitamin H eine unterstützende Wirkung zu

entfalten. Auch bei Eisenmangel-Anämien, die auf übliche Therapie nicht oder nur vermindert ansprechen, bei toxisch bedingten Panmyelopathien, bei chronischen Colitiden und sogar bei Perniciosa kann öfters ein günstiger Einfluß des Biotin erwartet werden. Im allgemeinen kommt man bei den genannten Indikationen mit einer Dosierung von täglich 5 - 10 mg per os oder intramuskulär aus. Meist beträgt die Behandlungsdauer 1 - 3 Wochen. Mitunter gelingt es schon nach weniger Tagen eine überraschende Wirkung zu erzielen.

Verträglichkeit - Nebenwirkungen

Da sich weder im Tier-Experiment noch in klinischen Versuchen Erscheinungen einer Hypervitaminose nach größerer Biotin-Zufuhr gezeigt haben, war von vornherein mit Nebenwirkungen irgendwelcher Art durch massive Dosierung nicht zu rechnen. In der Heidelberger Univ.Hautklinik wurden täglich Einzelgaben bis zu 30 mg und Gesamtgaben bis zu 770 mg Biotin für eine Kur verabfolgt. Auch bei Nachkontrolle der Arbeit Thiers wurden täglich bis 100 mg intramuskulär und 60 mg per os stets über 8 Tage bis zu 3 Wochen gegeben. Niemals wurden Unverträglichkeit weder bei peroraler noch parenteraler Verabreichung beobachtet und niemals sind uns Nebenerscheinungen wie Juckreiz-Verstärkung usw. mitgeteilt worden.

Veröffentlichungen

In unserem Prüfungsbereich sind nachstehend aufgeführte Arbeiten, die sich mit Biotin beschäftigen, bereits

erschienen:

Lemke: "Zur Ätiologie und Therapie der Dermatitis seborrhoides"
Monatsschrift für Kinderheilkunde, Bd.98, H.8, S.350-353

Kiessling: "Klinische Erfahrungen mit innerlichen und intramuskulären Biotingaben"
Dermatol.Wochenschr.1951,H.51, S.1246

Liebe: "Zum Krankheitsbild der Erythrodermia desquamativa-Leiner "
Kinderärztl.Praxis, 20:5, 216-23 (1952)

Koch: "Einfluß des Pyridoxin (Vit.B_6) und Biotin auf den Glykogengehalt der Rattenleber"
Internationale Zeitschr.f.Vitaminforschung, Bd.XXII, H.4, 1951.

Guttmann: "Die Behandlung chronischer Erkrankungen, insbesondere der multiplen Sklerose mit einer Polyvitamintherapie"
Psychiatrie, Neurologie und medizinische Psychologie", 5.Jahrg., H.10, S.362-369.

Folgende Publikationen stehen noch in Aussicht:

Vogtherr: "Über die Beeinflussung des Wasser- und Mineralhaushaltes durch Biotin"

Geimer: "Das Biotin (Vitamin H) und seine intracutane Applikation bei einigen Dermatosen"

Noack: "Über Erfahrungen mit Biotin in der Kinderklinik Stralsund".

Schlußbetrachtung

Aus den Ausführungen auf den Seiten 4 - 10 geht hervor, daß Biotin zur Behandlung von Dermatitis seborrhoides und seiner

schwersten Form: der Erythrodermia desquamativa Leiner geeignet ist. Auch bei diesem Formenkreis verwandten Affektionen hat sich Biotin bewährt. Seborrhoisches Ekzem spricht auf Vitamin H jedoch nicht an. Ebenso wenig lassen sich subakute und chronische Polyarthritiden mit Biotin beeinflussen. Bei auf übliche Therapie nicht ansprechenden Fällen von Perniciosa und Eisenmangel-Anämien kann Biotin mit Aussicht auf Erfolg versucht werden. Chronische Colitiden reagierten teilweise überraschend gut.

Unverträglichkeitserscheinungen sind auch bei höchster Dosierung von täglich 100 mg Biotin, oral oder parenteral verabreicht, nie beobachtet worden.

Damit dürfte die erste Frage, die wir uns eingangs vorgelegt haben "Wo stehen wir mit unseren Biotin-Erfahrungen?" hinreichend beantwortet sein.

Um aber auf die zweite Frage eine alles Für und Wider berücksichtigende Antwort geben zu können, muß man sich darüber im klaren sein, daß in normalen Zeiten mit geregelter und ausreichender Ernährungslage die auf Biotin-Mangel zurückgehenden Erkrankungen kaum zur Beobachtung kommen. Nur wenn schwangere Mütter sich falsch ernähren, wenn es also zu qualitativer und quantitativer Unterernährung kommt, ist mit einem Anwachsen der Dermatitis seborrhoides im Kleinkind- und Säuglingsalter zu rechnen. Es ist ferner zu berücksichtigen, daß Biotin-Mangelerscheinungen auch durch Verabfolgung von roher Leber und Niere via Mutter jederzeit leicht behoben werden können.

Ein größerer Bedarf an Biotin wird also nur dann zu erwarten sein, wenn Notzeiten hereinbrechen, die naturgemäß mit einer raschen und erheblichen Verschlechterung der Ernährungsverhältnisse einhergehen.

Aus diesen Gründen läßt sich unseres Erachtens die Einführung des Biotin als ausgesprochene Spezialität nicht rechtfertigen. Aufwand und Kosten für eine solche stehen sicherlich in keinem Verhältnis zum Bedarf.
Ob die Nachfrage nach unserem Vitamin H-Versuchs-Präparat auch so groß gewesen wäre, wenn es hätte bezahlt werden müssen, läßt sich objektiv nicht beurteilen.
Um jedoch den bestimmt nicht ausbleibenden Anforderungen nach Biotin entsprechen zu können, wird empfohlen, die therapeutischen Anwendungsformen des Präparates, Tabletten und Ampullen zu je 5 mg Wirkstoff in die Preisliste aufzunehmen, ohne jedoch irgendwelche Propaganda vorzusehen.

Dieber

Grenzach, den 13.Oktober 1954
W/Dr.Dbr./Ka

Translation

Red Corner Report

Dr. R. Konz, Dr. A. Ronco, Dr. C. Bally VI/Klin.

Basle, August 23, 1955

Biotin Ro 1-5492

Results of the Clinical Study 1947-1955*)

Compounds: Ro 1-5492/2 = Ampuls 1 cc, 5°/oo Solution
Ro 1-5492/3 = Tablets 5 mg.

In the past nine years, biotin has been tested in expanded clinical studies in the Basle area and to some extent in Betley. The synthetic process developed by Roche made it possible to supply adequate amounts of the new substance for therapeutic testing, and dermatologists in particular began to be increasingly interested in the compound as a result of in vivo findings.

Reports emanating from depleted areas in Eastern Europe aroused the interest of physicians. Clinicians in Czechoslovakia were the first to report that severe cases of erythroderma desquamativa Leiner-Moussous had been cured in relatively short time. Their findings were subsequently confirmed by clinical investigators in other European countries (Switzerland, Germany, France, Italy, Yugoslavia, Austria etc.) as well as in the Near East (Israel).

It was found that the various forms of dermatitis seborrhoides in the newborn and in infants of the first trimenon as defined by Moro ("Eczema Infantum and Dermatitis Seborrhoides", Berlin, 1932) respond to biotin in more or less the same way. This would indicate that Leiner's erythrodermia which is the generalized form of this disease does not occupy a unique position. The localized forms with symptoms appearing most often on the body and/or face or on the hairy part of the head (crusta lactea, seborrhea) likewise respond to biotin.

Of the total 211 erythrodermia cases suitable for evaluation, 181 were cured with biotin; the corresponding figures for seborrhoid dermatitis with localized symptoms are 152 out of 192 cases treated. Biotin was used with analogous results in a similarly large number of less well-defined cases which are, however not included in the evaluation. Not only do these results attest to the reliability of the compound's effect, they confirm, at the same time, the etiologic or pathogenetic unity of the described symptoms.

As a rule, daily dosage comprised 2-4 tablets and/or 1-2 ampuls at 5 mg. In these doses, biotin brought about disappearance of the general symptoms and skin changes in app. 1-3 weeks; only rarely was it necessary to continue treatment beyond this time, usually when the face or head were involved. Occasionally, concomitant treatment with Vitamin B-complex e.g. in the form of Becozym "Roche" is indicated. Biotin administered in the above-mentioned as well as in higher dosage was always tolerated well locally and generally.

The investigators are almost unanimous in their opinion that biotin is preferable to all other remedies and therapeutic measures commonly employed in the treatment of dermatitis seborrhoides. They are in favor of making the compound commercially available pointing out the relatively high incidence of the disease in its various forms, the tendency of local as well as generalized complications (generalization, secondary infections) and the refractory nature of the disease which is sometimes a serious problem for the physician. On several occasions, clinicians have deplored the fact that they cannot prescribe biotin in the ordinary way; they find it difficult to understand why we delay introducing the compound on the market.

After the first or second trimenon, the effectiveness of biotin declines rapidly. For this reason, the drug is not expected to be generally successful in the treatment of older children or adults. In addition, results have been disappointing until now in all other dermatoses regardless of the patient's age. In the majority of these cases, biotin proved completely ineffective, but occasionally a partial or supportive effect was seen in patients with acne vulgaris, rosacea, true seborrhea and seborrhoid eczema; there are isolated reports of good results in cases of panmyelopathy, anemia, colitis and arthritis. To-date we have unfortunately not succeeded in having these findings systematically checked by other investigators.

It would appear then, that biotin therapy as of now is indicated only in the various types of dermatitis seborrhoides. Even under normal conditions, this illness is not uncommon in Central and Western Europe and often difficult to attack with available therapeutic methods. But biotin brings about a rapid cure in almost all cases without causing any complications.

The clinical study of the effects of biotin in dermatitis seborrhoides can be considered completed. At present, nothing would be gained by continuing the experiments. Only practical experience will show whether the compound has therapeutic possibilities beyond the present area of indication. Its development is not furthered if the drug continues to be available free of charge.

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Biotin in Dermatitis Seborrhoides and Leiner's Disease

(+)-Biotin Ro 1-5492 has been distributed to 135 physicians in 20 countries, mainly in the form of 5 mg tablets (Ro 1-5492/3) and 1cc ampuls also containing 5 mg (Ro 1-5492/2) To-date, app. 25,000 ampuls and 100,000 tablets have been sent out.

Interest centered on the treatment of dermatitis seborrhoides in the newborn and in infants of the first trimenon (More: "Eczema infantum and dermatitis seborrhoides", Berlin, 1932). It has been established that both the localized--crusta lactea and seborrhea-- and the generalized form of erythrodermia desquamativa Leiner-Moussous are more rapidly and more reliably effected by biotin than by all other available methods of treatment.

Our evaluation based on 85 statements by our clinical investigators on the application of biotin in dermatitis seborrhoides and 70 reports on its use in erythrodermia desquamativa Leiner *) shows results to be very encouraging: 152 out of 192 positive, 131 out of 211 cases positive and only 20 and 24 negative results. Approximately the same success quotient was obtained in an equally large group of cases which are, however, not suitable for inclusion in the evaluation. The term "positive" treatment result refers to cases which were cured at a more rapid, at times considerably more rapid rate than ever observed with the previously applied therapeutic (or dietetic) measures or those cases which had previously responded inadequately to treatment.

The above figures indicate that with regard to biotin response basically no differences exist between dermatitis seborrhoides and erythrodermia desquamativa Leiner. This may also be taken as one more indication that these two dermatoses represent one etiologic and pathogenetic unit without any distinct lines of demarcation.

A great number of reports from the Central and Western European countries show that biotin was just as effective in countries with normal living conditions as it had been found to be in the deprived areas (East European countries, postwar Germany and Austria).

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*) Several of the cases described as erythrodermia desquamativa Leiner may actually have been seborrhoid dermatitis in the strict sense of the word; some dermatologists use these terms synonymously.

The investigators report the same positive results as described in the earlier publications originating from the Czech. clinics. There is sufficient evidence then that seborrhoid dermatoses in infants is by no means uncommon in our areas, and that biotin is the drug of choice; this finding claims practically unanimous support in the entire testing area.

Seborrhoid dermatitis until now required as a rule extensive treatment (over a period of weeks or months) especially in its generalized form i.e. Leiner's erythrodermia where complications are to be expected (mostly severe intestinal infections, respiratory infections and various forms of otitis) which often prove fatal to the infants; cases responding to biotin, on the other hand, are frequently cured within 1-2 weeks, occasionally requiring 3-4 weeks or more of treatment. Scaling and reddening of the skin subside within the first 3-5 days of therapy; after 1-1 1/2 weeks eruptions have almost completely disappeared in the majority of cases.

Investigators are somewhat divided with regard to the most effective mode of treatment; some believe parenteral application is indicated on the theory that disturbed gastrointestinal function often goes hand in hand with seborrhoid dermatitis and tends to impair reception of the active substance; others perceive these gastrointestinal symptoms to be the result of a biotin deficiency-induced change in the intestinal flora and therefore recommend oral medication. Occasionally, the treatment combines oral and parenteral administration.

Daily dosage: generally (in combination treatment every 2 days) 1-2 ampuls cont. 5 mg biotin Ro 1-5492/2 administered intramuscularly or 1-4 tablets cont. 5 mg biotin Ro 1-5492/3 administered per os. In the majority of cases, a 1-3 week treatment period was required, occasionally 4 weeks and in exceptional cases up to 2 months. If a therapeutic effect is not noticeable at that time, continued medication would be pointless.

Available data do not permit a definitive explanation of the unsuccessful application of biotin in seborrhoid dermatitis. It should be mentioned in passing that obscurities of nomenclature and overlapping in terminology occasionally led to the application of biotin in cases where it was in no way indicated, thus merely simulating lack of success. Aside from this, it would be most difficult to separate refractory cases from cases which respond promptly to biotin therapy.

Glanzmann makes reference to a "constitutional resistance" and differentiates between cases which have a good therapeutic response to biotin and the pure "deficiency types".

Observations by Lemke*) indicate that in addition to an actual biotin deficiency, impaired utilization of the active substance may be a factor in the therapeutic effectiveness in seborrhoid dermatitis; he succeeded in curing many recalcitrant cases which failed to respond to biotin alone by combining biotin with vitamins of the B complex. (Decamyn Roche). This finding is all the more remarkable since the vitamins contained in Decamyn were ineffective in the absence of biotin in the cases reported by Lemke. It seems that vitamin B6 and/or panthothenic acid have a special significance regarding this synergism with biotin; better results were obtained with the entire B complex than with the individual components. Lemke's findings were confirmed by H. Klingelhöfer, Dermat. Clinic, University of Heidelberg (Dermatologische Wochenschrift, 1951, 51, 1246).

It is important to watch for gastrointestinal disorders-especially in cases where treatment is begun relatively late-since the resorption of orally administered biotin is made more difficult. According to the literature, dyspepsia is the predominant cause of sudden death in Leiner cases failing to respond to therapy.

Dermatitic lesions which have cornmatized to a large extent are not expected to respond, also cases characterized by superimposed infections (predominantly staphylococcic) or by inflammatory changes in the respiratory tract (pneumonia) or by ear infection (otitis). Nevertheless, some success has been reported in cornmatized cases, particularly if the cornmatization was not too advanced or was not due to other causes. In cases complicated by local or generalized infection, supplementary biotin therapy proved decididly superior to antibacterial treatment alone.

In a representative paper, Martin (Clinique de Medecine des Enfants, Bordeaux) describes the results obtained in treating 27 cases of seborrhoid dermatitis in infants, 23 of these were cured with biotin. Affected areas in the hairy part of the head were most resistant to treatment; they occasionally required prolonged treatment with relatively high dosage. This finding is confirmed by other investigators. Other cutaneous changes healed in the usual way. At the same time, inflammatory changes of the oral mucosa and of the intestinal tract (diarrhea) showed good improvement.

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*) "Zur Aetiologie und Therapie der Dermatitis seborrhoides" (Monatsschrift für Kinderheilkunde, 1950, 98, 350)

The arrested development observed in some infants responded remarkably well to biotin therapy. Martin emphasizes the superior effect of biotin in the treatment of dermatitis seborrhoides.

Various investigators, in order to find an explanation for the effects of biotin in dermatitis seborrhoides, have determined biotin content in the mother's milk and in the blood and urine of the infants. As seen in Table 1, some of these investigators were able to show definitive, in part very big differences in the values of healthy compared to sick babies. Mother's milk appears to contain less biotin than cow's milk and particularly low values were found in the milk of mothers in deprived areas and/or of Leiner children. While the values reported by [illegible] as well as of [illegible] make it safe to assume that a primary or secondary biotin deficiency is the cause of Leiner's erythrodermia and of dermatitis seborrhoides, the very accurate determinations carried out by [illegible] who found biotin excretion in the urine to be practically normal in young patients suffering from seborrhoid dermatitis, suggest at most that utilization is impaired.

Although these laboratory findings do not permit definitive conclusions with regard to a connection between biotin deficiency and dermatitis seborrhoides in infants, the therapeutic results leave no doubt about the consistent and superior effectiveness of biotin in the various manifestations of this disease. The application of biotin represents a marked advance in the treatment of this infant disease so frequently seen in Central and Western Europe. This is especially true for the localized form of dermatitis seborrhoides, in particular, crusta lactea which has been difficult to treat in the past and which tending to lead, to complications (secondary eczematization and general infection) has caused the pediatrician considerable concern.

In connection with the above-mentioned complications, we like to draw attention to a manuscript just now received from Dr. S. Kohil*) (Zentralkrankenhaus der Arbeiter-Krankenkasse, Afula, Israel). The author had previously reported therapeutic success with biotin in dermatitis seborrhoides of young infants (Ann. Paediatrici, 1954, 183, 23). Her present report deals with 10 additional patients (5 girls and 5 boys, ranging in age from 2 1/2 weeks to 3 1/2 months) who were treated with biotin for normochromic or hyperchromic, macrocytic anemia, secondary symptoms of dermatitis seborrhoides.

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*) "Uber die Anämie bei Dermatitis seborrhoides und ihre Beeinflussung durch Biotin" (Ann. Paediatrici, in press)

Dosage: 5 mg of biotin per os daily for 14-21 days. In 8 of the 10 children, the improvement in the dermatological picture went hand in hand with more normal hematologic results. The other 2 children in whom dermatitis subsided without changes in the blood picture had suffered infections (diarrhea + otitis media, influenza + parenteral diarrhea) during their hospital stay. Improvement of the blood picture consisted of hemoglobin and erythrocyte increases. Even though the hematologic findings did not become completely normalized during the brief period of treatment, this presumably occurs as soon as the causative noxa (biotin deficiency) has been removed. The author believes that the etiologic correlation between biotin deficiency and anemia in dermatitis seborrhoides as well as the significance of biotin for hematopoiesis (in addition to other components of the B-complex, in particular vitamin B_{12} and vitamin B_{12}) have been demonstrated. Her experiences are supported by analogous findings reported by Glanzmann and by Frank (Ann. Paediatrici, 180, 172, 1953).

Biotin in General Dermatology

The efficacy of biotin in dermatitis seborrhoides is limited to newborn and infants during the first trimenon. Thereafter, or at the latest after the second trimenon, treatment becomes rapidly less successful. In older children and adults biotin is largely ineffectual.

From the earlier Prague areas, a paper by Mikulecky is available which describes experiments in other dermatoses "Possibilities of Therapeutical Use of Pyridoxine (Vitamin B_6) and Biotin (Vitamin H) in Dermatology" (S.E.S.K. Tschechische Dermatologie, 1949, 24, No.1, 22). The author reports on the successful application of biotin combined with vitamin B_6 or pantothenic acid in treating seborrhea of the face, seborrhoid alopecia and psoriatic erythrodermia; acne and psoriasis failed to respond to this treatment.

In other European areas (Switzerland, Holland, France) only occasional cases of seborrhea sicca, seborrhoid eczema and acne vulgaris were reported to react favorable to biotin therapy in daily dosage of 10-20 mg. Some of the patients who were cured or noticeably improved had been unresponsive to treatment for years; but it must be remembered that biotin was the supplementary medication in most of these cases; antibiotics, Vitamin A, antihistamines, desensitization, local treatment etc. as such did not lead to significant improvement. Some investigators see in biotin a sort of "specific adjuvant".

The author*) of a publication transmitted to us by Grenzach after completion of this manuscript believes that biotin has promoted healing in a number of adult patients treated for various dermatoses (see Table 2). This applies in particular to dyshidroses, scaling eczema, and various forms of pruritus. The author, though realizing that his material had been rather limited and therefore does not permit definitive evaluation, is of the opinion that biotin should be tried parenterally, preferably intradermally, in the usual dosage in these particular conditions.

Nutley has also carried out some prelimenary studies with biotin in the field of dermatology. However, they did not include dermatitis seborrhoides and Leiner's disease in young infants which are relatively unknown in the USA. Until now it has not been determined, whether this is due to a real difference in the conditions prevailing on the two continents--perhaps based on constitutional, climatic or dietetic factors. This theory is supported by the observations of an American sanitation officer in Germany who recently diagnosed erthrodermia exfoliativa (Leiner's disease) in 4 infants--children of American military personnel stationed in Germany**)

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*) Geiger, R.: "Untersuchungen über die therapeutische und pharmakodynamische Wirksamkeit des Biotins (Vit. H) bei einer Reihe dermatologischer Erkrankungen" (Der Hautarzt, 1955, 6, No. 5, 222-27)

**) R. O. Crotty "Erythroderma desquamativum (Leiner's disease)" (Arch. of Dermatology, May 1955, 71 5, p-537.

On the other hand, American dermatologists have tested biotin quite extensively in various types of dermatosis in adults. It was found that with very few exceptions biotin even when administered in high doses for a prolonged period was ineffectual in cases of [illegible], [illegible], [illegible], [illegible], neurodermatitis, [illegible], [illegible] and [illegible].

The Canadian authors Kalz, Kral and Forsey *) are reported to have obtained some, at least temporary, improvement with biotin i.m. in rosacea and although we do not feel too optimistic about these results, they in turn believe that additional studies are justified.

Biotin in Internal Medicine

Biotin has had only limited application in internal medicine. It has been used in a few cases of [illegible] following benzene poisoning or arsenic intoxication at the Evangelisches Krankenhaus in Vienna (Dr. Fleischhacker); biotin was given in a dosage of 3 tablets of Ro 1-5492/1 and/or 1 ampul of Ro 1-5492/2 i.m. or i.v. for 6-7 days. The investigators claim that the drug distinctly promoted erythropoiesis (previously uninfluenced by liver preparations) but the drug had less effect on the leukopoiesis than did choline, folic acid and/or vitamin B_6. The rise in erythrocytes did, however, come to a standstill (mostly at the 3 million mark) and this could not be overcome even though higher biotin doses were tried. It was observed that subsequent administration of liver preparations, B-vitamins or choline (which proved ineffectual prior to biotin therapy) later led to normalization of the red cell picture. It is of course difficult to judge whether these improvements were actually brought about by biotin therapy or whether they were spontaneous.

Biotin is also reported to have had a favorable effect on pernicious (achrestic) anemia which had failed to respond to liver preparations. It must be pointed out that the reticulocytosis was minimal and the increase in erthrocytes relatively insignificant; however, subsequent liver therapy is said to have restored the blood picture to normal within a short time. One case of Lichen ruber planus in which the blood picture resembled pernicious anemia is reported to have reacted well to biotin, since the dermatologist as well as hematological symptoms showed improvement. A case of [illegible] chloroanemia resistant to iron and liver therapy responded to biotin with a marked reticulocyte crisis (243‰) and an increase in erythrocytes from 1.8 to 3.4 millions. The subsequently resumed iron therapy led to rapid normalization of the red cell picture. However, the reported successes are contrasted by many reported failures.

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*) "Observations on Biotin" (J. Invest. Dermat. 1951, 16, 111)

Biotin was tried in several cases of chronic leukemic myelosis. The investigator claims to have noted a greater rise in leukocytes in the course of which immature elements were increasingly flushed out into the peripheral blood; based on this findings, he believes to have found a method of treating leukosis by artificially induced biotin deficiency (doses of egg white).

One case of chronic leukemic lymphadenosis remained completely unaffected by biotin therapy.

And, finally some success with biotin in daily doses of 5-10 mg per os, was reported in the treatment of colitis ulcerosa.

The above-mentioned studies are described in a report by S. Schmid *). Although Schmid's findings with regard to biotin's effect on anemias associated with dermatitis seborrhoides appear well documented we are doubtful that the conclusions drawn by the author would stand up under critical reexamination. Unfortunately we have not succeeded in convincing him nor other investigators to conduct a systematic study of the effects of biotin in these particular diseases.

Talow**) reports considerable and rather lasting improvement with daily doses of 50 mg biotin in previously resistant cases, namely:one case of postclimacteric polyarthrosis and 10 of 11 cases treated for bilateral coxarthrosis; however, bone changes (as determined by X-ray examination) remained uninfluenced. However, these findings could not be confirmed in subsequent clinical investigations.

Tolerability

Biotin (Ro 1-5492) was always tolerated very well both in oral and intramuscular application (daily doses of up to 100 mg). Even on prolonged administration, neither subjective nor objective side effects were observed.

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*) "Therapie mit Biotin" (Klin. Zeschr. inn. Med., 1951, 32, 19)
**) "Emploi therapeutique de la biotine en rhumatologie" (Rev. rhumat., 1951, 18, 543)

Basle, August 25, 1955

Translated from the German
Translator: I. J. Ryby
Nutley, Dec. 22, 1964.

Table 1

Comparative Survey of Biotin Determinations in Mother's Milk, Blood and Urine of Infants

Author	Values of Biotin Determination in Mother's Milk, Blood and Urine						
Svejcar & Homolka, 1950	Normal Values	Mother's Milk 1.8 γ%		Infant's Blood 0.04 γ%		Infant's Urine 1.5 γ%	
	Leiner Infants (breast fed)	0.88γ%		0.0017γ%		0.47γ%	
Freudenberg in Publ. Thelin, 1949	Biotin in Mother's Milk		Basle: 1.5-3.7 γ% ∅ =2.5 γ%		Lorrach (Deutschland): 0.053-0.42 γ% ∅ =0.2 γ%		
Nassau, Afulah; see also Textbook S. Karger, Basle, 1953, 3rd ed.	Mother's Milk:	1-6 Days post partum		0.4-0.8 γ%			
	Whole Milk:			12-16 γ%			
	Skim Milk:			7-8 γ%			
	Half Milk:			6.5-8 γ%			
	Infant Urine:	1st	2nd	3rd	4th	5th	6th day post partum
		0.8-1.35	0.9-1.8	1.2-3.1	1.3-2.1	0.7-1.4	2.1-4.4 γ%
Chiari (1952)	Biotin in the Blood:	Healthy Infants		Leiner + D. S.		Infants with Eczema	
		0.04-0.05 γ%		0.01-0.025 γ%		0.046-0.078 γ%	
				(Method: Barton & Wright)			
Berger (1950)	Urine of Normal Infants			24 Hours			
	2-4 Weeks old		0.642 γ%	1.601 γ			
	1-6 Months old		3.088 γ%	6.80 γ			
	Dermatitis seb.		1.48 γ%	5.24 γ			
	Eczema		4.482 γ%	7.334 γ			
	After 5 mg. i.m. of Biotin, the following amounts were excreted in 24 hours:						
	Normal Infants	7/10 of total amount					
	Eczema	1/2 of total amount					
	Dermatitis seb.	very small amounts					
		(Test: Snell, Eakin & Williams)					

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Table 2

Effect of Intracutaneous Doses of 5-15 mg. of Biotin on Various Dermatoses in the Adult. Duration of Treatment: 1-2 Weeks

Diagnosis	Number of Cases	Pruritus	Antipruriginous Effect		Clinical Effect		
			+	Ø	Cured	Improved	Unchanged
Dyshidrosis	18	16	14	2	14	2	2
Dyhidrosiform eczema	12	12	9	3	4	5	3
Eczema	11	8	6	2	4	2	5
Eczematoid	15	10	8	2	6	5	4
Lichon chron. Vidal	4	4	4	-	3	1	-
Diff. Neurodermitis	2	2	2	-	-	1	1
Pityriasiform Seborrhoid	3	2	1	1	2	1	-
Lichen ruber planus	4	4	4	-	2	-	2
Genitoanal pruritus	5	5	5	-	4	1	-
Pruritus corporis	3	3	2	1	2	-	1
Rhagades (corner of the mouth)	2	-	-	-	2	-	-
Acne vulgaris	14	2	2	-	2	-	-
Psoriasis vulgaris (circumscribed areas)	21	5	3	2	-	5	16
Multiform exudative erythema	1	-	-	-	1	-	-
Sclerodermia circumscripta	1	-	-	-	1	-	-
	116	73	60	13	45	30	41

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Im Arbeitsgebiet von Grenzach befinden sich in Vorbereitung:

Vogtherr: Ueber die Beeinflussung des Wasser- und Mineralhaushaltes durch Biotin.

Noack: Ueber Erfahrungen mit Biotin in der Kinderklinik Stralsund.

TO Mr. H. L. Newmark

DATE March 6, 1968

FROM Dr. Colin Dalton

SUBJECT Effect of Biotin on Lipid Metabolism.

1. Biotin was administered orally for 9 days at 1 mg/kg to the normal rat and serum free fatty acids, cholesterol and triglycerides were determined (Table 1). No significant changes were found.

2. Biotin was administered orally or subcutaneously at 1 mg/kg/day to Mongolian gerbils fed a normal or a hypercholesterolemic diet. Groups were sacrificed at 3 and 6 weeks and body weight, organ weight, serum lipids, adrenal and liver cholesterol and fecal lipid excretion were determined.

Data on lipid concentrations obtained following 3 weeks treatment are shown in Tables 2 and 3 and following 6 weeks treatment in Tables 4 and 5. No consistently significant changes were found in serum FFA, triglycerides or cholesterol in normal or hypercholesterolemic animals. Little change was observed in adrenal or liver cholesterol concentration.

The effect of Biotin treatment on body and organ weight is shown in Tables 6 and 7. Little change in body, adrenal or liver weight was found in the hypercholesterolemic groups. Significant body weight loss was observed in the normal diet groups following injection and oral administration of Biotin.

Fecal excretion of lipid during the final 24 hour period of each test was determined (Table 8). No significant changes were found.

Colin Dalton

Colin Dalton, Ph.D.

Table 1. Effect of Biotin* on Lipid Metabolism in the Rat

Treatment	Serum FFA μEq/L ± S. E.	Serum Cholesterol mg/100 ml ± S. E.	Serum Triglycerides mg/100 ml ± S. E.
Control	776 ± 45	73.1 ± 1.4	87.4 ± 4.8
Biotin	724 ± 23	71.8 ± 3.2	70.6 ± 6.9

* Biotin was administered orally at 1 mg/kg for 9 days.

C. Dalton

Table 2. Effect of Biotin[1] on Lipid Metabolism in Mongolian Gerbils

Treatment	Serum Triglycerides mg/100 g ± S. E.	Serum Cholest. mg/100 g ± S. E.	Serum FFA µEq/L ± S. E.	Adrenal Cholest. mg/100 g ± S. E.	Liver Cholest. mg/100 g ± S. E.
Control	63.5 ± 3.5	228 ± 20	1057 ± 42	2.03 ± 0.12	0.31 ± 0.03
Biotin 1 mg/kg p.o.	46.8 ± 4.4*	205 ± 9.2	1048 ± 49	2.39 ± 0.05*	0.36 ± 0.01
Biotin 1 mg/kg s.c.	57.2 ± 2.4	358 ± 15**	1032 ± 47	2.35 ± 0.10	0.32 ± 0.01

[1] Daily treatment for 3 weeks.

Significant change from control *p < .05, **p < .01.

Table 3. Effect of Biotin[1] on Lipid Metabolism in Mongolian Gerbils Fed a Hyperlipemic Diet[2]

Treatment	Serum Triglycerides mg/100 g ± S. E.	Serum Cholest. mg/100 g ± S. E.	Serum FFA µEq/L ± S. E.	Adrenal Cholest. mg/100 g ± S. E.	Liver Cholest. mg/100 g ± S. E.
Control	81.6 ± 7.2	668 ± 37	1019 ± 17	5.0 ± .26	5.50 ± 0.31
Biotin 1 mg/kg p.o.	114.4 ± 25.7	859 ± 69	958 ± 55	5.1 ± .18	5.18 ± 0.19
Biotin 1 mg/kg s.c.	63.2 ± 3.2	739 ± 45	1097 ± 65*	5.0 ± .05	4.98 ± 0.09

1 Daily treatment for 3 weeks

2 Diet consists of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic Acid.

Significant change from control * $p < .01$.

Table 4. Effect of Biotin[1] on Lipid Metabolism in Mongolian Gerbils

Treatment	Serum Triglycerides mg/100 g ± S. E.	Serum Cholest. mg/100 g ± S. E.	Serum FFA µEq/L ± S. E.	Serum Cholest. mg/100 g ± S. E.	Adrenal Cholest. mg/100 g ± S. E.
Control	99.6 ± 8.7	145.6 ± 8.6	1006 ± 27	0.33 ± .017	2.04 ± .05
Biotin 1 mg/kg p.o.	97.6 ± 6.3	142.0 ± 9.9	1062 ± 150	0.40 ± .013*	1.93 ± .05
Biotin 1 mg/kg s.c.	136.0 ± 17.6	185.2 ± 12.6*	816 ± 30	0.34 ± .024	2.06 ± .08

[1] Daily treatment for 6 weeks.

Significant change from control * $p < .05$.

Table 5. Effect of Biotin[1] on Lipid Metabolism in Mongolian Gerbils Fed a Hyperlipemic Diet[2]

Treatment	Serum Triglycerides mg/100 g ± S. E.	Serum Cholest. mg/100 g ± S. E.	Serum FFA µEq/L ± S. E.	Liver Cholest. mg/100 g ± S. E.	Adrenal Cholest. mg/100 g ± S. E.
Control	160 ± 14.7	559 ± 13	770 ± 45	5.69 ± .50	5.43 ± 0.20
Biotin 1 mg/kg p.o.	219.2 ± 42.8	479 ± 69	784 ± 81	5.54 ± .32	5.02 ± 0.18
Biotin 1 mg/kg s.c.	238.4 ± 23.6	584 ± 56	671 ± 36	4.36 ± .41	5.04 ± 0.10

[1] Daily treatment for 6 weeks.

[2] Diet consist of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic acid.

Table 6. Effect of Biotin[1] on Body and Organ Weight in Mongoligan Gerbils

Treatment	NORMAL DIET			HYPERCHOLESTEROLEMIC DIET[2]		
	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.
Control	65.0 ± 2.3	59.5 ± 2.9	2.95 ± 0.07	71.6 ± 3.7	64.3 ± 3.4	5.58 ± 0.17
Biotin 1 mg/kg p.o.	58.4 ± 2.3	58.8 ± 2.5	3.1 ± 0.09	69.6 ± 3.1	66.9 ± 3.4	5.59 ± 0.06
Biotin 1 mg/kg s.c.	55.0 ± 4.2	70.7 ± 6.5	3.2 ± 0.06*	66.0 ± 3.5	68.9 ± 4.9	5.11 ± 0.21

[1] Daily treatment for 3 weeks.

[2] Diet consists of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic acid.

Significant change from control * $p < .05$.

Table 7. Effect of Biotin[1] on Body and Organ Weight in Mongolian Gerbils

Treatment	NORMAL DIET			HYPERCHOLESTEROLEMIC DIET[2]		
	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.	Body Wt. g	Adrenal Wt. mg/100 g ± S. E.	Liver Wt. g/100 g ± S. E.
Control	70.0 ± 8.9	57.2 ± 2.3	3.19 ± 0.07	62.5 ± 1.7	74.5 ± 4.2	5.82 ± 0.2
Biotin 1 mg/kg p.o.	64.0 ± 1.8*	57.3 ± 3.4	3.34 ± 0.03	61.2 ± 2.9	76.0 ± 1.5	5.87 ± 0.4
Biotin 1 mg/kg s.c.	56.8 ± 3.5*	59.7 ± 2.8	3.69 ± 0.24	60.0 ± 2.7	76.6 ± 3.5	5.65 ± 0.2

[1] Daily treatment for 6 weeks.

[2] Diet consists of 98% Purina Lab Chow; 1.5% Cholesterol; 0.5% Cholic acid.

Significant change from control * $p < .05$.

Table 8. Effect of Biotin* on Fecal Lipid Excretion in Normal and Cholesterol-Fed Gerbils

TREATMENT	Total Fecal Lipid mg/24 Hours ± S.E.			
	NORMAL DIET		HYPERCHOLESTEROLEMIC DIET	
	Control	Treated	Control	Treated
Biotin p.o. 3 weeks	6 ± 1	8 ± 2	45 ± 7	45 ± 6
Biotin p. o. 6 weeks	4 ± 1	7 ± 1	50 ± 10	34 ± 4
Biotin s.c. 3 weeks	6 ± 1	10 ± 3	45 ± 7	30 ± 6
Biotin s.c. 6 weeks	4 ± 1	22 ± 9	50 ± 10	33 ± 4

* Biotin administered 1 mg/kg/day for the time indicated.

C. Dalton

Neumann-'43
Bib No. 963

Proc. Soc. Exptl. Biol. Med. 52:257-260

14110

Biotin Deficiency in Rats Fed Purified Diets Containing Succinylsulfathiazole and p-Aminobenzoic Acid.*

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Only a few reports have been made concerning the effects of succinylsulfathiazole (sulfasuxidine) on the nutrition of animals fed purified diets.[1-5] Welch[1] found no favorable effect on growth of rats when *p*-aminobenzoic acid was added to a purified diet containing sulfasuxidine. In none of the other studies has *p*-aminobenzoic acid (or inositol) been included in the basal diet in attempts to determine the effect of sulfasuxidine on the nutrition of animals.

The effects of sulfanilylguanidine (sulfaguanidine) have been given considerably more attention.[6-11] Through the use of sulfaguanidine or sulfasuxidine deficiencies of biotin,[8,10] folic acid,[2,10] and vitamin K[4] have been reported. The evidence strongly indicates that the vitamin deficiencies which result are due to a lowering by the drugs of microbial synthesis of these essential nutritional factors in the alimentary tract. Black *et al.*[4] found that *p*-aminobenzoic acid counteracts the hypoprothrombinemia and growth inhibition that occurs when sulfaguanidine is fed. Other

* Supported by a grant from the Graduate Research Fund of Indiana University.

1 Welch, A. D., *Federation Proc.*, 1942, **1**, 171.

2 Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **145**, 713.

3 Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

4 Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P., *J. Biol. Chem.*, 1942, **145**, 137.

5 Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

6 Black, S., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 308.

7 Mackenzie, J. B., Mackenzie, C. G., and McCollum, E. V., *Science*, 1941, **94**, 518.

8 Dann, W. J., *J. Biol. Chem.*, 1941, **141**, 803.

9 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 56.

10 Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

11 Light, R. F., Cracas, L. J., Olcott, C. T., and Frey, C. N., *J. Nutrition*, 1942, **24**, 427.

studies[1,4,6,7,11,12] also have indicated that sulfaguanidine is antagonized by *p*-aminobenzoic acid.

In connection with other studies on rats fed both sulfasuxidine and *p*-aminobenzoic acid, we have found evidences of biotin (and a vitamin K[13]) deficiency. This finding constitutes possible additional evidence of a difference between sulfasuxidine and sulfaguanidine as respects their relation to *p*-aminobenzoic acid. Owing to our inability to secure more pure biotin for further study of this problem, we are reporting the results on biotin at this time.

Experimental and Results. Young piebald rats (McCollum strain), 40 to 50 g in weight, were confined to individual cages. The cages were cleaned frequently because the feces of rats fed sulfasuxidine are so soft the screen floors quickly become soiled, thus providing opportunity for coprophagy. The basal diet contained: casein (Smaco, vitamin-free), 18; glucose (Cerelose), 72; salts, 5; corn oil, 3; cod liver oil, 2; and a synthetic vitamin mixture† containing thiamine hydrochloride 10 mg; pyridoxine, 10 mg; riboflavin, 20 mg; nicotinic acid, 200 mg; calcium pantothenate, 200 mg; *p*-aminobenzoic acid, 200 mg; inositol, 400 mg; 2-methyl-1,4-naphthoquinone, 600 mg; and choline chloride, 1200 mg. When used, sulfasuxidine‡ was added at a level of 1% replacing an equal amount of glucose.

Rats fed the basal diet without sulfasuxidine show good growth and appear to be in a moderately good state of nutrition. During the first 6 weeks they gain about 20 g per week. After 10 to 12 weeks they still appear to be almost like stock animals. In contrast, rats fed the basal diet with sulfasuxidine added gain about 10 g per week during the first 5 weeks. From the 5th to 8th weeks about one-half die and the rest eventually succumb. There is a marked variation in ability to survive. Perhaps this is due to differences in ability to utilize the deficient nutritive factors probably being formed in a limited but variable degree by intestinal microörganisms.

The growth inhibition by the sulfasuxidine is considerably reduced by feeding daily 0.5 ml of a liver preparation§ representing 1.7 g liver.

In Table I it is shown that biotin, either as a concentrate or as the pure methyl ester, effects a significant growth response in rats supplemented daily with 0.5 ml of the liver preparation. In all cases the rats had been restricted to the diet at least 2 weeks before supplementation with the liver. The biotin was not given until liver had been fed 2 weeks. As shown by Daft *et al.*,[8] who used a purified diet deficient in *p*-aminobenzoic acid, certain liver extracts are useful in producing mild degrees of biotin deficiency. In our studies the biotin was administered orally in 1γ daily doses per rat for only 6 or 8 days, after the rats had been on the diet 4 to 13 weeks as indicated. The controls received equal amounts of the biotin solvent, 0.5 ml of 50% ethanol daily.

As shown in Table II, rats fed 1 or 2 γ of a biotin concentrate per day from the time they were restricted to the diet showed a greater growth response when given 0.5 ml of liver preparation daily than did those not given the biotin. Moreover, the total weight of the biotin-fed group was greater than their controls at the time liver supplementation was begun. These results indicate mild biotin deficiency in rats fed both *p*-aminobenzoic acid and sulfasuxidine. But the better growth noted in the biotin-fed animals previous to liver feeding may have been due to "detectable quantities" of folic acid, or other factors in the concentrate, because in another experiment 9 rats showed no appreciable growth response when given pure biotin alone (1 or 2 γ daily for 6 days) after 3 weeks on the basal diet. None of these were given liver.

After 4 to 6 weeks nearly all rats developed

[12] Wood, W. B., Jr., *J. Exp. Med.*, 1942, **75**, 369.

[13] Wakim, K. G., Day, H. G., Krider, M. M., and O'Banion, E. E., unpublished data.

† Most of the synthetic vitamins were supplied through the courtesy of Dr. D. F. Robertson, Merck and Co.

‡ The sulfasuxidine was kindly furnished by Dr. W. A. Feirer, Sharp and Dohme, Inc.

§ Prepared from a concentrate generously supplied by the Lederle Laboratories. Four ml of this concentrate is diluted to 40 ml with water to give the preparation as used.

TABLE I.
Effect of Biotin on Growth of Sulfasuxidine-fed Rats after Receiving Liver Concentrate 2 Weeks.

Biotin* supplement	Wks on diet before biotin added	No. of rats, sex	Avg total wt when biotin was added g	Avg gain 1 wk before g	Avg gain 1 wk after g	Avg gain 2 wk after g
5000†	9	2 (0♂)	155	9	15	—
0	9	2 (2♂)	160	8	5	—
5000†	13	3 (0♂)	168	10	23	32
0	13	2 (1♂)	160	10	13	22
Methyl ester	4	3 (2♂)	116	5	24	33
0	4	3 (1♂)	140	7	11	23

* Purchased from S.M.A. Corporation.
† The biotin concentrate contains "detectable quantities of folic acid." (Personal communication from S.M.A. Corporation.)

TABLE II.
Effect of Liver Concentrate on Growth of Sulfasuxidine-fed Rats Receiving Biotin from the Beginning of the Experiment.

Biotin* supplement	Wks on diet before liver added	No. of rats, sex	Avg total wt when liver added g	Avg gain 1 wk before g	Avg gain 1 wk after g	Avg gain 2 wk after g
200†	12	4 (0♂)	145	1	25	41
0	12	2 (0♂)	128	1	15	37
0	9	3 (2♂)	105	3	15	33
0	5	2 (1♂)	91	8	18	35

* Purchased from S.M.A. Corporation.
† The biotin concentrate contains "detectable quantities of folic acid." (Personal communication from S.M.A. Corporation.)

a mild dermatitis characterized by fine, flaky scales on the feet, notably on the anterior surfaces of the hind feet. Alopecia, especially of the abdomen, occurred in over one-half the rats. In a few cases total abdominal denudation occurred. No noticeable improvement in the dermatitis or alopecia followed administration of biotin or liver, but the biotin supplement was given in small doses and for only 6 to 8 days. Daft *et al.*[3] noted remission of dermatitis in rats given biotin for 14 days. "Spectacle eye" was not distinguished with certainty but it appeared to be present in mild form in several animals. There was no definite abnormality in gait as reported by Nielsen and Elvehjem[14] and others. According to these workers a rather severe degree of deficiency, as produced by egg white, must exist before that syndrome appears.

Discussion. Our studies give some support to Welch's[1] conclusion that *p*-aminobenzoic acid, in the amounts fed, does not abolish the growth inhibiting action of sulfasuxidine. Whether the degree of biotin deficiency in our studies would have been greater with the exclusion of *p*-aminobenzoic acid from the diet was not determined. However, it is probable that the *p*-aminobenzoic acid did exert some antagonistic action since we have evidence that it inhibits, but does not totally block, the production of hypoprothrombinemia in rats fed sulfasuxidine.[13] Our animals ingested 1 to 2 mg of *p*-aminobenzoic acid per rat daily as calculated from food consumption data. Black *et al.*[4] found that 150-300 γ per day was sufficient to counteract the hypoprothrombinemia and growth inhibition produced by sulfaguanidine fed at a 0.5% level.

We believe the relationship of *p*-amino-

[14] Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 405.

benzoic acid to the action of sulfasuxidine and sulfaguanidine in the alimentary tract needs to be investigated on a quantitative basis. As shown by Wood,[12] working with 6 different sulfonamides and pure cultures of *B. coli*, the bacteriostatic potency of a sulfonamide is directly proportional to its ability to counteract the antibacteriostatic action of *p*-aminobenzoic acid. If, therefore, sulfasuxidine should happen to be appreciably more bacteriostatic than sulfaguanidine a greater amount of *p*-aminobenzoic acid might be required to exert a comparable antibacteriostatic action when the former is fed. Since Martin[10] has found that biotin improves the growth of sulfaguanidine-fed rats receiving *p*-aminobenzoic acid it must be concluded that, at the levels fed, the antagonistic action of the latter is not complete in the case of sulfaguanidine. Similarly our studies indicate that sulfasuxidine lacks considerable of being totally inhibited by *p*-aminobenzoic acid even at the seemingly large levels we have fed.

Summary. Biotin deficiency occurs in rats fed a purified diet containing sulfasuxidine and *p*-aminobenzoic acid. Growth appears to be a sensitive indicator of biotin deficiency in the rat.

14111

Sugar Alcohols. XXIII. Effect of Sorbitol Feeding on Successive Generations of Rats.

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In former studies[1] two of the authors showed mannitol and sorbitol to be innocuous when fed in comparatively large amounts to the *Rhesus macacus* monkey and man over long-time periods. The laxative action of large quantities of mannitol limits its value as a dietary adjunct. Sorbitol, however, does not possess this disadvantage and at present is enjoying a widespread use as a carbohydrate-like item of diet. It seemed fitting, therefore, to study the effect of sorbitol feeding on successive generations of animals.

Material and Methods. Crystalline sorbitol was used in one group of studies. In the other, a commercial sorbitol syrup was employed. This syrup is a non-crystallizing aqueous solution of sorbitol having a polyhydroxylic compound content of about 83%. It is manufactured by the electrolytic reduction of glucose and is known under the trade name of "Arlex."* Each of these substances mixed with Purina chow meal was fed to young white rats of our laboratory stock and also animals of the Duke University strain. Controls were fed dextrose and the basal diet. Each compound composed 5% of the total food intake. Males and females were kept separated until they were at least 100 days old and then mated. This was repeated through 3 generations. Growth curves of the rats fed the 3 types of food in the 3 generations were plotted. Periodically animals were killed for gross and histologic study. At the end of 1 year the capacity of the livers to store glycogen was determined. Eighty first generation animals fed the experimental diet from the time of weaning were used and the experiment was extended over 20 months.

Results. The growth curves of the first generation animals fed the 3 kinds of food were identical within the normal variations of the experiment. The offspring of these animals showed similar growth rates in the second and third generations. The litter sizes were about the same. The average liver-glycogen[2] content

[1] Ellis, F. W., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1941, 141, 147.

* Generous supplies of crystalline sorbitol and "Arlex" were furnished by the Atlas Powder Co. of Wilmington, Del.

[2] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, 100, 485.

Nielsen-'42
Bib No. 972

J. Biol. Chem. 145:713-714.1942.

THE GROWTH-PROMOTING EFFECT OF FOLIC ACID AND BIOTIN IN RATS FED SUCCINYLSULFATHIAZOLE

Sirs:

In previous experiments[1] it was shown that succinylsulfathiazole (sulfasuxidine[2]) reduced the growth of rats maintained on a synthetic ration to the same extent as sulfaguanidine. In this note we wish to report the effectiveness of biotin and especially folic acid in counteracting the growth inhibition produced by succinylsulfathiazole.

Weanling rats 18 days old from Sprague-Dawley and our stock colony were used. The basal ration had the following percentage composition: sucrose, 72; casein, 18; corn oil, 5; Salts 4,[3] 4; and succinylsulfathiazole, 1. Choline hydrochloride was added to this basal ration at 1 gm. per kilo of ration. The B vitamins were fed daily in supplement dishes at the following levels: thiamine 20 γ, riboflavin 20 γ, pyridoxine 25 γ, and calcium pantothenate 100 γ. 2 drops of haliver oil were given each rat per week.

In a preliminary series, rats were placed on the basal ration and the basal ration with 0.25 and 0.5 gm. of 1:20 liver powder added daily. The lower level prevented loss of weight and the higher level allowed normal growth. Many of our animals maintained on the basal ration and receiving only the synthetic vitamins developed spectacle eye, which indicated a mild biotin deficiency as noted in a previous paper.[4] The animals would reach a weight plateau or lose weight after 4 to 5 weeks on the basal ration and at this time therapy was started; thus each animal served as a control.

A concentrate of the eluate factor of Snell and Peterson (folic acid) was prepared according to the procedure of Hutchings, Bohonos, and Peterson,[5] but was purified only to the superfiltrol eluate stage.[6] Three rats were given 5 mg. of this concentrate (the equivalent of 0.25 gm. of solubilized liver extract) and three rats were given 5 mg. of the concentrate and 1 γ of biotin per day. In all the animals definite growth responses were observed, ranging from 13 to 20 gm. for the 1st week of therapy.

[1] Black, S., Overman, R. S., Elvehjem, C. A., and Link, K. P., *J. Biol. Chem.*, 145, 137 (1942).

[2] Sulfasuxidine was kindly supplied by A. D. Welch of Sharp and Dohme, Inc., Glenolden, Pennsylvania.

[3] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 138, 460 (1941).

[4] Nielsen, E., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, **48**, 349 (1941).

[5] Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, **141**, 521 (1941).

[6] We are indebted to E. B. McQuarrie for this preparation.

The feeding of biotin in addition to the concentrate resulted in better growth responses. However, when biotin was fed or injected alone, the spectacle eye condition improved but the growth responses were very small, due undoubtedly to a limited intake of folic acid.

We obtained from Dr. E. E. Snell of The University of Texas, Austin, a folic acid preparation which was found to be 15,000 times as potent in folic acid as solubilized liver. At present we have obtained growth responses in fifteen rats with various levels of this folic acid preparation. The growth response for the 1st week of therapy ranges from 12 to 31 gm. We have found that 10 γ per day of this preparation are as effective as 100 γ. The therapy has been continued in several animals and in all cases additional increases in weight have been obtained. The deficiency produced by feeding succinylsulfathiazole has been largely overcome by the feeding of folic acid and biotin.

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Received for publication, September 8, 1942

Nisenson-'57
Bib No. 986

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J. Pediat. 51: 537-548. 1957.

SEBORRHEIC DERMATITIS OF INFANTS AND LEINER'S DISEASE: A BIOTIN DEFICIENCY

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IT IS believed that Leiner's disease and seborrheic dermatitis of infants are degrees of the same disease. Leiner's disease, or erythroderma desquamativa, is probably a generalized and greatly intensified seborrheic dermatitis.

The cause of seborrheic dermatitis is not well understood. Some[1] have thought that it is due to an infection with a yeastlike organism, Pityrosporon, others[2] that it is of parasitic origin or is due to hyperactivity of the sebaceous glands. Another explanation is that it results from the lack of a special dietary factor or vitamin which is contained in liver and casein. Reiches[3] believes that it is transmitted directly from an adult, usually the parent, to the infant.

The treatment of seborrheic dermatitis has usually consisted of a low-fat, high-casein diet, liver injections, and local applications of either sulfur, ammoniated mercury, tar, vioform, or selenium sulfide. The treatment of Leiner's disease has been similar to that of seborrheic dermatitis. The same high-protein, low-fat diet is used. Cleansing of the skin is done with Phisoderm, and, locally, petrolatum with or without salicylic acid has been applied. Antibiotics usually are necessary for the treatment of secondary infection.

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Recent clinical and experimental work point definitely to a deficiency of biotin, previously called vitamin H, as the cause of seborrheic dermatitis in infants and Leiner's disease. A group of cases treated with biotin are reported here, bearing out this contention.

In reviewing the literature in this regard, it was found that Paul Gyorgy[4] wrote an excellent article on the dietary treatment of scaly dermatoses of the seborrheic type. By feeding rats a diet rich in egg white, a disease was produced which bears the characteristics of a seborrheic dermatitis, similar to the gross appearance in infants called seborrheic eczema. The possibility that egg-white injury in rats might be related to seborrheic dermatitis in man furnished the stimulus for the chemical study[5] of the curative factor, vitamin H, or biotin. Gyorgy's experiments[6] involved about 7,000 rats. The lesions following egg-white injury in rats consisted of intertrigo and confluent pigmented brown scales on the back, resembling cradle cap in infants.

In exceptional cases the disease involved the skin of the whole body of the rat, which resembled the typical exfoliative dermatitis seen in Leiner's disease.

Administration of the specific curative factor even without change in the basal diet of the rat restored normal conditions in two to four weeks. This

specific curative factor was identified as biotin. The best and most potent sources were found in liver, kidney, yeast, and, to a limited extent, in cow's milk.

It is of interest to note that other seborrheic-type lesions can be produced in rats on a diet deficient in pyridoxine and riboflavin, not identical to those due to biotin deficiency although they are all of the scaly, desquamative type.

DESCRIPTION OF TERMS

Seborrheic dermatitis occurs most commonly in infants under 4 years of age. It often begins with cradle cap, a superficial scaly eruption on the cheeks and intertrigo of the axillae, groin, and neck and sometimes around the umbilicus. It is essentially a disease of scaling on an inflammatory base, with very little evidence of vesiculation and oozing.

As a rule, it itches but little, there is no eosinophilia, and skin tests are negative. However, it may be associated with atopic dermatitis and, although not primarily an allergic disease, can and does occur in allergic infants. On the other hand, it may occur as a distinct entity with no other evidence of atopic dermatitis.

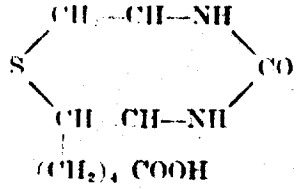
Leiner's disease, which has the characteristics of a widespread seborrheic dermatitis, was first described by Leiner[7] in 1908, when he reported 43 cases. Forty-one of the infants were breast fed and two were bottle fed. Fifteen of these infants died.

The disease begins almost always at the end of the first or the beginning of the second month. It is a peculiar universal skin disease, which consists of a slight inflammation of the whole cutis, a desquamation of the epidermis, and a seborrhea of the scalp.

In uncomplicated cases, there is little itching, no eosinophilia, and no allergic antibodies to be found in the blood. Diarrhea is common in Leiner's disease and is often serious. Secondary infection is frequent and nutrition may become poor. There is often a low serum protein.

BIOTIN

Biotin was first recognized as a growth factor for yeast and isolated by Kögl and Tonnis[8] in 1936 from egg yolk. In 1940, DuVigneaud, Gyorgy, and associates[9] demonstrated that biotin, or vitamin H, was the same as the anti-egg-white injury factor, and isolated vitamin H chemically.[10] Gyorgy had carried out extensive studies on vitamin H as previously described in rats, and DuVigneaud and associates[11] showed that vitamin H was identical with biotin. Harris[12] synthesized biotin in 1943 and gave it the following formula.



The characteristic syndrome of seborrheic dermatitis was produced by Gyorgy in rats fed on a high raw egg-white diet. Rabbit and monkey also showed characteristic dermatitis on high egg-white diet.[14] The presence of a substance, avidin, in raw egg white was found to be the cause of biotin deficiency.

Avidin, a glycoprotein, unites with biotin to form a stable compound which cannot be broken down by proteolytic digestion, but only by heating. Avidin, so combined, prevents the absorption of biotin from the intestinal tract

and provides an experimental way of producing a deficiency of biotin. Avidin is the first known example of an antivitamin.

The requirements of biotin for man are difficult to establish since a large part of the biotin is supplied by the intestinal bacteria,[15] primarily *Esch. coli*. It is postulated that the absence of these bacteria may be responsible in infants for biotin deficiencies. Other sources of biotin are found mostly in liver, yeast, kidney, and egg yolk.

DISCUSSION AND REVIEW OF LITERATURE

The American literature is devoid of any articles describing the use of biotin in seborrheic dermatitis and Leiner's disease. However, the European literature, beginning in 1950, contains a number of articles dealing with the efficacy of biotin in these conditions. It is also evident that Leiner's disease is a much more common disease in Europe than here, and this is probably due to differences in diet. Evidence of this was the marked increase in Leiner's disease after World Wars I and II, when meat, egg yolk, and liver were difficult to obtain in the European countries.

It also became clear that seborrheic dermatitis of the young infant is an illness which bears no relationship to the seborrheic dermatitis of older children and adults.[16] The treatment of seborrheic dermatitis in the older age group with biotin was of no value.

One of the first articles reported was in the Czechoslovakian literature by Palacky and Strycek[17] in which sixteen cases of Leiner's disease and one case of seborrheic dermatitis were treated. These authors concluded that the favorable response to the treatment with biotin and meat hydrolysates or with a combination of the two seemed to be proof of the deficiency of biotin as the etiological factor in these conditions. In addition, hypoproteinemia and deficiency of gamma globulin were found in these cases.

Svejcar and Homolka,[18] in 1950, reporting from the Children's Clinic in Prague, cured twenty-six breast-fed infants with Leiner's disease by use of biotin. It was they who noted an increase in the number of cases of Leiner's disease after World War II, similar to what had occurred after World War I.

In view of the fact that the children afflicted by the disease were mostly breast fed, they expressed the opinion that the frequency of the disease in their country was caused by a low content of biotin in the mother's milk. This lack was explained by the imperfect nutrition after the war, particularly in such foods as beans, peas, liver, and kidneys.

The serious course of the disease was materially influenced by biotin, which shortened it to a few days. In 1948, not one infant receiving biotin died. The decided action of biotin led them to confirm the assumption of Gyorgy that biotin is the deciding factor in the etiology of seborrheic dermatitis in infants, especially in its most severe clinical form, Leiner's disease.

The amount of biotin found in the blood and urine of children with Leiner's disease was lower on the average, than in normal children. Their patients were treated with daily injections of 0.1 mg., or the nursing mothers were given 4 mg. per day, orally. Amelioration was observed in two to three days and a complete cure in seven to fourteen days. In addition,

penicillin was given to eliminate the secondary infections. During treatment, the biotin excretion in the urine increased as well as the biotin level in the blood.

In five cases of Leiner's disease in bottle-fed infants, the intake of biotin was sufficient, but there was an increased loss of biotin in the urine and feces due to more severe secondary infections and diarrhea. When the infection was controlled, the biotin levels in the urine and feces fell, showing that more of it was retained.

The authors conclude that Leiner's disease in the breast-fed infant is caused by a lack of biotin in the milk of the mother and is sometimes aggravated by secondary infection. In the artificially fed baby, the lack of biotin is caused by general toxic, infective influences which increase the requirement for the biotin and inhibit the possibility of its use in the tissues.

Sara Kokil,[18] in 1954, reported twenty-four cases of seborrheic dermatitis from Germany; twelve of the infants were breast fed and twelve bottle fed; they ranged in age from 2 weeks to 3½ months. Seborrheic dermatitis of the young infant is an illness of its own which bears no relationship to the allergic eczema of infancy or the seborrheic dermatitis of older children and adults. It is a winter disease in Germany, appearing every year from November to April, and is probably due to a lowering of the biotin content in breast milk and cow's milk during that season. The amounts of biotin become insufficient to guarantee normal keratinization. Biotin is a specific against seborrheic dermatitis. Oral or parenteral doses of 5 mg. per day will cure seborrheic dermatitis in two to three weeks. Similar affections of older children are uninfluenced by biotin.

Theelin[19] reiterates the observation that experiments with human beings and animals make one think that the lack of biotin is the cause of seborrheic dermatitis. He describes the favorable results in a case with one injection of 5 mg. of biotin, followed by one week of oral therapy of 2 mg. daily.

Sydenstricker and associates[20] were able to produce a fine scaly desquamation of the skin and a grayish pallor in four adult volunteers fed a diet poor in biotin, of which 30 per cent consisted of egg white. The diet was supplemented by riboflavin and vitamin B complex. The urinary biotin excretion fell to low levels. Treating these volunteers with daily injections of biotin improved the skin and general well-being of the subjects.

In 1951, Martin[21] discussed his results in France. Six cases of localized seborrhea of the buttocks or scalp were definitely improved by oral administration of biotin. He mentions that the dosage of biotin for the infant is unknown. He gives six to twelve daily injections of 5 mg. of biotin. Occasionally, in the severe form, he gives it twice daily. In the milder forms, he recommends oral doses of 2 to 3 mg. per day for several weeks. He refers to Theelin[19] as giving daily intramuscular doses of 5 mg. for fifteen days.

Martin describes his results in four other severe cases. The first infant, breast fed, with Leiner's disease, received daily injections of 5 mg. of biotin for one week, followed by 2 mg. orally, daily for one week. The skin was normal three weeks after the onset of treatment. A second case of [illegible] gave [illegible] improvement of the [illegible] on 3 mg.

orally for five days. The lesions became worse when the drug was stopped. Biotin given intramuscularly for eight doses produced remarkable improvement.

In the third case, widespread seborrhea followed a diarrhea subsequent to Aureomycin therapy for staphylococcus pleurisy. This case resembles our Case 6, in which the diarrhea responded to vitamin B complex. Martin treated this patient with ten injections of biotin daily, of 5 mg. each; fifteen days later the skin was clear.

In the fourth case, a premature infant with poor weight gain and growth, extensive seborrhea cleared after five injections of biotin, accompanied by excellent weight gain.

Martin finds that approximately fifteen days after biotin treatment, the seborrheic skin desquamates and is replaced by normal skin.

In the treatment of seborrheic dermatitis, Lemke,[22] in 1950, stated not only that biotin is indispensable, but that there is a synergism between biotin and vitamin B complex, and that other factors of the B complex are also important in the cure. In one infant, B complex injections produced no improvement until biotin was injected along with the B complex, and a dramatic cure then occurred in four days. In two other cases, the simultaneous injections of vitamin B complex and biotin produced a marked improvement in four days. In two further cases, injections of the nursing mother with biotin and B complex produced dramatic improvement in the breast-fed infant.

To further corroborate the interrelationship of biotin and vitamin B complex, James[23] treated cradle cap with daily oral doses of 1 mg. of riboflavin over a fourteen-day period, with very satisfactory results.

In further studies with the various components of the B complex, Lemke[22] reported that when one child received two injections of biotin plus nicotinamide, there was no improvement until oral B complex and oral biotin were given afterward for fourteen days. Using panthothenic acid and biotin injections, there was improvement in another case, but improvement was most marked when the mother was given liver injections. In four other children, vitamin B_6 was given alone. There was improvement in two of these cases. Biotin was then given in conjunction with the vitamin B_6; two patients were cured, one improved, and in one there was no improvement. Lemke concludes that seborrheic dermatitis is due to an absence of both biotin and the total vitamin B complex. He treats his patients with three injections of 5 mg. of biotin two days apart, plus one ampule of B complex at the time of each injection, both of which were given either to the breast-fed infant or to the nursing mother. His investigations are of further interest in that he demonstrates that liver, which is a good source of biotin, gives no definite results in seborrhea when fed to these infants. This is probably because the infant is unable to separate biotin from liver. However, when the nursing mother was fed 150 grams of cooked liver or kidney for ten days, or 1,500 grams of liver in three to five days, the skin lesions of the breast-fed infant healed in approximately eight days. One kilogram of liver contains 2 mg. of biotin, and this, according to Lemke, should be sufficient for complete improvement of Leiner's disease in

eight days, when ingested by the nursing mother. He demonstrated that nursing mothers were able to cure their breast-fed infants completely by receiving only two 5 mg. injections of biotin. Evidently, small doses of biotin injected in the nursing adult produces biotin for the nursing infant as well as vitamin B complex.

Petrocini and Debernardi,[3] reporting from Italy in 1954, treated seven cases of Leiner's disease and five cases of seborrheic dermatitis in infants with biotin, with excellent results. Three cases of atopic dermatitis failed to respond. In four cases, their doses intramuscularly were 5 mg. daily for six to eleven days. In eight cases, they used 10 mg. of biotin orally, daily, for twenty to thirty days. They found parenteral therapy more effective than oral therapy.

In view of this array of material on the use of vitamin B complex and biotin in the treatment of seborrheic dermatitis and Leiner's disease, several patients were treated with these drugs.

CASE REPORTS

The patients were treated at Children's Hospital of Los Angeles, in private practice, and one case was reported from the Orange County General Hospital. The striking results that were obtained in this group of cases were convincing evidence that a deficiency of biotin was of prime importance in the causation of these two diseases.

The first six cases of seborrheic dermatitis were treated with injections of vitamin B complex, with moderate improvement. Subsequently, biotin* was given, which was even more effective.

*Biotin was supplied by Hoffmann-La Roche Inc., Nutley, N. J., in 5 mg. tablets for oral use and 1 c.c. ampules of 5 mg. each for parenteral use.

Seborrheic Dermatitis Treated With Vitamin B Complex.—

CASE 1.—M. G., male, aged 1½ months, had a rash for one week, with diffuse scaly erythema of scalp and face and intertrigo of axillae and navel. He was being fed evaporated milk and rice cereal. Treatment consisted of an Alacta formula, 10 per cent sulfur ointment, and 1 c.c. of Lederplex intramuscularly, with marked improvement in one week.

CASE 2.—L. P., male, had onset of rash at 2 months of age. There was seborrhea of scalp in the postauricular area. Creases of the groin and axillae were erythematous. The infant was treated with Nutramigen, local sulfur ointment, and 1 c.c. of Lederplex intramuscularly given on four occasions. Very definite improvement was seen after each injection.

CASE 3.—L. L., male, developed a rash at 6 weeks of age. There were seborrheic dermatitis of face and shoulders, scaliness of scalp and eyebrows, and erythema of axillae. He was on evaporated milk formula. Treatment consisted of sulfur ointment, skimmed milk, and 1 c.c. of Lederplex intramuscularly. There was marked improvement in skin eruption.

CASE 4.—H. V., male, at 1½ months of age had diffusely crusted lesions on scalp and rash on back and face. He was treated with Sobee formula and Pragmater at 3 months of age. Seborrhea was still present and the axillae were still erythematous. After 1 c.c. of Lederplex intramuscularly, the skin showed definite improvement.

CASE 5.—N. M., male, had seborrheic eruption of face, neck, scalp, and postauricular areas, beginning at 5 weeks of age. He was treated with Sobee formula at 2 months of age, but the rash became worse, with scaling of eyebrows and face and persistence of diffuse erythema. Treatment with skimmed milk, sulfur ointment, and injection of 1 c.c. of Lederplex gave definite skin improvement.

These five cases, where Lederplex was given for a short time only, revealed the benefit of B complex in seborrheic dermatitis. None of these infants was breast fed. The lesions improved, but there was not complete cure in all of the cases.

Another interesting case is reported to show the production of seborrheic dermatitis and diarrhea due to the long-continued use of antibiotics. Diarrhea and seborrhea were benefited partly by vitamin B complex.

CASE 6.—N. D., male, developed *Esch. coli* meningitis at the age of 1 week. He was treated with Chloromycetin, streptomycin, and sulfadiazine for several weeks; then because of the recurrence of the infection, he was given Terramycin and triple sulfonamides orally. While on this regime, he developed a very severe diarrhea. Cultures of the stools were negative for pathogenic organisms. There was no skin eruption, except for a red perianal rash which was resistant to local therapy.

On the belief that this diarrhea was due to destruction of the normal bacterial flora, with resultant vitamin B deficiency, Solu-B injections were given daily. The diarrhea subsided in a few days and the perianal rash healed. Because of the persistence of an elevated spinal fluid cell count and positive Pandy test, the child was treated with streptomycin intramuscularly, which he received for six weeks, and oral triple sulfonamides which he received for three and a half months.

Lederplex injections were given three times a week during the entire period of drug therapy. During this time, there was no diarrhea, but the child developed seborrheic dermatitis of the scalp, axillae, and the postauricular areas. These lesions persisted despite local therapy and Lederplex injections and disappeared only when all antibiotics were discontinued.

Biotin-Treated Cases.—

Nine cases of seborrheic dermatitis and two cases of Leiner's disease were treated with biotin, with good to excellent results. These cases are presented briefly. The amount of biotin given was considerably less than that described in the literature because of the small supply obtained.

CASE 7.—M. M., male, 1 month old, had seborrhea of the scalp and eyebrows, roughness of cheeks, and erythematous denuded areas of the groin and right axilla. The child was given two 5 mg. injections of biotin three days apart, and the skin cleared in five days. This child was being fed an evaporated milk formula.

CASE 8.—F. S., female, 3 months old, had extensive crusted cradle cap. The child was on an evaporated milk formula. One injection of 5 mg. of biotin was given and Pragmatar was applied locally. The scalp cleared in a week.

CASE 9.—S. G., male, 1 month of age, had an erythematous and crusted scalp. The face was rough and scaly. One injection of 5 mg. of biotin was given and the skin improved. At 3 months of age, the scalp was still scaly, but not as severely so, and the child had several bouts of diarrhea.

CASE 10.—R. W., male, at 3 months of age had seborrheic eczema of the scalp, with extensive erythema and denudation of the skin of the neck, axillae, groin, and retroauricular areas. Biotin was given orally, 1 mg. three times a day, for one week. The response to oral biotin was only fair, with persistence of cradle cap, scaliness of the axillae, and dry flaky skin of the back. An injection of 5 mg. of biotin was given at 4 months of age and the scalp and skin cleared.

Diarrhea was present at 4 months of age and recurred at 6 months. The rash reappeared to a slight extent. The diarrhea responded to skimmed milk and the rash to Vioform ointment.

This case is of interest in that it shows a better response to injected

biotin than to oral biotin. It also shows the coexistence of diarrhea and seborrheic eczema.

CASE 11.—J. H., female, was breast fed for one month. At 1½ months of age, extensive scaling of scalp and eyebrows appeared. There was erythema of the axillae and groin. One injection of 5 mg. of biotin was given. Two weeks later the skin was considerably improved. A second injection of 5 mg. was given. The response was good, with only slight residual scaliness of the scalp.

CASE 13.—B. L., female, had onset of rash at 1½ months, with roughness of face, scaling of scalp, redness of axillae and groin, and crusting behind the ears. The infant had been fed an evaporated milk formula. She was given an injection of 5 mg. of biotin at 2 months of age, and the skin improved, but the scalp remained scaly. Diarrhea was present during this period. At 2½ months of age, the skin and scalp became worse, and 1 c.c. of Lederplex was given, with improvement of skin, but not as dramatic as with biotin. At 3 months of age, a second injection of biotin, 5 mg., was given. The skin improved again and the neck cleared, but the rash recurred after a few days. Two more injections were given a week apart, with improvement of the skin, but again there was recurrence of the rash after a few days. The child was then given daily injections of both biotin, 5 mg., and Lederplex, 1 c.c., for four consecutive days, with marked improvement of the skin and scalp.

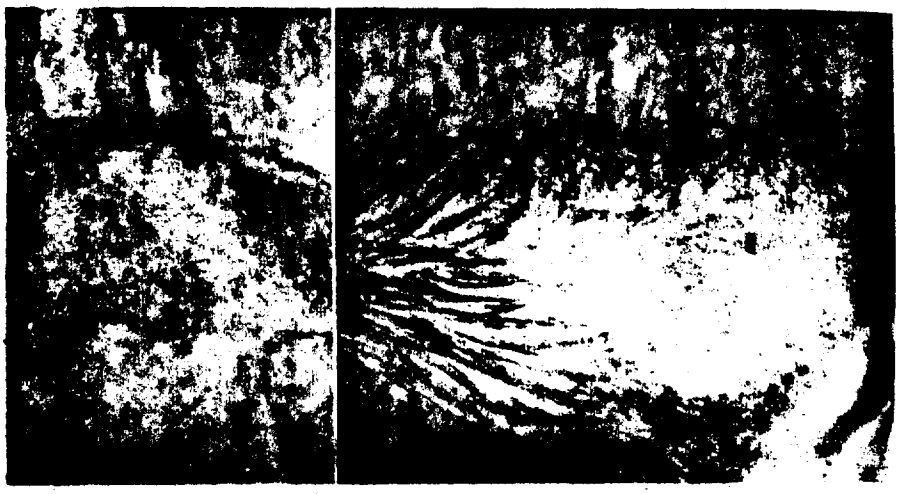


FIG. 1. FIG. 2.

Fig. 1.—Typical seborrheic erythematous lesion of axilla.
Fig. 2.—Seborrheic scaling of scalp.

CASE 12.—C. N., male, was on a diet of Lactum, meat, vegetables, and fruit. At 1½ months of age the scalp was scaly and there was angry, erythematous, denuded involvement of the axillae and groin. The child was given 5 mg. of biotin intramuscularly and the diet was changed to Dalactum. One week later the skin showed improvement, with less erythema. Diarrhea was present at this time. A second injection of biotin, 5 mg., was given one week after the first. Two weeks after the second injection the skin and scalp were entirely clear. (Figs. 1 and 2.)

CASE 14.—E. T., female, at 10 days of age, had an erythematous eruption

in the gluteal areas, spreading up the back. At 3 weeks of age, there was seborrhea of the scalp, with massive crusting, and moderate crusting of the face, trunk, and postauricular areas. The child was treated with a soybean formula, penicillin injections, saline soaks to the scalp, and Pragmatar ointment. The formula was changed to Dryco. There was slight improvement during the stay in the hospital. (Figs. 3 and 4.)

allergic sibling. Oral biotin was given daily in doses of 2 to 3 mg. for four weeks, with improvement of the skin, disappearance of the erythema, and minimal residual crusting of the scalp.

Cases of Leiner's Disease.

Case 16.—C. B., female, had onset of rash at 4 weeks of age, with cradle cap which gradually became thick and matted. The lesions spread to the chest and back with scaling and oozing.

Fig. 3.

Fig. 4.

Fig. 3.—Scaling and erythema of neck.
Fig. 4.—Scaling, erythematous lesions of face and scalp in seborrheic dermatitis.

After discharge, the child developed diarrhea, and the diet was changed to rice and Nutramigen. Five milligrams of biotin was given intramuscularly and repeated in two days. The formula was changed to skimmed milk, and sulfur ointment was applied locally. Two weeks after the second injection, the skin was clear, except for some redness of the neck and residual crusting behind the ears.

Case 15.—M. R., male, at 2 months of age had seborrhea of scalp and postauricular areas. Erythema of axillae and groin was present. Stools occasionally were loose. The child was given meat-base formula because of an

At 7 weeks of age, the entire body was covered with dry yellow flaky lesions and she was admitted to Children's Hospital of Los Angeles. The child had the appearance of a boiled lobster. (Fig. 5.) There was a fine, branny, scaly, dry rash covering a red, angry underlying epidermis which involved all of the skin surface, except the palms and soles. There was no weeping or itching.

The child also had diarrhea, with many green, loose stools. She was first fed soy milk, and the skin became worse. The stool culture revealed Paracolon and the infant was treated with neomycin and triple sulfonamides.

Fig. 5. Boiled lobster appearance of Leiner's disease.

Fig. 6.

Fig. 7.

Fig. 6.—Diffuse scaly erythematous eruption in Leiner's disease.
Fig. 7.—Scaly [illegible]

with little improvement. The diet was changed to skimmed milk and Casec. The diarrhea did not improve and another stool culture showed Proteus.

The skin of the axillae cleared, but the other areas were essentially the same. Lederplex was given orally and followed by three daily injections with no change in the condition of the skin. Two injections of biotin of 5 mg. each were given, three days apart. The diarrhea improved considerably for the first time after these injections, and the skin showed improvement. Vioform ointment was applied. The skin cleared entirely a month after the injections of biotin.

CASE 17.—C. S., male, had onset of erythematous papular rash at 1 month of age over trunk and face. At 2 months of age, the child was fed Mull-Soy and the rash improved, but he began having diarrhea and losing weight. The milk was changed to whole milk. At 3 months of age, the child developed a generalized seborrheic eruption involving the scalp, face, ears, and trunk. At 4 months of age, he was admitted to the Orange County General Hospital with a diffuse erythematous scaly eruption, with considerable desquamation. (Figs. 6 and 7.)

The child was fed a soy milk formula with lamb and rice. He was given injections of penicillin and treated orally with Gantrisin and later with Achromycin.

Biotin was given by injection, 5 mg. every other day for four days, and a salicylic-sulfur ointment was applied to the skin.

Stool cultures were negative for pathogens.

There was marked improvement of the skin eruption about a week after the biotin injections.

CONCLUSION AND SUMMARY

There is evidence, both in the results of the cases reported here and in the European literature, that seborrheic dermatitis and Leiner's disease, [illegible] are variants of the same disease, are associated with biotin deficiency. However, the basic requirements of biotin for the infant are unknown.

Nine cases of seborrheic dermatitis and two cases of Leiner's disease showed marked improvement with biotin. The dosages used in our cases, although effective, was perhaps inadequate when compared with the dosage reported in the literature.

The doses recommended, therefore, for Leiner's disease and severe cases of seborrheic dermatitis are 5 mg. of biotin intramuscularly daily for seven to fourteen days. For milder cases, 2 to 4 mg. of biotin orally for two to three weeks are recommended by some authors, and 10 mg. daily for twenty to thirty days by another. The results were better with parenteral than with oral administration of biotin.

Five infants with seborrheic dermatitis were given injections of vitamin B complex alone, with moderate improvement in the skin lesions.

Since there is an interrelationship between biotin and vitamin B complex, and since vitamin B complex enhances the effect of biotin, it may be that better results would follow concomitant daily injections of vitamin B complex along with the biotin.

Feeding the nursing mother with a total of 1,500 grams of liver over three- to five-day periods, or giving her two to four injections of 5 mg. of biotin will seemingly be as effective.

There is also a relationship between the diet of the nursing mother and the incidence of these diseases, as shown by the increased number of cases of Leiner's disease following the wars. The improvement of the diet of both mother and infant as well as the

diminution of breast feeding in this country may account for the infrequency of Leiner's disease.

Foods high in biotin content are liver, egg yolk, kidney, and yeast. These foods are most effective when fed directly to the nursing mother, who can utilize biotin better than the infant.

Biotin is seemingly without value in seborrheic dermatitis of the older child and adult, which suggests a disease of different etiology.

The technical assistance of Mr. Daryl Davis, photographer, and Dr. Ralph Campbell, is gratefully acknowledged.

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Oppel-'42
Bib No. 1022

Amer. Jour. Med. Sci. 204(6):856-875. 1942.

STUDIES OF BIOTIN METABOLISM IN MAN*

PART I. THE EXCRETION OF BIOTIN IN HUMAN URINE

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As early as 1929 it was suggested by Findlay and Stern[10] that certain skin and nervous system changes in children, known as Swift's disease or pink disease, might be due to a lack of some essential material in the diet. This suggestion was made because

* This investigation has been aided by a grant from the Josiah Macy, Jr. Foundation.

of the similarity between the clinical and pathologic changes in these children and those produced in rats as a result of feeding a diet of high egg white content. Although many years elapsed before the mechanism of "egg white injury" was clarified, the idea that a vitamin essential for man might be involved has been repeatedly mentioned. György, who gave the name vitamin H to the anti-egg white injury factor, has suggested several times that it might play a rôle in man.[11,13,14] During the present year Sydenstricker, Singal, Briggs, De Vaughn, and Isbell[21] reported that they had produced such a vitamin deficiency in man by egg white feeding and had cured it with a biotin concentrate.

The study of the factors involved in egg white injury in animals has been made principally by Boas,[2] Parsons, Lease, and Kelly,[19] and György,[12] and their work has been recently reviewed by du Vigneaud.[4] The universal biologic nature of the protective factor was indicated by the finding of du Vigneaud, Melville, György, and Rose[6] that vitamin H is identical with coenzyme R, a respiration enzyme essential for the growth of *Rhizobium trifolii*, and biotin, a stimulant for the growth of yeast. Workers from these same groups later isolated the vitamin from liver.[5] It now appears that the syndrome of egg white injury results from the formation of a heat labile compound between biotin and a substance in egg white[15] called avidin,[9] or antibiotin.[24] This compound is not absorbed from the intestinal tract and thus deprives the animal of biotin.[8] This is the only condition under which rats have been made biotin-deficient, but chickens have been made deficient by a low biotin diet alone.[1,16,17] Biotin-deficient chicks develop within a few weeks swelling and redness of the eyelids, cracking and bleeding of the skin of the feet and around the mandible, and loss of feathers. The skin lesions in the rat, which have a general similarity to those in the chick, have been regarded as a seborrheid desquamative type and they are accompanied by spasticity of the muscles and a kangaroo-like posture.

In the present study an attempt was made to learn something about the use of biotin in man by studying its metabolism. The work so far completed is reported in 3 parts. The first surveys the excretion of biotin in urine. The second gives data on the relationship between biotin intake and output, and the third describes and discusses the significance of a material with biotin-like properties which has also been found in the urine.

Methods and Results. The determinations were carried out by a yeast growth method described by du Vigneaud, Hofmann, Melville, and György (5). In this test a synthetic medium from which practically all the biotin has been removed is inoculated with a standard quantity of yeast culture, and 10 cc. amounts are added to 2 series of flasks. To one series known amounts of biotin are added, and the yeast is allowed to grow for 16 hours at 30° C. The amount of yeast growth in each flask is then determined by measuring its turbidity with a photo-electric colorimeter and from these

values a standard curve, or growth curve due to known amounts of biotin, is constructed. The second series of flasks is used to construct the growth curve for the substance to be tested. The biotin content of the unknown solution is calculated by simple proportion from the position on the growth curve of the unknown that corresponds to the point of half maximum growth on the standard curve. To form our standard curve, pure biotin was added in amounts of .1, .03, .01, .003, .001, .0003, .0001, .00003, .00001, .000003 micrograms per flask. The urine was added in amounts of .3, .18, .1, .056, .03, .018, .01, .003, .001 cc. per flask. These quantities of biotin or urine were made up to a volume of 2 cc. before they were added to the medium. Each 100 cc. of medium was inoculated with .6 mg. of 24-hour culture of *Saccharomyces cerevisiæ* strain 139.

When the biotin content of a urine was determined in this way the same value was found on repeated determinations. Twenty-four hour samples, collected in bottles to which 10 cc. of glacial acetic acid had been added, were found to contain the same amount of biotin after standing in the icebox for a month as on the day they were collected. There has been no evidence of biotin deterioration in any of the specimens studied. Neither was any of the biotin in urine found in the combined form in which it occurs in foods such as liver and yeast. In these materials biotin is present in a complex union from which it is separated only by autolysis, digestion, acid hydrolysis, or autoclaving. The urine value was not changed when the sample was mixed with concentrated HCl and evaporated to dryness or autoclaved 2 hours with concentrated H_2SO_4.

TABLE 1 BIOTIN ELIMINATION FOR NORMAL SUBJECTS

Subject No.	Micrograms per liter	Micrograms per 24 hours	Urine vol. in cc.
	Men		
1	12.5	23	1810
2	46.7	44	950
3	12.7	24	1850
4	30.4	27	883
5	17.5	30	1700
6	7.8	23	2990
7	32.2	50	1560
8	23.4	26	1100
9	69.0	111	1620
10	39.0	41	1050
Variation	7.8–69.0	23–111	883–2990
	Women		
11	89.1	46	520
12	20.4	48	2330
13	55.0	66	1200
14	44.6	49	1080
15	39.9	33	820
16	30.2	64	2120
17	28.2	39	1350
18	47.4	51	1090
19	64.6	106	1640
20	9.6	14	1500
Variation	9.6–89.1	14–106	520–2330

Other experiments were carried out to see if the material we were testing differed in any way from biotin derived from other sources. No difference was found except that only part of the material in the urine could be completely inactivated by egg white. The significance of this finding is discussed in Part III of this paper.

Most of the normal subjects excreted from 20 to 50 micrograms of biotin per 24 hours, but there were a few whose daily output was more or less than this. This is shown in Table 1, where the elimination by a group of 10 normal men and 10 normal women who ate their usual diets is listed. There was no significant difference between the average values for the 2 groups, and the range extended from 14 to 111 micrograms, with an average figure of 45.7 micrograms per 24 hours.

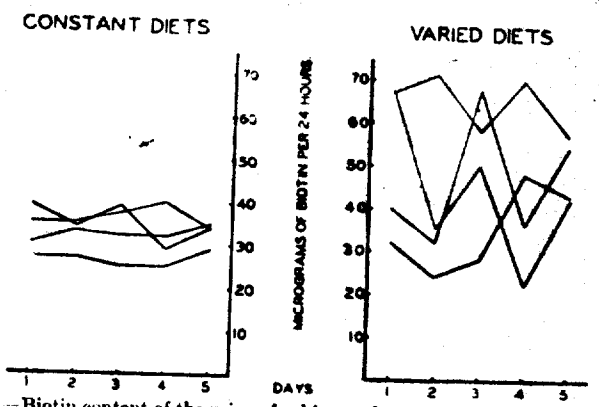

FIG. 1.—Biotin content of the urine of subjects who ate constant diets and varied diets for 5 days.

From day to day there was a moderate variation in biotin eliminated when the same subject ate an unrestricted diet. In contrast to this, there was little daily variation in the biotin content of the urine when the same diet was eaten each day. The opportunity to make such observations was provided by 4 patients who, because of various metabolic studies, ate the same diets every day for 5 or more days. In these diets not only the calories, minerals, protein, fat, and carbohydrate were kept constant, but the same breakfast, lunch, and dinner were eaten each day. In Fig. 1 the biotin excretion of these subjects on constant diets is plotted with the excretion values for a group of subjects who ate varied diets, and a rather striking difference is shown.

A study was also made of the elimination of biotin at different periods throughout the day. From 6 subjects on unrestricted diets the urines were collected so that the biotin excreted from one mealtime to the next could be measured separately. This value was divided by the number of hours between the collections of

urine, to obtain the average hourly excretion for the period. These results are shown in Fig. 2. The hourly excretion was usually, though not always, higher during the day than at night and was different for each subject. One hospital patient who received practically all her food for the day at 11 A.M. by stomach tube provided an ideal experiment for us. Her biotin excretion throughout the day showed a uniform pattern and was not increased immediately after the feeding. This is also shown in Fig. 2. The tube feeding she received contained the uncooked whites of 4 eggs, and we were interested to see whether these prevented the absorption of much biotin. The eggs were therefore left out of the feeding for 5 days but no significant change in the biotin content of the urine occurred.

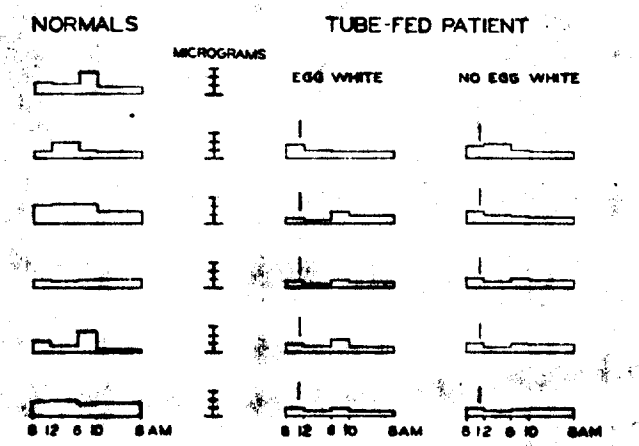

FIG. 2.—Hourly biotin elimination throughout the day. The area of each block indicates the amount of biotin eliminated during that period. The normals ate their ordinary meals. The tube-fed patient received her food at 11 A.M. as indicated by the line above each diagram.

When a test dose of crude biotin was taken by one subject there was a prompt flooding of the urine with it. A normal male, who usually excreted about 40 micrograms of biotin a day and who eliminated 3.18 micrograms per hour during the morning, drank an aqueous solution containing 1864 micrograms of crude biotin at noon. During the following 6 hours his urine contained 560 micrograms (93 micrograms per hour). During the 24-hour period after he took the biotin he eliminated 793 micrograms. These results are shown in Fig. 3.

The biotin content of the urines of 60 patients from the pavilions of the New York Hospital was studied to see if any of these showed a striking difference from the normal subjects. Cases with a wide variety of pathologic conditions were chosen, but those with skin or nervous disease processes were especially sought. Patients

with malignant disease, blood disorders, endocrine conditions, infections, and a variety of miscellaneous conditions were studied. An attempt was also made to find those whose food intakes had been

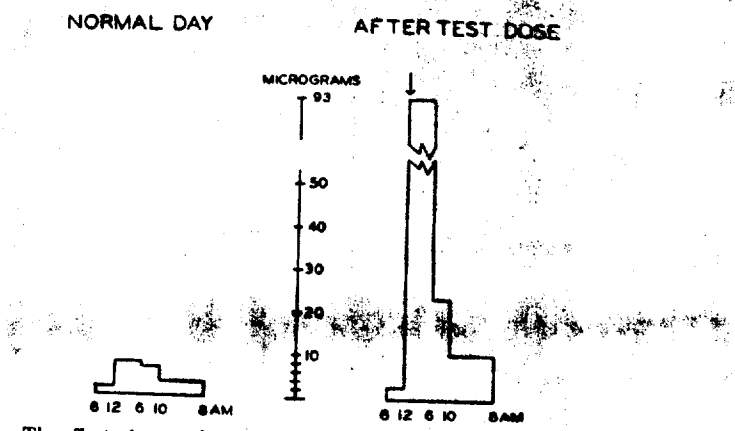

FIG. 3.—The effect of a test dose of 1800 micrograms of crude biotin on the hourly output in the urine. The arrow indicates the time of the dose.

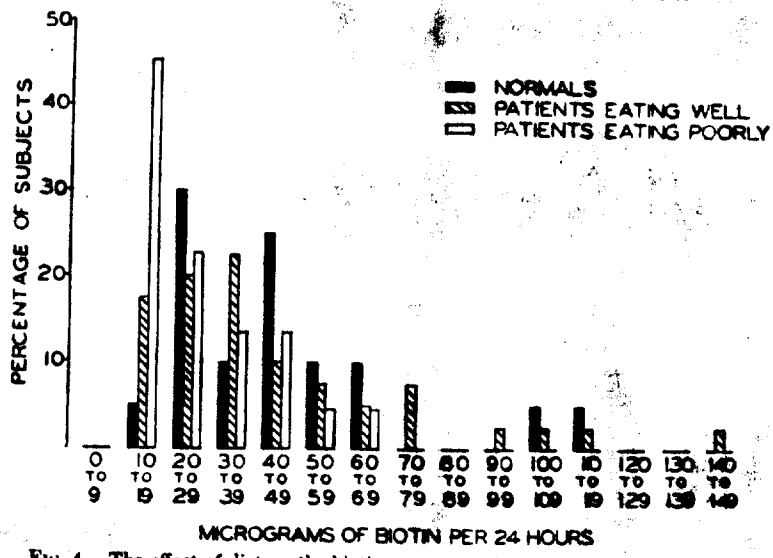

FIG. 4.—The effect of diet on the biotin content of the urine. Nearly 50% of the patients who ate poorly excreted only 10 to 19 micrograms of biotin per 24 hours.

greatly restricted by disease or treatment. Practically all the values fell within the normal range. In the majority of instances where the food intake of the subject had been conspicuously poor the biotin excretion values were low. This is graphically shown in Fig. 4,

where the biotin excretion values for the normal subjects, patients who ate well, and patients who ate poorly, are shown in separate columns.

Discussion. The conclusions allowed by these results are limited by the fact that our urinary biotin values can be related to biotin intake only in a general way. The amounts of biotin in so few foods are known that we cannot yet calculate its content in human diets. Isolated determinations of the biotin content of urine have been reported, but there has been no previous study of the significance of the urinary biotin. More specific information than we have here is gained from complete biotin balance studies, and such studies on human subjects are reported in the next part of this series. A few facts appear to be established from these results, however, and there have been some reports on biotin metabolism in animals with which they can be correlated.

There are 2 types of biotin metabolism in animals. Chickens are apparently dependent on their diets for biotin and become deficient when the supply is low. Eakin, McKinley, and Williams[8] found that on ordinary diets these fowl excrete from 10 to 20% of their dietary biotin in free form and an additional 15 to 25% in a combined form which is liberated by hydrolysis. These figures represent the total biotin output since the excretion from the kidneys of these birds is present in their feces. It would appear that the rat is more difficult to make biotin deficient through ordinary dietary means than the chick, and he probably derives biotin from some other source than his food.[4] Biotin from this non-dietary source as well as dietary biotin might be found in the rat's urine, but unfortunately studies of biotin excreted by rats have not been reported.

The experiments here reported clearly indicate that the biotin content of human urine is influenced by the amount of biotin in the diet. This is shown in several ways. When the subjects were grouped into those who ate well and those who ate poorly, a corresponding difference in biotin elimination was shown. For some individuals this did not hold true but there is no reason to believe that every large meal contains a large amount of biotin or that a small meal cannot. A correlation between biotin intake and urinary output is further shown by a comparison between the urine values of subjects who ate varied diets and of those who ate constant diets. In the former group the output is varied; in the latter it is quite constant. Moreover, the biotin content of urine was markedly increased by the ingestion of a test dose of crude biotin.

While the above experiments show that biotin in food affects the urine value, there are others that point to some source of the vitamin for man besides his food. Throughout these studies we have been constantly searching for a patient who excreted no biotin in the urine and we have analyzed the specimens of every patient we could find on a poor diet. Many of these were in the terminal stage of

chronic illnesses and had eaten nothing for days. In spite of this effort we have been unable to locate a patient without biotin in the urine. In fact, we have found very few who excreted less than our lowest normal. This is quite in contrast to other vitamin excretion studies and suggests that human subjects are not entirely dependent on food for their biotin. Evidence is presented in Part II of this paper to show that they derive a good deal of it from synthesis by bacteria in the intestinal tract.

In the study of patients we selected those who showed skin and nervous diseases since these body systems are the ones altered in biotin-deficient animals. Patients with malignant diseases were also investigated because of the findings, by other workers, of a high biotin content of tumor tissue[2] and of the procarcinogenic effect of this vitamin.[7] The rest of the patients were chosen to see if any alteration due to fever, anemia, increased metabolism, cirrhosis, or other abnormalities might influence biotin metabolism. No group of patients showed abnormally low values and only an occasional case excreted less than our normals. It cannot, however, be finally concluded that some error of biotin metabolism does not occur in these diseases. Such an error might be demonstrated by a more exact test than the determination of the biotin content of a 24-hour urine specimen, collected from a subject on an unrestricted diet.

Conclusions (Part I). In a study of the biotin excretion in human urine it was found that:

1. Normal subjects on unrestricted diets excreted from 7 to 89 micrograms of biotin (as measured by yeast growth stimulation) per liter of urine or from 14 to 111 micrograms of biotin per 24 hours.
2. With ordinary diets the biotin elimination varied from day to day, and throughout the day. There was a striking increase immediately after a large dose of crude biotin was ingested.
3. Biotin excretion was greater in the urine of those who ate well than in the urine of those whose intake of food was poor. It was relatively constant for those who ate the same food every day.
4. The biotin excretion of a large number of patients with a variety of disease conditions was studied. The values found were within the normal range and even during periods of starvation were not abnormally low.

PART II. THE RELATIONSHIP BETWEEN THE BIOTIN CONTENT OF THE DIET AND ITS OUTPUT IN THE URINE AND FECES

In Part I of this paper a general survey of the biotin content of urine was made and values for normal subjects were given. It was found that patients with a wide variety of morbid processes excreted relatively normal amounts of the vitamin. A general comparsion of the abundance of the diet and the urinary biotin level was made and showed that the excretion was usually low when the

diet was poor. There appeared to be a level below which the value did not fall even during starvation. This low level was also reached, however, by some normals on a normal diet.

To investigate further the metabolism of biotin in man, balance studies have been made on a small group of patients. Most of these studies were done in the Metabolism Pavilion of the Russell Sage Institute of Pathology at the New York Hospital, and the routine of that Institute was generally adhered to, as described below. Nothing to date has indicated that the diseases for which these subjects were hospitalized or the treatments they were given created any disturbing effect on the experiments.

Methods and Results. To regulate the biotin intake each patient was kept on a constant diet and ate the same three meals throughout the balance period. Different subjects ate different diets and various combinations of diets were used. In the first series of experiments a constant diet was eaten every day. In a second series of experiments two diets of widely different biotin content were alternated. These latter diets were identical in the protein, fat, carbohydrate, calcium, and phosphorus content and the principal difference between them was that 50 gm. of liver and 110 gm. of steak were taken with alternate luncheons. After several days on either program, during which the collection of urine and stool specimens was begun, a dose of 0.2 gm. carmine was given and this was repeated at the end of the experimental period, which was usually 5 days later. In this way the stool specimens corresponding to the experimental period were marked.

The urines were analyzed by the procedure previously outlined. The diets required considerable preparation before analysis. A duplicate of the day's food was thoroughly ground and suspended in water, and the mixture was shaken mechanically for 1 hour. It was then evaporated to dryness on a steam bath and the dry residue was ground and suspended in water to which 0.25 cc. concentrated H_2SO_4 was added for each 5 cc. of solution. This mixture was autoclaved at 20 pounds pressure for two hours, and then adjusted to pH 4 to 4.5, filtered with suction, and the filtrate made up to volume. Ten grams of the ground diet residue were made up to an ultimate volume of 100 cc.

The stool specimens for the whole period were mixed, dried, and hydrolyzed in exactly the same way as the diet except that 100 mg. of the ground stool were made to a final volume of 20 cc.

Because only a limited number of experiments of this type were feasible, certain refinements of the biotin analysis technique were made to increase as much as possible the accuracy of the results. In all cases the tests were run in triplicate so that an error of any single determination would be found. Furthermore, the specimens in each balance period were all analyzed on the same day so they could be calculated from the same standard curve. It had been previously found that on certain days the standard curve, independently or sometimes with the unknown's curve, would not occupy its usual position on the chart. On such days the values were inaccurate. Errors of this type could be largely avoided by triplicate analyses and were probably completely avoided by using only those curves on which the standard fell within narrow limits, arbitrarily chosen.

The diets used are listed in Table 2. Diet 1 consisted of powdered milk and was found to contain 60 micrograms of biotin. When the hydrolysate of it was mixed with egg white in the proportions of Diet 1c, biotin activity was completely inhibited in the test tube. Diet 2 has a low calcium and phosphorus content but otherwise corresponds to the food consumed by

many individuals who are only moderately active. Diets 3, 4, and 5 are average, general diets, and the biotin values for them probably correspond to that of the average American diet. From the values found in these analyses, it appears that liver, which was present only in Diet 5, is one of the few foods which significantly alter the intake of this vitamin for the day.

TABLE 2.—COMPOSITION OF DIETS USED

	Diet No.					
	1	1a	2	3	4	5
Micrograms of Biotin	60	0*	30-34	34-37	32-35	54-65
Weight (gm.) Wet	....	....	840	1212	1998	1873
Weight (gm.) Dry	214	....	320	342	406	390
Calories	1000	....	1543	2015	2014	2017
Protein (gm.)	57	....	55	75	75	75
Fat (gm.)	60	....	60	66	76	76
Carbohydrate (gm.)	71	....	185	283	246	246
Calcium (gm.)	....	....	0.1	0.6	0.9	0.9
Phosphorus (gm.)	....	....	0.6	1.0	1.3	1.3

* Diet No. 1 plus 18.3 gm. of powdered egg white.

The diet of powdered milk and egg white was given to 2 subjects, on one of whom it had to be discontinued after 2 days. She had previously finished a control period of 3 days on powdered milk alone. During these 5 days her biotin excretion values in the urine were 29, 31, 38, 33, and 20 micrograms. The other subject was not given a control period but took the milk and egg white combination 5 days, during which he excreted 30, 24, 36, 24, and 46 micrograms. These values were not significantly different from those he previously and subsequently showed on a general diet. The stools on these subjects were collected individually and varied so much in regularity, because of the constipating nature of the food, that they could not be compared with the other figures.

In all the rest of the experiments the results were much more satisfactory and were in general agreement. They are shown on Figs. 5 and 6 and on Table 3. Fig. 5 shows graphically the relative amounts of biotin in the diet and in the urine of two subjects on constant diets. Although these subjects ate different foods, they consumed approximately the same amount of biotin daily and each of them excreted a relatively constant amount in the urine. One subject excreted slightly less biotin than she consumed but the other excreted considerably more than he ingested each day. In other balance periods the urine not infrequently contained more biotin than the diet.

Fig. 6 shows the relationship between dietary biotin and urinary biotin when diets containing 35 and 65 micrograms were taken on alternate days. Under these circumstances the urinary biotin values were not constant as were those on Fig. 5 but showed daily variations which followed the fluctuations in biotin intake quite closely.

On Table 3 the figures for the biotin balance periods investigated are listed in detail. The daily intake in the diet and output in the urine is listed. The average daily value for output in the feces is given since all the feces put out during the balance period were mixed and analyzed together. In 2 cases where the urines were not obtained, only the diet and fecal values are given. It is at once evident from this table that the biotin content of the stool far exceeded that of the diet, and from three to six times as much biotin was eliminated in the urine and feces of these subjects as they could have absorbed from their food. Biotin synthesis by the bacteria in the intestine apparently furnished a reservoir from which biotin could be absorbed. There is some suggestion that a correlation existed

between the amount of biotin in the feces and the quantity in the urine. When Subjects B (Periods 3 and 4) and C (Period 1) consumed the same amounts of biotin, the stool and urine values of the former were both considerably higher than those of the latter. No correlation between the biotin content of the diet and that of the feces is suggested by these data.

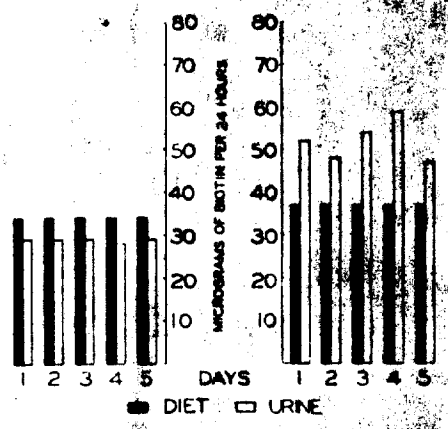

FIG. 5.—The relationship between the amounts of biotin in the diet and the urine of 2 subjects on constant diets.

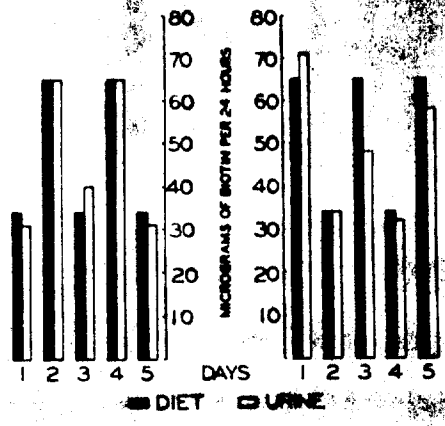

FIG. 6.—The relationship between the amounts of biotin in the diet and the urine of 2 subjects who ate diets of 35 and 65 micrograms of biotin on alternate days.

Discussion. The biotin contents of the diets used in this study probably indicate the usual range of biotin intake by normal men and women. Amounts varying from 30 to 65 micrograms a day appear exceedingly small when they are compared with the amounts of other members of the vitamin B complex in ordinary diets. The daily human consumption of the latter is often expressed in milli-

TABLE 3.—BIOTIN EXCHANGE IN THE SEVERAL METABOLISM PERIODS

Subject and number of metabolism period	Daily biotin intake in micrograms	Daily biotin output in micrograms			
		Total	Stool	Urine	
	Diet Number 2				
C. Period 1	34			29	
	34			29	
	34			29	
	34			28	
	34			29	
	34	116	87	29	Average
B. Period 1	33		86		Average
L. Period 1	30		99		Average
	Diet Number 3				
B. Period 3	34			73	
	34			53	
	34			44	
	34			50	
	34			48	
	34	217	163	54	Average
B. Period 4	37			52	
	37			48	
	37			54	
	37			59	
	37			47	
	37	243	191	52	Average
	Diet Numbers 4 and 5				
C. Period 4	34			31	
	65			65	
	34			40	
	65			65	
	34			31	
	46	128	82	46	Average
C. Period 5	65			71	
	34			34	
	65			48	
	34			32	
	65			58	
	83	187	105	48	Average
C. Period 6	62			60	
	35			29	
	62			65	
	35			34	
	62			76	
	51	154	98	53	Average
W. Period 1	32			37	
	54			39	
	32			42	
	54			77	
	32			47	
	54			65	
	32			48	
	41	181	127	54	Average

grams. This relatively small biotin intake by man, however, corresponds to a similar small biotin requirement of other biologic species. For example, optimal growth of yeast occurs when biotin is present in a concentration of only .0001 micrograms per cc., while thiamine must be present in a concentration of .04 micrograms per cc. to produce optimal yeast growth.[23] The biotin requirement of animals is also very small. Hegsted, Mills, Briggs, Elvehjem, and Hart[16] found that chicks require only 7 to 10 micrograms of biotin per 100 gm. of ration, a value surprisingly similar to that of our human diets. Our diets contain 2 to 4 micrograms of biotin per 100 gm. of wet ration, or from 10 to 16 micrograms of biotin per 100 gm. of dried diet.

The biotin content of the diet loses some of its importance because of the synthesis of biotin in the human intestinal tract. A number of bacteria, including *E. coli*, have been shown by Landy and Dicken[18] to synthesize biotin during growth in artificial media, and they appear to form more of it than they use. Burk[3] found his rats were excreting more biotin in their stools than he was feeding them. The high biotin content of the stools of our subjects thus did not come as a complete surprise. This colonic source of biotin may explain why a subject can put out more biotin in his urine than is taken in his diet, and why we found a relatively normal biotin excretion by fasting human subjects and by those who took egg white for short periods. Such findings suggest that the quantity of biotin provided from this source is adequate for the needs of man, and there is thus reason to doubt that human subjects require any biotin in their food or can be made deficient by a lack of it.

Since human subjects receive biotin from 2 sources, the significance of biotin in the urine becomes doubly complex. There is clear evidence that biotin from both sources appears in the urine. In the case of food biotin, this is shown by the striking daily variation of urinary biotin which occurred when subjects ate diets of high and low biotin content on alternate days. In the case of fecal biotin, by the continued biotin excretion which accompanies starvation and by the fact that the urine often contains more biotin than the diet. Apparently in man there is a relatively constant level of biotin excretion in the urine based on biotin synthesis by colonic bacteria, and superimposed on this, sudden variations occur due to changes in biotin content of the diet.

If this interpretation is correct, there is still no reason to doubt that the biotin content of the urine will become significantly diminished when the supply of the vitamin to the individual is greatly reduced. To produce the reduction, however, it is necessary to eliminate the supply from bacterial synthesis as well as the supply from the food. This apparently can be accomplished with diets of high egg white or avidin content. Recently Sydenstricker, Singal, Briggs, De Vaughn, and Isbell[21] have reported levels of 3.5 to 7.5

micrograms of biotin in the urine of human subjects whom they believed to be clinically deficient. These are significantly lower than any values we have found. There is also evidence, which is presented in the following part of this paper, to show that qualitative differences of the biotin content of the urine exist, and that the value of one of the biotin-like materials found in the urine may be as significant as the total biotin level.

Conclusions. (Part II). A small group of hospital patients have been kept on diets of known biotin content and the excretion of the vitamin in their urines and feces has been studied. The following conclusions have been drawn:

1. Diets of average normal composition contained from 30 to 40 micrograms of biotin a day, which increased to 65 micrograms when liver was included.

2. On a daily biotin intake of constant value, the biotin excretion in the urine was constant for each subject. The average daily figures for the different subjects, however, varied quite widely.

3. When diets of 35 and 65 micrograms of biotin were taken on alternate days, the urine showed corresponding daily fluctuations.

4. The average daily biotin content of the feces greatly exceeded the biotin content of the diets. There seemed to be some correlation between the biotin content of the feces and the content of the urine. There was no correlation between the biotin content of the feces and the content of the diets used.

5. The total biotin output in the urine and the feces was 3 to 6 times as great as the intake in the diet.

PART III. THE EXCRETION OF TWO BIOTIN-LIKE SUBSTANCES IN URINE

The previous parts of this series report some observations on the metabolism of biotin in man, made by determining the amounts of this vitamin in diets, feces, and urine specimens. The analyses were made by a yeast growth method, and since this lacks certain desirable specific characteristics, we did many experiments throughout the work to test the properties of the material. These experiments have shown that the material in our specimens which gives the yeast growth response characteristic of biotin, can be divided by the avidin reaction into two parts, and there are thus two substances with biotin-like properties. A study of these substances is the basis of this part of our report.

Method of Analysis. The measurement of biotin was described in detail in Part I. A comparison is made between the potency of an unknown material in factors that stimulate yeast growth, and that of a solution of known biotin content. A medium from which practically all of the biotin has been removed is used, and after it has been uniformly inoculated with yeast culture it is added to the unknown and standard solutions, which are made up in varying dilutions in flasks. After incubation the turbidity of

all the flasks is measured colorimetrically and curves of the yeast growth caused by the known and unknown solutions are formed by plotting the dilution of the solution against the turbidity of it. As the biotin content of the standard solution in micrograms per cc. is known, the strength of the unknown solution can be calculated by determining what dilution of it causes a turbidity equal to that of some point on the standard growth curve. The point of half maximum growth on this curve has been arbitrarily selected as the place where the comparison is made.

Avidin is the protein responsible for the development of egg white injury in animals. It apparently produces this syndrome by combining with the biotin in their diets to form a compound which is not absorbed from their intestines. Eakin, Snell, and Williams (9*a*) have shown that it also prevents yeast from utilizing the biotin for growth in pure and crude solutions. Its properties have been described by these authors (9) and by Woolley (24), and its combination with biotin appears to be a specific one. Its crystallin preparation was recently described (20).

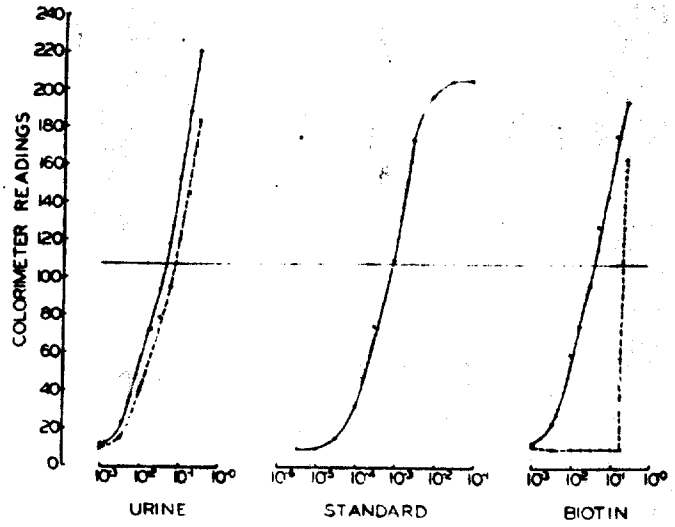

FIG. 7.—The difference between the effects of avidin on the growth factors in urine and in a biotin solution of corresponding strength. The untreated urine and biotin solutions are indicated by solid lines; the avidin treated, by interrupted lines. The growth curves are formed by plotting the dilution of the solution as the abscissa and the turbidity (colorimeter reading) as the ordinate. The standard solution contained 1 microgram of biotin per cc.

When it was found that the urine contained a material which would stimulate yeast growth on biotin-free media, attempts were made to inactivate it with avidin. A number of different urines were tested after quantities of avidin had been added that exceeded many times that needed to prevent completely the growth activity of a corresponding amount of biotin in aqueous solution. In other experiments a constant amount of avidin was added to each flask which made up the growth curve, and thus the ratio of biotin to avidin changed progressively throughout the series. When either procedure was used the avidin caused only a partial inhibition of the growth-stimulating material in the urine. The height of the growth curve was lowered and the curve was changed to a position which corre-

sponded to that of a solution of lower biotin content. In no case, however, has the growth effect of the urine been completely inhibited.

On Fig. 7 curves are shown in which the growth effects of urine and urine plus avidin are compared with solutions of pure biotin. A biotin solution was selected which gave a growth effect identical to that of the urine used. One cc. of the same avidin solution was added to all the flasks both of the biotin and the urine series. The difference between the effects of the avidin on the biotin and on the urine can be seen by comparing these curves.

The avidin has completely inhibited the growth in all flasks containing $.18 \times 10^{-1}$ micrograms of biotin, or less, and the curve falls abruptly at this point. It partly inhibited the growth in the one flask containing more biotin than this. In the urine series none of the flasks was completely inhibited by the avidin. The growth in all of them, however, was inhibited to a similar degree so that a new growth curve was formed. This falls parallel to the curve for the urine without added avidin. The growth effect of the material in urine not inhibited by the avidin can be calculated, in terms of biotin activity, from the position of this new curve.

By testing a urine sample with and without avidin in this way, values are obtained for: a, all of the biotin-like growth material in urine; and, b, the amount of biotin-like material in urine which will not combine with avidin. The difference between these two values represents the amount of material in the urine that did combine with the avidin. This latter material is probably biotin.

In urine the amounts of biotin and of the biotin-like material which fails to combine with avidin are easy to measure, because both of them are present in relatively large amounts. In suspensions of diets, stools, and other biologic materials there is only a little of the non-combinable material, and the strongest possible suspensions must be tested to find it. These suspensions are colored, because they have been hydrolysed with H_2SO_4 to liberate the biotin, and a color correction must be made. We have found it practical to minimize this color correction by incubating the yeast 30 hours, instead of the usual 16 hours, so that the contents of the flasks are more turbid.

Properties of the Non-avidin Combining Growth Material. It was at first thought that the failure of avidin to inactivate all of the biotin in urine might be due to the presence of some interfering substance, most likely urea. Pure biotin was therefore added to a urea solution, which corresponded in strength to the urea in one of our urine samples. No interfering effect of the urea occurred and the biotin was completely inactivated by avidin. In other experiments pure biotin was added to urine specimens and it was found that avidin completely inactivated it without changing the amount of the other growth substance present. When urine was diluted, the same percentage of non-avidin combining growth substance always remained regardless of the degree of dilution. No evidence was thus found that anything in the urine prevented the avidin-biotin combination.

A few tests were carried out to see if the two growth substances in urine could be distinguished by any other reaction than the avidin test. It had been previously shown that HCl and H_2SO_4 hydrolysis, with concentrations of acid which do not destroy biotin, does not alter the total amount of growth substance in the urine measured by this test. Woolley[34] has shown that avidin is relatively stable to changes of temperature and pH. We adjusted urine samples to a

pH range of from 1 to 12 and added to them avidin solutions of corresponding pH. The amounts of biotin and non-combinable biotin-like substance in the urine were the same irrespective of the pH at which they were mixed. When urines adjusted to the same pH range were autoclaved, some of the non-combinable material changed to combinable material in the neutral range. This observation was originally made by Burk.[3] Attempts to destroy biotin in urine with bromine and H_2O_2 have not been satisfactory.

A few experiments have been made to see if other substances which affect growth would alter the growth activity of the material in urine. In the presence of avidin the amount of non-combinable biotin in urine was not altered by adding folic acid, or other pure vitamins to it. Solutions of liver extract and yeast extract did increase the amount of non-combinable material under the same conditions.

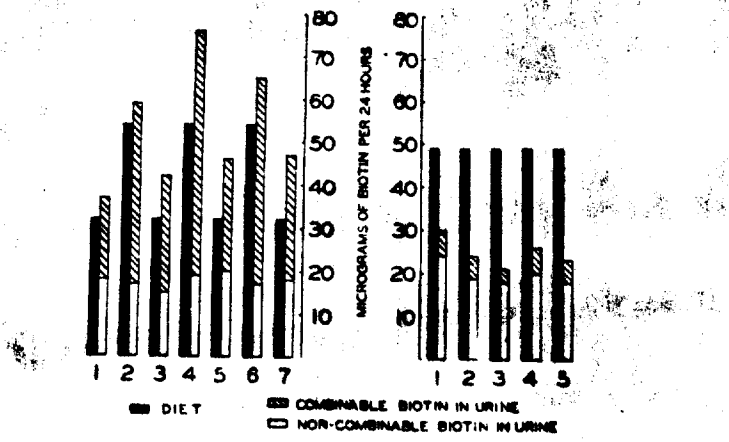

FIG. 8.—Excretion of biotin and non-avidin combining growth material in the urine. *Left:* Subject who consumed 35 and 55 micrograms on alternate days for 7 days. *Right:* Subject who consumed 47 micrograms of biotin and large amounts of egg white every day for 5 days.

Excretion of the Non-avidin Combining Growth Substance. Not only all of the human urines studied, but the urines of dogs, rabbits, and rats have been found to contain this new biotin-like growth factor. Urine is the most plentiful source of it we have found, but we do not know why it appears in the urine in such large amounts. The diets and stool specimens of patients on whom we did biotin balance studies contained so much less of it than their urine, that it could not have been absorbed from their intestines. A few experiments have been made to see what factors do alter its excretion.

The urines of 2 subjects who were eating diets containing 35 and 55 micrograms of biotin on alternate days were examined. The amount of biotin in the urine varied directly with the amount in the food. The excretion of non-avidin combining material, however,

remained quite constant. To 2 other subjects test doses of 150 micrograms of biotin were given during a period when they were maintained on diets of constant biotin content. This dose caused a flooding of the urine with biotin, but did not alter the excretion of the non-combinable material.

In another series of experiments an attempt was made to reduce the intake of biotin by giving avidin or egg white alone and with sulfanilylguanidine or succinyl-sulfathiazole. In some of the human subjects the excretion of biotin in the urine was decreased by these procedures, but it did not disappear completely. The excretion of the non-combinable biotin was not shown to be altered. We have not had an opportunity to study the urine of human subjects who showed signs of biotin deficiency, but two rats that were made biotin-deficient by avidin feeding, excreted no combinable biotin in their urine. Unfortunately, they were not studied when they consumed normal diets, so that we do not know whether or not their non-combinable biotin excretion had been changed.

The results of two typical experiments on human subjects are shown in Fig. 8.

Discussion. Of the various procedures used to identify biotin, the stimulation of yeast growth in a biotin-free medium and the inhibition of this effect by avidin are among the most specific. These tests are used to measure the amount of this vitamin in pure and crude materials, and any substance which gives both of these reactions has been considered to be biotin or some closely related compound.

Our results seem to show clearly that there is something in crude biologic materials, which gives the biotin effect on yeast growth in the presence of avidin. We have not been able to distinguish it from biotin by any other reaction, and we do not think it is any other known vitamin. The most striking thing about it, however, is that urine contains large amounts of it, though only small amounts are absorbed from the intestine. This suggests that it is formed in the animal body and one is tempted to believe that it is a product of biotin metabolism. Metabolic products of other vitamins, some with altered biologic properties, are known to appear in the urine.

Procedures intended to alter the metabolism of biotin in the body have so far failed to change the excretion of the non-combinable biotin-like substance in the urine. It seems clear that moderate variations in biotin intake and doses of biotin which greatly exceed that amount normally consumed do not alter the excretion of the non-combinable material. It is not yet clear, however, that its excretion is unchanged during periods of biotin starvation. We have not had an opportunity to make proper quantitative measurements under such conditions. The biotin absorption did not appear to be completely prevented in any of our human subjects, and the rats which appeared to be absorbing no biotin were not studied under other conditions.

While it thus appears that two substances which give the yeast growth effect of biotin can be distinguished, it is not clear how these substances are related. For this reason we have used the term biotin for the mixture of these materials in Parts I and II of this paper. In Part III, however, we have designated the materials as true biotin and non-combinable biotin for convenience, though we realize that their exact identity is not established and that the non-combinable biotin may not be a single compound. In future studies of biotin metabolism it seems important to use avidin to separate these two materials. We have done this in enough instances to form some idea of what the new procedure will show and how it will affect the results already reported.

In Part I it is stated that normal subjects excreted from 14 to 111 micrograms of biotin per 24 hours, depending on the nature of the diet. These values can be broken down into those for true biotin and non-combinable biotin. In instances where this was done the correlation between biotin intake and output was shown to be due to the fact that the daily output of non-combinable biotin is constant and uninfluenced by diet, whereas the value for true biotin fluctuates with the amount in the diet and thus provides the best index of biotin absorbed. Under those conditions where the urinary biotin values were low, the breakdown of the figures revealed that the non-combinable material accounted for such a large percentage that small variations in the excretion of true biotin were masked. When more such cases and patients with various diseases are studied with the avidin technique significant differences in biotin excretion may be found which did not show up before.

In Part II of this paper, in which the results of biotin balance studies are reported, the values for diets and stools represent true biotin, for we have not found significant amounts of non-combinable biotin in either of these. The biotin values for the urine will have to be divided, but this division does not alter the fact that the biotin in the urine and feces still exceeds by many times that present in the food, so that an important synthesis of biotin by the intestinal bacteria remains established.

While it would appear that the application of the avidin technique will amplify some of the work reported in Parts I and II of this series, we feel that more should be learned about non-combinable biotin before this is undertaken. Much remains to be known about its properties and physiology. At the same time we feel that the information already gained should be reported, as it may be helpful to other workers in the field. The present report is thus submitted with the realization that it will probably undergo some amplification and possibly some revision.

Conclusions.* (Part III). 1. The material in urine which gives the biotin test by the yeast growth assay can be separated into an avidin-combining and non-avidin-combining fraction.

* For Conclusions of Part I, see page 863; of Part II, page 869

2. Both of these fractions have been found in all human urines tested and in the urines of the dog, rabbit, and rat.

3. The avidin-combining fraction is probably biotin, and its excretion in the urine varies with the amount of biotin in the diet. Small amounts of it were excreted by patients taking diets containing large amounts of egg white. None of it was excreted in the urines of two biotin deficient rats.

4. The non-avidin-combining fraction has been found in all urine tested. The quantity in the urine was not changed by altering the biotin content of the food or by oral doses of biotin. Only minute amounts of the non-avidin-combining biotin were found in diets and stool specimens.

The author gratefully acknowledges the many suggestions and criticisms made by Dr. Vincent du Vigneaud and other members of the Department of Biochemistry of the Cornell University Medical College and the coöperation of Dr. C. P. Rhoads and his associates at Memorial Hospital, who furnished us with specimens from patients. The technical assistance of the Misses Margaret Whittemore, Betty Prescott, and Annette Pisanelli is also gratefully acknowledged.

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Oppel-'48
Bib No. 1023

Am. J. Med. Sci. 215: 76-83, 1948.

STUDIES OF BIOTIN METABOLISM IN MAN*

IV. Studies of the Mechanism of Absorption of Biotin and the Effect of Biotin Administration on a Few Cases of Seborrhea and Other Conditions

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Biotin remains one of the group of B vitamins that has not been responsible for clinical deficiencies in man. This could readily be explained by the fact that intestinal synthesis produces very adequate amounts of biotin, as was suggested in Part II of this series of papers.[11] Since that report was published in 1943, others[3,4,5,9,10] have shown that almost all of the B vitamins are synthesized in the human colon. These include thiamine, folic acid, and others, the lack of which causes deficiency states. Thus it would seem possible that unusual circumstances might occur that would lead to a biotin deficiency. The facts that a normal constituent of the diet, namely raw egg white, interferes with biotin utilization, and that sulfa drugs inhibit bacterial growth in the colon, suggest possible ways to interfere with this protective mechanism. These facts have been utilized to produce biotin deficiency in animals. The present experiments were designed to study their significance in man. A search has also been made for evidence of states of biotin deficiency with the aid of a tolerance test and some suspected cases have been treated with biotin.

There have been 3 attempts to produce biotin deficiency in humans. In all of them, avidin in some form was used. The first reports were made in 1942 by Sydenstricker, Singal, Briggs, DeVaughn and Isbell.[13] They used 7 human volunteers but were able to complete their experiments with only 4. The diet given was designed to be low in vitamin B members, though no estimates of its biotin content were made. It contained 200 gm of dehydrated egg white which was taken in solution in 3 equal portions with each meal. Usually the egg white furnished more than 30% of the total daily caloric intake. Changes were noted during the 3rd and 4th weeks when all subjects developed a fine scaly dermatitis. This cleared, but skin or mucous membrane changes developed later. After the 5th week mental or nervous symptoms resembling experimental thiamine deficiency developed. The urines at about this time showed a marked reduction of biotin content, from a normal of 29 to 62 μg. per 24 hours to about 3.5 to 7. This rose rapidly after the injection of a biotin concentrate, about 150 μg. of which were required daily for relief of symptoms.

The second report of an attempt to produce biotin deficiency in man was made by Rhoads and Abels in 1943.[12] This experiment was done with the hope that a biotin deficiency might delay the course of mammary carcinoma in 1 patient and of leukemia in another. Diets containing 46 to 60 μg. of biotin were used and they were supplemented by raw frozen egg white, dried egg white and avidin concentrates. For the first 12 weeks, the equivalent of 1000 to 1200 units of avidin was used (1 unit of avidin inactivates 1 μg of biotin). This was later increased by 500 to 750 units for the next 16 weeks and finally by 1000 to 1250 units for the last 2 weeks. In spite of these large amounts of avidin, no decrease of the urinary ex-

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

cretion of biotin occurred* and no signs of deficiency disease simulating those described by Sydenstricker and his co-workers[15] appeared.

Kaplan[7] gave egg white or avidin concentrate to 10 patients with malignant disease. A general diet as free as possible from food rich in biotin was used, and was supplemented with from 36 to 42 egg whites per day. Later in the study, an avidin concentrate capable of inactivating 2000 μg. of biotin per day was used. Most of the patients were observed for many months, and none of them showed any signs of a biotin deficiency. Urinary excretion studies were not reported.

The failure of 2 of these 3 groups of workers to produce a biotin deficiency by administering large amounts of avidin is difficult to explain, and the discrepancy between the results is also confusing. Several possibilities suggest themselves. Avidin is a relatively insoluble material and it may not have been available for this reason in the experiments by Rhoads and Abels,[12] and by Kaplan.[7] Since avidin is thought to prevent the absorption of biotin from the intestine, another explanation would be that the avidin-biotin complex is not stable in the human intestinal tract. All of the patients who could not be made biotin-deficient had malignant disease, and the possibility exists that they utilize biotin differently for this reason. Our first group of experiments was done to test these possibilities.

Methods and Results. A biotin tolerance test was devised. Twenty-four hour urines were collected from patients on an unrestricted diet, and after a control day, a dose of 500 μg. of biotin was given which caused a marked increase in urinary output above the normal. Several days after the first dose, the same quantity of biotin was given with 250 mg. of avidin, an amount which completely inactivated this much of biotin in the test-tube. The avidin was given in various ways. It was dissolved in ammonium sulfate, it was mixed with biotin in milk, and powdered biotin and avidin were mixed and given in capsules. Four patients were used. Two of them had pernicious anemia, and were shown to have no free hydrochloric acid in their gastric contents. The other 2 patients had malignant disease. One had a bronchogenic carcinoma and the other had chronic myelogenous leukemia.

The results of these experiments are given in Table 1. They show that the avidin, by all methods of administration, effectively prevented the biotin from being excreted by all the patients and that avidin and biotin react in the human intestinal tract of patients with achlorhydria or malignant disease in the same way that they do in normal animals.

A study of biotin absorption from the colon was next made. Two subjects who had shown a striking increase in the urinary output of biotin after an oral test dose were given a dose by rectum. The biotin was dissolved in a few cc. of water. It caused an increase in the urinary output that was smaller than that which followed an oral dose, but large enough above the normal to indicate definite absorption (Table 2). The effect of sulfa drugs on the suppression of synthesis of biotin was also studied. We thought that a biotin tolerance test might indicate some degree of unsaturation in patients who had taken sulfa drugs for varying periods of time. In 6 normals, the amount excreted on the biotin day was 4 to 10 times that eliminated on the control day and varied from 245 to 337 μg. Two patients, 1 of whom took sulfadiazine for 2 weeks, and the other for 2 months, were tested. No evidence of biotin unsaturation could be demonstrated. A similar procedure was carried out on a patient who took sulfasuxadine in doses of 9 gm. per day for 16 days. His tolerance test showed a decidedly smaller output of biotin after the sulfasuxadine period than before, and suggested some degree of biotin unsaturation. However, his urinary output on the 16th day of sulfasuxadine was normal (Table 2).

* The method of biotin assay used measured both the true and the non-combinable biotin, described in Part III of this series.

Two other patients were studied with much more detail. They were kept on a diet free from liver, kidneys and sweetbreads but otherwise unrestricted. Such diets probably contain less than 65 μg. of biotin per day.[8] With each meal they drank a glass of milk containing the whites of 2 raw eggs. This much raw egg white contains enough avidin to inactivate about 115 μg. of biotin. In addition, they were given sulfasuxadine, sulfathalidine, or streptomycin by mouth to arrest bacterial growth and biotin synthesis in the ecius. Since vitamin K is also synthesized by colonic bacteria, they were given oral vitamin K and their prothrombin times were observed. Each day's urine was saved. Every 4th night a dose of carmine was given which could be identified in the stools, and each 4 days' stool specimens were pooled, dried and analyzed for biotin according to the procedure previously described.[11] The results of the study on 1 patient are shown in Figure 1.

This patient, who was 46, had a hepatoma of the liver. He ate poorly but took the milk and egg white faithfully. He was out of bed and his general condition remained fairly good until the day before the end of the experiment when he developed an interperitoneal hemorrhage from which he died. He showed a marked decrease of biotin synthesis in the stools and a fall of the urinary output to a low

TABLE 1.—EFFECT OF BIOTIN AND BIOTIN PLUS AVIDIN ADMINISTRATION ON THE URINARY OUTPUT OF BIOTIN

Day	Biotin per 24 hrs. (μg.)	Remarks
1	28	
2	140	500 μg. of biotin in milk
3	23	
4	26	500 μg. of biotin and 250 mg. of avidin in milk
5	25	
6	33	500 μg. of biotin and 250 mg. of avidin in $(NH_4)_2SO_4$ in milk
7	24	
1	38	
2	237	500 μg. of biotin in capsules
3	68	
4	88	500 μg. of biotin and 250 mg. of avidin in capsules
5	30	
1	16	
2	19	
3	25	
4	211	500 μg. of biotin in capsules
5	24	
6	20	
7	38	500 μg. of biotin and 250 mg. avidin in milk
8	29	
9	25	
10	366	500 μg. of biotin in capsules
11	60	
12	48	
1	14	
2	14	
3	15	
4	125	500 μg. of biotin in capsules
5	24	
6	23	
7	21	500 μg. of biotin and 250 mg. avidin in capsules
8	17	
9	21	
10	139	500 μg. of biotin in capsules
11	31	
12	24	

level. There were no clinical signs suggestive of a biotin deficiency, possibly because the experiment did not last long enough.

A second patient was selected with the hope that he could be carried on the program long enough to be made deficient. He was [illegible] chronic myelogenous leukemia, recently brought into remission by Roentgen ray therapy. He was not strong but was out of bed and ate fairly well. At first he was given 9 gm. of sulfasuxadine a day because 15 gm. had caused diarrhea in the other patient. On

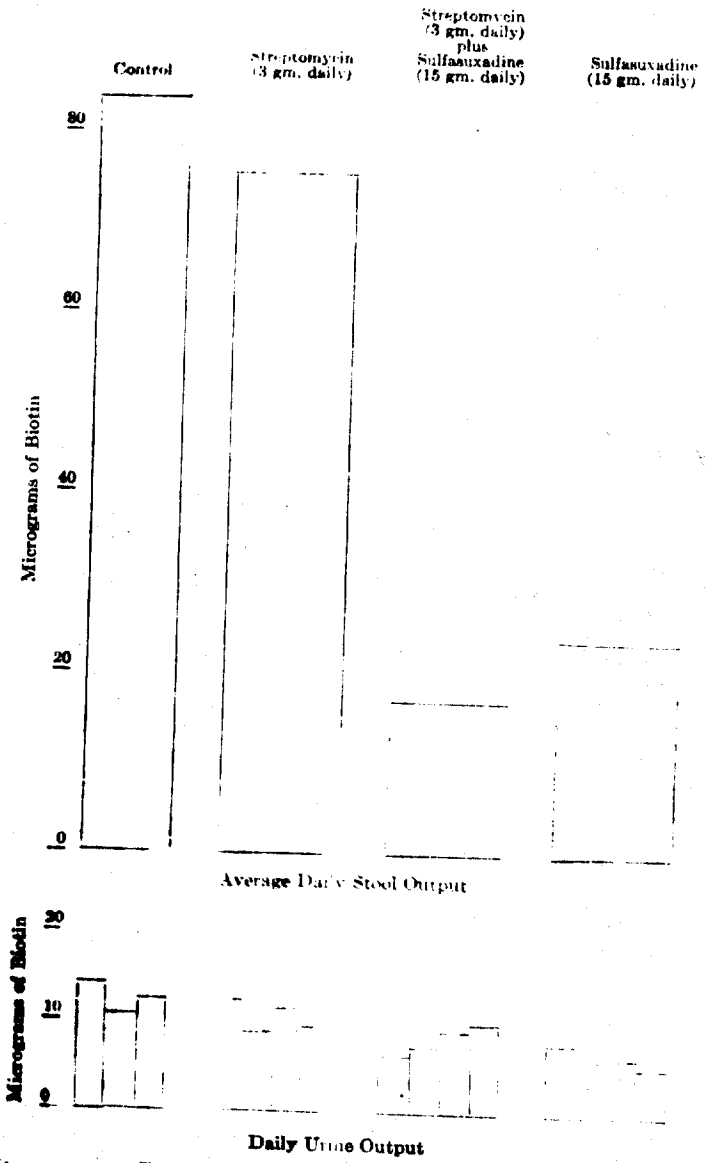

FIG. 1.—Effect of drugs on biotin synthesis.

the 5th and 6th days, his (undiluted) prothrombin time became prolonged (the diluted prothrombin time remained normal) and the sulfasuxadine was stopped and 3 mg. of vitamin K were given parenterally. Two days later, his prothrombin time was normal and 6 gm. of sulfathalidine was begun. This was later increased to 8, 10 and finally 12 gm. a day. The patient had no diarrhea, his appetite and strength improved and he gained [illegible] There were no clinical signs of a [illegible]

formation. The experiment was discontinued after 46 days. A tolerance test at this time showed a slightly lower value than the test done before the sulfa drug was given (Table 2).

While these metabolic studies were underway, another group of patients was observed for evidence of biotin deficiency. For the past 15 or more years various workers have suggested that certain disease states may be due to a lack of biotin (vitamin H), but the first report of a clinical case with [illegible] in the [illegible]

TABLE 2.—BIOTIN TOLERANCE TESTS

Diagnosis	Urinary output of biotin (μg.) Day of test: 1 (500 μg. of biotin)	2	3		Remarks
Normal	25	245	62		
"	78	337	71		
"	44	259	66		
"	42	311	46		
"	29	294	75		
"	38	303	74		
A.S. heart disease	57	244	53		
" " "		104*	54		* 1 mg. biotin by rectum
	42	263	62		
Bacterial endocarditis	34	148	58		Before sulfasuxadine
	43	95	41		After 16 days of sulfasuxadine
" "	46	416	138		After 2 weeks of sulfadiasine
	59	113*	69		* 500 μg. biotin by rectum
Lung abscess	64	378	64		After 8 weeks of sulfadiasine
Cirrhosis	45	407	92		
"	24	208	84		
"	56	293	106		
Peptic ulcer	23	232	79		
" "	54	279			
Neoplasm	17	203	41		
"	10	124*	18		* Incomplete specimen
"	28	140	23		
Leukemia	40	168	24		Before sulfa drugs
"	15	125	24		After 46 days of sulfasuxadine and sulfathalidine
"					
Seborrheic dermatitis	39	132		123*	* After 20 days 250 μg. biotin daily
" "	15	119		157*	* After 14 days 250 μg. biotin daily
				180*	* After 29 days 250 μg. biotin daily
" "	15	114		342*	* After 20 days 250 μg. biotin daily
" "	10	156		120*	* After 13 days 250 μg. biotin daily
" "	52	231	72		
Atopic dermatitis	41	153	34		
Alopecia totalis	24	258		286*	* After 19 days 250 μg. biotin daily
Alopecia areata	40	52			
Myotonia congenita	33	233	50	517*	* After 7 days 1 mg. biotin subcutaneously daily
Aplastic anemia	49	244	50		
Pernicious anemia	38	239	68		
" "	25	211	24		
" "	52	352		209*	* After 19 days 250 μg. biotin daily
Non-tropical sprue	31	331	25		

eccentric dietary habits, had consumed 2 to 6 dozen raw eggs per week as part of an otherwise poor diet which included large amounts of wine. When admitted to the hospital, his entire face, ears, shoulders, dorsum of the forearms, hands and lower legs were fiery red and covered with medium sized scales. On the ward diet, the rash promptly cleared and it did not return when egg white and a low biotin diet were later given. The next report of human biotin deficiency was made during the last year by Chavarria[2] and co-workers from Costa Rica. They observed that children with vitamin B deficiency of severe degree developed canities and alopecia which disappeared when the other manifestations of the deficiency state were cured by a good diet. They thought that the addition of 0.25 mg. of biotin 3 times a day accelerated the return of the hair to normal.

Our studies were made principally of patients who might be expected to have evidence of vitamin B deficiency or who had skin or hair changes resembling those in biotin-deficient animals. Biotin deficiency has now been produced in the chicken, duck, rat, mouse, hamster, rabbit, dog, pig, calf and monkey, and the picture is quite similar in all of them. It is mainly a loss of hair (or feathers), a seborrheic type of skin rash and, in the late stages, the development of a sudden and severe paralysis. The latter has been shown to respond to potassium administration and resembles periodic family paralysis.[13,14] There have been no lesions in the central nervous system.

We used the following program in our study of patients. A biotin tolerance test was done to look for evidence of low biotin excretion in the urine or biotin unsaturation of the tissues. In some instances, a stool was analyzed to see if biotin was being synthesized. To several patients, 250 μg. of biotin a day was given for 4 to [illegible] weeks to see if it would improve the [illegible]ical condition. On these patients, a [illegible] hour urine was examined 1 week or 2 [illegible]er therapy began to see if the biotin was being utilized or excreted in the urine. The results seemed to indicate in every instance that the dose was adequate. In a few instances larger doses were administered but the test always showed that about half of the biotin administered was eliminated in the urine. The results of these procedures are shown in Table 2.

Most of the patients responded to the tolerance test in the same way as the normals, excreting about half the test dose or more. A few patients excreted relatively small amounts after a test dose which may indicate that their tissues were unsaturated. It is of considerable interest that 4 of the 5 patients with seborrhea had low values, excreting less than a third of the test dose. These patients all received biotin daily for 1 month or more with the following results. The first patient had seborrhea and mild dermatitis of the scalp which seemed to improve in about 1 week and did not recur for several months. The second patient had an extensive dermatitis of the face and scalp. This showed slight improvement for 1 month, after which it relapsed while under treatment. The third patient had a mild dermatitis on many areas of the body and developed an acute generalized dermatitis from the overzealous use of local remedies. While this reaction was subsiding, he was given biotin and only bland local therapy on which he did well. When the skin was healed, he still had a very oily skin on the face and scalp. The fourth patient had seborrhea of the scalp and a dermatitis in the interscapular regions. Neither condition changed under therapy. The fifth patient had a classical seborrhea of the scalp with dermatitis at the hair-line, ears and nasolabial fold. It did not improve in 4 weeks of treatment. In addition to the 5 patients with seborrhea, biotin was also given to 2 patients with alopecia areata and 1 with atopic dermatitis without beneficial effect. They also received 250 μg. daily for 1 to 2 months. Stools were analyzed on most of these skin patients and all were found to have biotin in normal concentration.

In view of the report from Costa Rica,[3] we were very hopeful that a patient with pernicious anemia and premature white hair would benefit from biotin administration. She was 39 years old and had been studied at this hospital for 20 years. At the beginning of her illness, the diagnosis was not clear, but later it was found that she could be kept in good general health and a normal blood count could be maintained by large doses of liver or folic acid. Neither of these treatments had affected her gray hair. This began shortly after the onset of her anemia and had progressed gradually until it became marked. All of the hair on her body had become smaller in diameter and had completely lost its pigment. It had been that way for 5 years when our experiment was made. She showed biotin present in the stool and a normal urinary excretion and response to a tolerance test. After 19 days of 250 μg. of biotin a day, she eliminated 209 μg. of her daily dose, a result which seemed to indicate quite a high degree of saturation of the body with biotin. Two months of biotin therapy had no definite effect on the canities. There had been no hair loss and no new hair grew. There have been reports that patients with pernicious anemia are deficient in several of the B vitamins by chemical test.[1] Our 2 other patients with pernicious anemia and 1 with non-tropical sprue showed a normal response to the biotin tolerance test.

In the third paper of this series,[11] it was shown that 2 biotin-like materials are excreted in human urine. One combined with avidin and thus reacted like true biotin. The other failed to combine with avidin and it was suggested that it might be a breakdown product of biotin which was excreted in the urine. The urinary excretion of this non-combinable material was measured throughout all these experiments. It showed no variations that could in any way be related to variations of biotin intake or urine value. The biotin values of 15 urines were measured by *S. cerevisiæ* plus avidin and *L. casei* methods. Values for true biotin were the same by both methods but there was no non-combinable material in urine when measured by the *L. casei* assay. These findings do not support the idea that non-combinable biotin is a metabolic product of biotin.

Discussion. The theoretical problem involved in producing a biotin deficiency in man with avidin seems to be to prevent the absorption from the human intestinal tract of about 50 μg. of biotin a day from food and several times that amount formed by intestinal synthesis. Nearly complete fixation of the biotin is probably necessary, since the daily minimal requirement is doubtless satisfied by only part of this amount. Avidin in sufficient quantity to combine with 1000 to 2000 μg. of biotin did not accomplish this fixation in either the experiments of Rhoads and Abels[12] or those of Kaplan.[7] According to the calculations, they gave more avidin than Sydenstricker and his associates,[16] which makes it apparent that the problem is not based on avidin dosage only. The relative ratios of biotin to avidin in these experiments cannot be calculated, since the biotin content of all the diets is not known and it is possible that this ratio and other factors, such as the form in which biotin is given,[6] are also important. Our experiments show that avidin inhibits the use of biotin by man and animals in the same way and there is nothing so far to indicate that the production of a biotin deficiency in man with avidin is not primarily a problem of using proper quantities and ratios.

In our experiments with sulfa drugs, ordinary dosages have not seriously impaired biotin synthesis. The type of drug and factor of dosage are important, and large doses of sulfasuxadine almost completely stopped biotin synthesis in 1 subject's colon. The 20 μg a day he was eliminating in the feces are partly accounted for by the biotin contained in the 6 egg whites and in the food biotin which was fixed by the avidin in the egg whites. It would thus seem that synthesis was almost completely stopped. Whether

this inhibiting action of non-absorbable sulfa drugs can be maintained long enough to produce a biotin deficiency state in man, remains to be seen. It has been done in animals in less than 6 weeks.[8] More work is needed to see how much harm can result from the inhibition of vitamin synthesis in man by sulfa drugs.

The study of patients with the aid of a tolerance test showed a low response by 4 of the 5 with seborrhea. This finding lends support to the idea, which has been proposed by several workers, that human seborrhea is related to biotin deficiency. Treatment of these patients with biotin has shown no consistent evidence that the treatment was of value. In a few other conditions with skin and hair disturbances, biotin has also failed to have any definite effect. No harmful effect of biotin administration for several months has been observed, and the results of urine output studies indicate that larger doses of biotin probably would not have done more good. The results thus do not indicate that any of the conditions studied are related to a lack of biotin.

Summary and Conclusions. [illegible] oral dose of 500 μg. of biotin [illegible] human subjects as a tolerance test caused a marked increase in 24 hour urine output. In a group of normals, this varied from 245 to 337 μg. The same dose given with 250 μg. of avidin caused no increase of urinary output. Biotin given by rectum caused an increased urinary output.

2. Sulfa drugs in ordinary doses did not seriously interfere with the synthesis of biotin by bacteria in the colon, but large doses of sulfasuxadine caused almost complete inhibition of biotin synthesis.

3. A tolerance test showed that 4 out of 5 patients with seborrhea had a low urinary output after a test dose. Biotin administration to these and to a few other patients with skin disorders had no definitely beneficial effect.

4. Marked variations in the urinary output of biotin were associated with no consistent change in the urinary output of non-combinable biotin-like material. The urine values for biotin in assays done with *S. cerevisiæ* and *L. casei* were the same. With *L. casei*, no non-avidin combining material was found.

The technical assistance of Mrs. Marion Goldberg Laeger is gratefully acknowledged. Supplies of biotin and avidin were kindly furnished by Dr. Elmer L. Severinghaus, Director of Clinical Research of Hoffmann-La Roche, Inc., Nutley, N. J.

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Boll. Soc. Ital. Biol. Sper. 25:918-921. 1949.

Orlandi-'49
Bib No. 1024

EFFECT OF THE H FACTOR ON SALICYLATES IN BLOOD AND URINE OF CHILDREN.

- E. Orlandi and T. Rabaglia.
(Institute of Pediatric Clinic, University of Parma)

Parma Section - Meeting of June 7 1949

Dry, Butt, Scheifley (1) first indicated the synergism of the action between salicylates and paramin-benzoic acid. Muratore and Putignano (2), Annoni (3), Ginonhiac (4), Peluffo and Ravera (5), M. Litter and D. Litter (6), confirm that the H factor increases the salicylate taxis (tropism) and vary the urinary elimination.

The explanation of the synergism phenomenon is still a hypothesis. Preluffo an Ravera consider three possibilities: 1) the increase of the salicylate taxis is due to the introduction of phenolic-hydroxyl into a molecule of the paramin-benzoic acid and to one of its eventual deamination; 2) that paramin-benzoic acid contracts the diuresis volume considerably increased by salicylate of Na; 3) that competitive action at the conjugation in the hepatic area should be stabilized with glycuronic acid between salicylic and paramin-benzoic acids.

On the basis of their accurate experiments on various free salicylic fractions and combined eliminations in urines after the administration of only salicylic acid and of a combination of salicylic acid and H factor, the authors exclude the first and the second hypothesis and adhere to the third, which is the most acceptable.

Muratore and Putignano put forward the hypothesis of a hepatic protective action and formulate, above all, in consideration the regulation of the thrombin operated from the same H factor.

In a recent publication (April 1949) M. Litter and D. Litter confirm the increase of salicylate taxis with the H factor and present a hypothesis, that H factor acts on the intestinal absorption and contracts the diuresis. Dry, Butt, Scheifler did not give as evidence the derivative of the phenolic type in the blood serum after H factor administration; Muratore and Putignano came to the same conclusion using Keller's method (which consists of using as ferric reagent the ferro-nitrate in an acid ambient).

We have performed systematic measurings of the salicytemic and salicyluric curves for the purpose of therapeutic application for rheumatic fever

in children, and for the contribution to study the machanism of the interesting phenomenon of syngerism.

We have used the method of U. Pellegrini and T. Rabaglia (7) for salicylemia and of T. Rabaglia(8) for salicylurine. We have first of all, brought into evidence, how only the administration of factor H provokes the appearance in the blood serum of traces of the phenolic type substances colored with ferric salts in the ambient medium of HCl acid. We have confirmed then, that the content in the blood serum of phenolic derivatives, after administration of the medicamentosous combination of salicylate of Na and paraminbenzoic acid is higher than that obtainable from the administration of the salicylate of Na only. Moreover it is observed, that the administration of the factor H does not contract in combination with salicylate of Na, the diuresis volume (see table I). Curves of salicylate have clearly confirmed that the factor H slows the speed of salicylate of Na elimination. While, in fact 1 gr. of silicylate of Na administred orally is eliminated, in children for 90 % during first 10 hours, the elimination percentage in the presence of factor H (0.2 gr.) is of 60 % (see tables II and III).

TABLE I. - Diuresis volumes and urine densities.

Name age weight	After administration of 1gr. of salicylate of Na + 2 gr. of $NaHCO_3$					Total elim within 10h.	After administration of 1 gr. of the salicylate of Na + 2 gr. $NaHCO_3$ + 0.200 gr. of factor H					Total
	1 h.	3 h.	5 h.	8 h.	10 h.		1 h.	2 h.	3 h.	5 h.	10 h.	
P.S. a. 10	1013	1010	1015	1020	1026	cc	1020	1010	1020	1030	1030	
Kg. 19	60cc	110cc	170cc	100cc	50cc	490	125cc	140cc	115cc	50cc	45cc	175
A. G. a. 13	1018	1020	1028	1032	1030		1020	1028	1025	1025	1021	
Kg. 43	100cc	10cc	105cc	80cc	70cc	455	100cc	55cc	125cc	140cc	85cc	505
S. N. a.10	1018	1020	1020	1019	1020		1079	1021	1019	1020	1020	
Kg. 24	90cc	80cc	160cc	90cc	120cc	480	80cc	70cc	95cc	100cc	50cc	390

TABLE II. - Behavior of salicytilate and of the urinary elimination in patients treated only with salicylate of Na (1 gr. of Na + 2 gr. $NaHCO_3$).

Name age weight	% of medicine eliminated by urine					Salicylate after 1 h. % of mg
	after 1 h.	3 h.	5 h.	6 h.	10 h.	
P.S. a. 10, Kg. 19	15.4	41.2	70.4	85.0	90.3	158.7
A.G. a. 13, Kg. 43	21.6	44.0	61.7	75.2	82.9	169.4
G.N. a. 9, Kg. 25	19.8	38.8	56.4	71.6	86.9	176.6

TABLE III - Behavior of salicylate and salicyluric action in patients treated with Na salicylate of Na (1 gr. of Na salicylate + 2 gr. of $NaHCO_3$) and factor H (0.200 gr.)

Name age Weught	% of medicine eliminated by urine					Salicylate after 1 h. mg.
	after 1 h.	3 h.	5 h.	8 h.	10 h.	
P.S.a. 10, Kg. 19	24.2	48.8	65.3	60.7	63	215.86
A.G.a. 13, Kg. 43	14.6	28.1	41.3	55.3	62	319.33
G.N. a.9, Kg. 25	12.5	28.6	42.8	56.3	61	271.16

Salicylate determinatios for endovenous administration of salicylate of Na have revealed that the addition of the factor H not only permits, as in oral administration, an salicylatic increase of an absolute value, but also a radical modification in the development of curves. While in endovenous administration of the Na silicylate the values of salicylemia decrease rapidly (blood-samples taken after a few minutes of endovenous administration reach the cutve already in the decreasing phase), the salicylemic values in the presence of factor H ascend, reach the maximum, then decrease.
Or, the presence of the factor H not only increases, but "graduates step-wise" in time the salicylemic values, and gives to endovenous curves a course analogous to the curves of oral administration (see Table IV).

This clear variation of the salicylemic curves was constantly encounterd, even in a unique case, where the factor H does not give an increase of salicylemic values.

TABLE IV. - Salicylemic values after endovenous administration

Name age	endovenous 1 gr. of salicylate of Na			endovenous 1 gr. of salicylate of Na+0.200 gr. of factor H at intervals of 15'		
	after 30'	60'	120'	30'	60'	120'
O. A., a. 14	198.0	158.7	144.5	217.6	247.9	210.5
C. F., " 8	262.2	230.1	133.8	215.4	335.3	121.3
M. A., " 10	194.4	146.2	137.3	78.4	133.8	51.7
P. S., " 9	207.7	149.8	101.6	240.8	315.7	213.3
B. R., " 12	187.3	133.8	107.0	214.0	251.4	144.5
S. R., " 16	198.0	149.8	117.7	235.4	267.6	171.2
P. A., " 7	187.3	133.8	90.9	198.0	278.3	133.8
S. B., " 12	171.2	133.8	112.3	214.0	283.6	165.9
C. G., " 9	208.7	149.8	117.7	230.1	272.9	181.9

Conclusions. Based on the results of our experiments, we conclude:

1) of the stated explanatory hypotheses on the synergism effect, the Litter hypothesis on the contraction of the diuresis should be excluded;

2) that the formation of phenolic type compounds from the factor H can not be excluded, but the increase in the concentration of phenolic derivatives of the blood serum, after the medicamentous combination administration of Na salicylate and of factor H, would be highly superior to that attributed to the above-mentioned transformation; the syngerism of salicylate action of the Na and of the factor H remains therefore, fully confermed;

3) that the action mechanism of the named syngerism is in the extra-renal point, it exists also in endovenous administration and therefore (at least for endovenous administrations) the advanced intestinal absorption factor of Litter should be excluded;

4) that endovenous curve variations, in the case of medicamentous combination administration, comparing similar curves of salicylate administration only, it would take into account the probable rapid effect of factor H on a regulation mechanism of salicylemic taxis and probably show a hepatic effect according to the hypothesis of Peluffo, Ravera and Putignano.

Nevertheless in the child's therapy reflexes, we are of the opinion, that the addition of factor H to salicylate of Na would be advisable for medicine administration to be oral or endovenous.

Particularly useful is a successful combination of two medicaments, when, in case of gastric intolerance or other reasons, endovenous administration of medicine must be applied for the purpose of delaying the rapid fall of the salicylemic taxis.

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ABSTRACT

Effect of biotin on salicylates in blood and urine of children.

E. Orlandi and T. Rabaglia (Univ. Parma, Italy).

Boll. soc. ital. biol. sper. 25, 918-21(1949).

The large increases in phenol concn: in blood and urine when Na salicylate and biotin (I) are given simultaneusly cannot be due to sudden conversion. It appears that I acts on a salicylate regulatory mechanism situated in the liver. The salicylate-intolerance of the gastric app. can be avoided by intravenous injections of the combination of salicylate and I. M. E.

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Orlandi-'49
Bib No. 1024

Boll. Soc. Ital. ~~Biol. Sper.~~ 25:918-921. 1949.

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INFLUENZA DEL FATTORE H SULLA SALICITEMIA E SULLA SALICILURIA NEL BAMBINO. — E. Orlandi e T. Rabaglia.

(*Dall' Istituto di Clinica Pediatrica dell' Università di Parma*).

Sezione di Parma Seduta del 7 giugno 1949.

Dry, Butt, Scheifley (1) hanno segnalato per primi il sinergismo di azione fra i salicilici e l'ac. paramino-benzoico. Muratore e Putignano (2), Annoni (3), Ginoulhiac (4), Peluffo e Ravera (5), M. Litter e D. Litter (6), confermarono che il fattore H aumenta il tasso salicitemico e varia la eliminazione urinaria.

Sulla spiegazione del fenomeno di sinergismo si è ancora nel campo delle ipotesi. Peluffo e Ravera considerano tre possibilità: 1) che l'aumento del tasso salicitemico sia dovuto all' introduzione dell' ossidrile fenolico nella molecola dell' ac. paramino-benzoico e a una sua eventuale desaminazione; 2) che l' ac. paramino-benzoico contragga il volume della diuresi notoriamente aumentata dal salicilato di Na; 3) che si stabilisca un' azione competitiva nella coniugazione in sede epatica con ac. glicuronico fra l'ac. salicilico e l'ac. paramino-benzoico.

In base alle loro accurate esperienze sulle varie frazioni saliciliche libere e combinate eliminate nelle urine dopo somministrazione di solo ac. salicilico, e dell'associazione ac. salicilico e fattore H, gli AA. escludono la prima e la seconda ipotesi, e affermano essere la terza quella maggiormente accettabile.

Muratore e Putignano avanzano l'ipotesi di un'azione protettiva epatica che essi formulano sopratutto in considerazione della regolarizzazione della trombinemia operata dallo stesso fattore H.

In una recente pubblicazione (aprile 1949) M. Litter e D. Litter confermano l'aumento del tasso salicitemico con fattore H ed emettono l'ipotesi che il fattore H agisca sull'assorbimento intestinale e contragga la diuresi. Dry, Butt. Scheifler non avevano posto in evidenza derivati di tipo fenolico nel siero di sangue dopo somministrazione di fattore H; Muratore e Putignano con il metodo Keller (che usa come reagente ferrico nitrato di ferro in ambiente acido) erano giunti alla stessa constatazione.

Noi abbiamo eseguito misure sistematiche di curve salicitemiche e di salicilurie, sia ai fini di una applicazione terapeutica nella febbre reumatica nel bambino, sia per contribuire allo studio del meccanismo dell'interessante fenomeno di sinergismo.

Abbiamo usato il metodo di U. Pellegrini e T. Rabaglia (7) per le salicitemie e di T. Rabaglia (8) per le salicilurie. Abbiamo anzitutto messo in evidenza come la somministrazione di solo fattore H provochi la comparsa nel siero di sangue di traccie di sostanze di tipo fenolico colorabili con sali ferrici in ambiente acido per HCl. Abbiamo confermato poi che il contenuto nel siero di sangue di derivati fenolici, dopo somministrazione dell'associazione medicamentosa salicilato di Na e ac. paramino-benzoico, è maggiore che non quella ottenibile dalla som-

ministrazione di solo salicilato di Na. Si è notato inoltre che la somministrazione di fattore H non contrae, in associazione con salicilato di Na, il volume della diuresi (vedi tab. I). Le curve di saliciluria hanno nettamente confermato che il fattore H ritarda la velocità di eliminazione del salicilato di Na. Mentre, infatti, 1 g di salicilato di Na somministrato per os viene eliminato, nel bambino, per il 90 % nelle prime 10 h, la percentuale di eliminazione in presenza di fattore H (0.2 g) è del 60 % (vedi tab. II e III).

TABELLA I. — *Volumi diuresi e densità urinarie*

Nome	età / peso	Dopo somministrazione di g 1 salicilato di Na + g 2 $NaHCO_3$					Totali eliminati entro 10 h	Dopo somministrazione di g 1 salicilato di Na + g 2 $NaHCO_3$ + g 0,200 fattore H					Totale
		1 h	3 h	5 h	8 h	10 h		1 h	3 h	5 h	8 h	10 h	
P. S.	a. 10	1013	1010	1015	1020	1026	cc	1020	1010	1020	1030	1030	
	Kg. 19	60 cc	110 cc	170 cc	100 cc	50 cc	490	125 cc	140 cc	115 cc	50 cc	45 cc	175
A. G.	a. 13	1018	1020	1028	1032	1030		1020	1028	1025	1025	1021	
	Kg. 43	100 cc	100 cc	105 cc	80 cc	70 cc	455	100 cc	55 cc	125 cc	140 cc	85 cc	505
S. N.	a. 10	1018	1020	1020	1019	1020		1079	1021	1019	1020	1020	
	Kg. 24	90 cc	80 cc	100 cc	90 cc	120 cc	480	80 cc	70 cc	95 cc	100 cc	50 cc	390

TABELLA II. — *Comportamento della salicitemia e della eliminazione urinaria in pazienti trattati con solo salicilato di Na (g 1 salicilato di Na + g 2 $NaHCO_3$)*

Nome età peso	% del medicamento eliminato per via urinaria					Salicitemia dopo 1 h mg %
	dopo 1 h	3 h	5 h	8 h	10 h	
P. S., a. 10, Kg. 19	15,4	41,2	70,4	85,0	90,3	158,7
A. G., a. 13, Kg. 43	21,6	44,0	61,7	75,2	82,9	169,4
G. N., a. 9, Kg. 25	19,8	38,8	56,4	71,6	86,9	176,6

TABELLA III. — *Comportamento della salicitemia e della saliciluria in pazienti trattati con salicilato di Na (g 1 di salicilato di Na + g 2 di $NaHCO_3$) e fattore H (g 0,200)*

Nome età peso	% del medicamento eliminato per via urinaria					Salicitemia dopo 1 h mg %
	dopo 1 h	3 h	5 h	8 h	10 h	
P. S., a. 10, Kg. 19	24,2	48,8	65,3	60,7	63	215,86
A. G., a. 13, Kg. 43	14,6	28,1	41,3	55,3	62	319,33
G. N., a. 9, Kg. 25	12,5	28,6	42,8	56,3	61	271,16

Le determinazioni salicitemiche per somministrazione endovena di salicilato di Na hanno anche rivelato che l'aggiunta di fattore H non comporta solamente, come nella somministrazione per os, un aumento salicitemico in valore assoluto, ma anche una modificazione radicale nell'andamento delle curve stesse. Mentre nelle somministrazioni endovena di salicilato di Na i valori di salicitemia rapidamente decrescono (prelievi di sangue dopo pochi minuti dalla somministrazione endovenosa colgono la curva già in fase decrescente), i valori salicitemici in presenza di fattore H ascendono, hanno un massimo, poi decrescono. *Ossia la presenza di fattore H non solo aumenta, ma « gradualizza » nel tempo i valori salicitemici e dà alle curve endovena un andamento analogo alle curve per os* (vedi tab. IV).

Questa netta variazione di andamento delle curve salicitemiche fu costantemente riscontrata, anche nell'unico caso nel quale il fattore H non portò ad aumenti nel valore salicitemico.

TABELLA IV. *Valori di salicitemia dopo somministrazione endovena*

Nome e età	g 1 salic. Na endovena			g 1 salic. Na endovena + g 0,200 fattore H a distanza di 15'		
	dopo 10'	60'	120'	30'	60'	120'
O. A., a. 14	198,0	158,7	144,5	217,6	247,9	210,5
C. F., » 8	262,2	230,1	133,8	285,4	335,3	121,3
M. A., » 10	194,4	146,2	137,3	78,4	133,8	51,7
P. S., » 9	207,7	149,8	101,6	240,8	315,7	213,3
B. R., » 12	187,3	133,8	107,0	214,0	251,4	144,5
S. R., » 16	198,0	149,8	117,7	235,4	267,6	171,2
P. A., » 7	187,3	133,8	90,9	198,0	278,3	133,8
S. B., » 12	171,2	133,8	112,3	214,0	283,6	165,9
C. G., » 9	208,7	149,8	117,7	230,1	272,9	181,9

Conclusioni: in base ai risultati delle nostre esperienze riteniamo:

1) che fra le ipotesi esposte per spiegare il sinergismo di azione sia da escludere quella di Litter sulla contrazione della diuresi;

2) che la formazione di composti di tipo fenolico da parte del fattore H non possa essere esclusa, ma che l'aumento in concentrazione dei derivati fenolici nel siero di sangue, dopo somministrazione dell'associazione medicamentosa salicilato di Na e fattore H, sia di gran lunga superiore a quella ascrivibile alla trasformazione suddetta: resta pertanto pienamente confermato il sinergismo di azione salicilato di Na e fattore H;

3) che il meccanismo di azione di detto sinergismo è a sede extra-renale, sussiste anche nelle somministrazioni per via endovena ed è pertanto da escludere (per lo meno per le somministrazioni endovena) il fattore di assorbimento intestinale avanzato da Litter;

4) che la variazione dell'andamento delle curve endovena, nel caso della somministrazione dell'associazione medicamentosa, nei confronti delle analoghe curve per somministrazione di solo salicilato, fa ritenere probabile la rapida azione del fattore H su un meccanismo re-

golatore del tasso salicitemico e manifesta probabilmente un'azione epatica in accordo con le ipotesi di Peluffo, Ravera e Putignano.

Pertanto nei riflessi della terapia del bambino, riteniamo che l'aggiunta del fattore H al salicilato di Na sia consigliabile nella somministrazione del medicamento per « os » e per « endovena ».

Particolarmente utile riesce l'associazione dei due medicamenti quando, per intolleranza gastrica o per altre ragioni, si debba ricorrere alla somministrazione del medicamento per via endovenosa, al fine di ritardare la rapida caduta del tasso salicitemico.

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Paul-1973
Bib No. 1058

Curr. Sci. 42(6): 206-208. 1973.

EFFECTS OF AN ACUTE DOSE OF BIOTIN ON THE REPRODUCTIVE ORGANS OF THE FEMALE RAT

THE adverse effects of biotin deficiency on the reproductive capacity and general metabolism of mammals are well documented[1,2]. On the other hand, there are no reports regarding the effects of an excess amount of biotin either in acute or chronic form on the reproductive ability and metabolism of the mammal. In certain insects, however, an excess intake of biotin causes severe degenerative effects on the ovary which leads to complete sterility[3,4]. It was of interest, therefore, to study the effects of an acute dose of biotin on the reproductive cycle, morphology of the ovary and the hepatic and uterine glycogen of the adult female rat.

Colony bred adult female rats of the Holtzman strain (approximately 3 months old) were selected after observing their normal oestrus cycle for three cycles. Eighteen rats thus selected were given biotin at a dose of 5 mg/100 g body weight dissolved in 0·2 ml of 0·1 N NaOH subcutaneously in two injections, morning and evening, at the dioestrus stage of the cycle. Groups of 6 rats each were sacrificed 7, 14 and 21 days after biotin injections. Another group of 6 rats was given an equal volume of 0·1 N NaOH at the dioestrus stage and was sacrificed 7 days after the injections. The entire experiment was performed under standardized light and dark, temperature and feeding conditions which are designed for best reproductive performances. The oestrus cycle of all the animals was studied daily on the basis of the microscopic examination of the vaginal smears. The leucocyte concentration of the vaginal smears was determined by the use of a haemocytometer after appropriate dilutions with normal saline. The histology of the ovary was studied following routine techniques and staining with haematoxylin and eosin. The liver and uterine glycogen was estimated according to the method of Montgomary[5].

The results of the present investigation showed that the NaOH-treated controls maintained normal oestrus cycles and the vaginal leucocyte numbers varied normally in relation to the stages of the oestrus cycle (Fig. 1). Since NaOH did not affect the normal oestrus cycle upto 7 days after treatment, running the controls upto 21 days was not considered necessary. The biotin-treated rats showed complete irregularity of the cycle and the vaginal leucocyte numbers progressively increased up to 14th day and sharply declined thereafter (Fig. 1). An increase in leucocyte infiltration in the vaginal lumen normally occurs during dioestrus stage in relation to the appearence of the luteal tissues in the ovary. This luteal phase of the cycle can be considered as an infertile stage of the mammalian reproductive cycle.

Therefore, biotin-induced irregularities of the oestrus cycle and massive infiltration of leucocytes in the vaginal lumen may be considered as an indication of its sterilizing effect. Biotin treatment

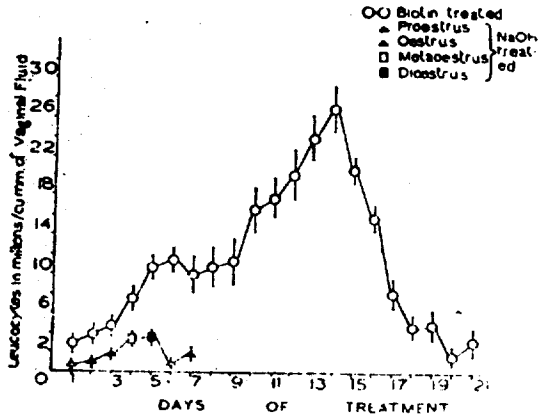

FIG. 1. Effect of biotin and NaOH on the leucocyte concentration in the vaginal fluid.

did not significantly affect the weights of the ovary, uterus, liver and normal body weight gain. The histological examination of the ovarian sections indicated that biotin treatment enhanced the process of the formation of corpora lutea, but a large number of the corpora lutea and stroma showed atrophic changes (Fig. 2).

The concentration and total liver glycogen reduced significantly 14 days after biotin treatment, but returned to the control level after 21 days (Table 1). The concentration and total uterine glycogen showed a slight reduction only 21 days after biotin treatment (Table 1). Terroine[1] has

TABLE I

The effect of biotin on the liver and uterine glycogen

Treatment	Liver glycogen		Uterine glycogen	
	Concentration mg/ 100 mg tissue	Total mg/liver	Concentration mg/ 100 mg tissue	Total mg/uterus
NaOH-treated control for 7 days	1·334 ±0·093	109·5 ± 1·12	0·136 ±0·112	0·469 ±0·028
7 days after biotin injections	0·567 ±0·038	43·4 ± 0·81	0·124 ±0·009	0·633 ±0·029
14 ,,	0·369 ±0·064	25·1 ± 0·58	0·144 ±0·019	0·465 ±0·025
21 ,,	1·232 ±0·095	103·8 ±1·52	0·083 ±0·010	0·305 ±0·036

There were six rats in each group.

reported that biotin deficiency in contrast to several other vitamins does not modify the glycogen content of the liver or muscle. Hence the observed reduction of hepatic and uterine glycogen is probably not a direct effect of biotin treatment. It has been suggested that progesterone inhibits glycogenic effect of oestrogen in the liver and uterus of the rat[6,7]. It is likely, therefore, that biotin is exerting its effect on hepatic and uterine glycogen through

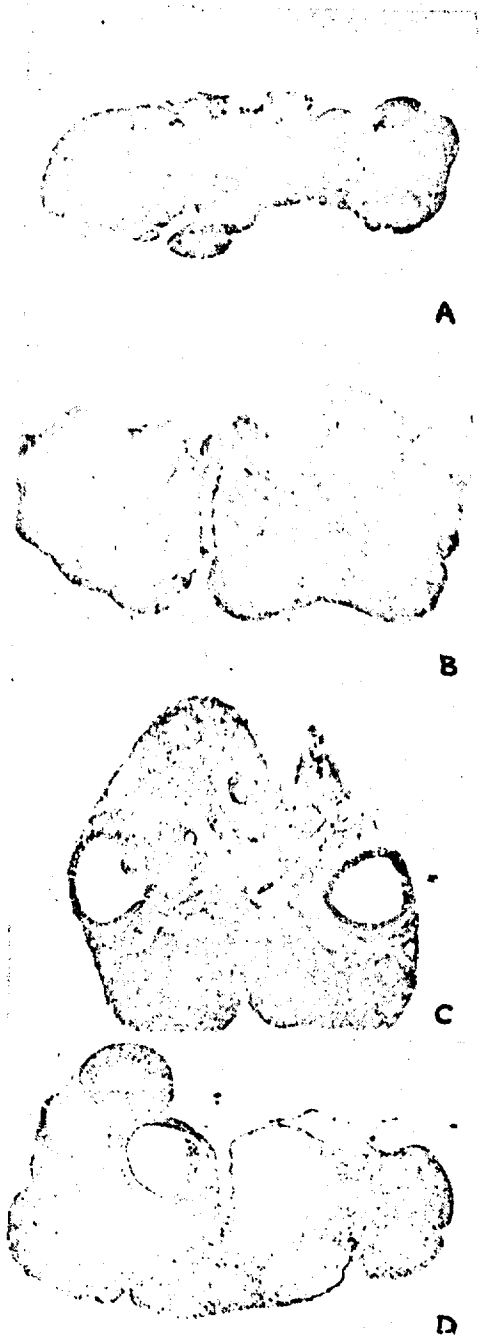

FIG. 2. Effect of biotin on the histology of the ovary. A, B, C and D—NaOH treated control (at oestrus), 7, 14 and 21 days after biotin injection respectively under low power.

an altered ratio of progesterone and oestrogen release from the ovary. The histology of the ovary of the biotin-treated rats tend to support this hypothesis.

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EFFECT OF BIOTIN ON THE STATE OF THE HAIR ROOTS AND SEBUM SECRETION IN WOMEN WITH DIFFUSE ALOPECIA

Pawlowski-'65
Bib No. 1060

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The double-blind method used indicated no effect of biotin on the state of hair roots or on quantity of sebaceous secretion in women with diffuse alopecia.

The clinical similarity of changes in diseases in animals with biotin deficiency to sebaceous inflammation of the skin in people has prompted many studies on the use of vitamin H in cases of sebaceous disease. The results were dissimilar, often contradictory, and in general not encouraging (Petrykiewicz and co-workers). From data in the literature, it turns out that the therapeutic effect of biotin on man was not proved (György, Harris).

Based on the unproven and doubtful causal relation between sebaceous balding type in men and diffuse alopecia in women, many dematologists use vitamin H in the last cases. The mechanism of excessive diffuse loss of hair in women is, according to Braun-Falco, caused mainly by an increase in the percentage of dysplasic and dystrophic hairs. But Kostanecki and co-workers found a distinct increase in such cases in the number of telogenes at the corners of the forehead.

Sebum often accompanies excessive loss of hair, but the problem of its parallel in intensity to disorders in growth is debatable.

The physiological values of telogene numbers show great individual fluctuations. Recently Witzel and Braun-Falco calculated, from experiments carried out on 12 women, that these were independent of age and skin region for the hair on the head. There are no accurate methods of identifying sebum secretion. It appears that a weight evaluation is most conclusive, although the correct values are differently used by authors. The average amount of sebum secretion in the forehead is 0.3-0.4 mg/cm^2 in the course of three years, according to Kligman. But these values are subject to fluctuation on successive days of study. Pawlowski and Petrykeiwicz obtained similar results, but Kordas obtained almost a two-fold increase.

The aim of this study was an attempt at the objective evaluation of biotin on sebum secretion and hair growth in cases of diffuse alopecia in women.

Subjects and Procedure. The subjects included 46 women from 17 to 51 years of age (average age 32 years) who had had diffuse alopecia for from one month to 24 years.

A group of 28 persons received vitamin H for 28 days in daily doses of 10 mg. 18 persons in the control group received a placebo for the same length of time. The study was carried out using the double-blind method.

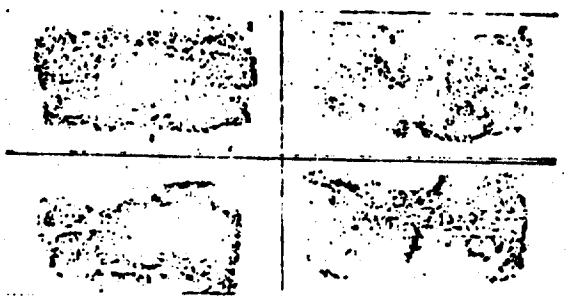

Fig. 1. Tissues treated with osmic acid vapor (case 12, group B).

Table 1. Results of Studies on the State of the Hair Roots and Sebum Secretion in the Group of People which Received Biotin

A #	% hair not growing		% hair growing properly		Sebum secretion in mg on 28 cm^2 area over three years	
	Bef. Treatment	Aft.	Bef. Treatment	Aft.	Before Treatment	After
1	24	25	—	—	5,5	4,0
2	47	44	41	34	5,0	3,5
3	12	11	86	86	3,0	2,5
4	10	10	86	88	2,0	5,0
5	18	20	74	62	2,0	1,0
6	44	38	50	54	4,5	3,0
7	45	42	43	48	4,5	2,5
8	10	66	90	40	2,0	3,5
9	2	2	58	58	1,0	3,0
10	17	19	65	62	0,5	3,5
11	4	5	—	—	3,5	6,0
12	19	15	79	80	4,0	2,0
13	3	16	—	—	7,0	4,0
14	41	17	51	76	—	—
15	43	39	47	52	4,0	3,0
16	18	16	80	80	3,0	3,0
17	17	20	—	—	4,0	2,0
18	38	25	—	—	5,0	4,5
19	23	16	71	61	4,0	0,5
20	26	24	—	—	5,0	4,0
21	29	66	51	39	3,0	1,0
22	34	30	66	66	1,0	1,0
23	31	25	60	63	4,0	1,0
24	65	42	30	57	—	—
25	12	8	76	55	2,5	3,0
26	55	25	45	75	3,0	1,0
27	37	33	44	65	2,5	4,0
28	14	30	86	66	3,5	1,0

Before beginning and at the end of treatment, the state of the hair roots was determined in the temporal-sinciput region (the method of Strauss and Pochi, as modified by Pawlowski and Petrykiewicz), as well as the amount of sebum secretion. The tissues used at the beginning of the study were treated with osmic acid vapor and were compared with the results obtained using the weight method. The state of the hair roots is presented, giving the percentage of hairs not growing, i.e. both telogene and katagene, as well as the percentage of hairs growing properly - anagene, in addition to dysplasic and dystrophic.

Results

The results of the studies are presented in three tables.

Table 2. Results of Studies on the State of the Hair Roots and Sebum Secretion in the Group of People to Whom a Placebo was Given

B #	% hair not growing		% hair growing properly		Sebum secretion in mg in 28 cm^2 area over 3 years	
	Bef. Treatment	Aft. Treatment	Bef. Treatment	Aft. Treatment	Before Treatment	After Treatment
1	22	25	68	66	4,5	1,0
2	32	8	68	91	4,5	4,0
3	15	10	68	78	6,0	3,0
4	14	3	75	95	1,5	1,5
5	7	2	79	95	1,5	1,5
6	57	5	37	93	2,0	1,5
7	25	7	64	92	3,0	4,5
8	16	16	78	74	1,5	4,0
9	23	23	70	70	3,5	3,0
10	17	13	69	83	3,0	2,0
11	5	7	95	83	1,0	1,0
12	53	56	38	41	2,5	6,0
13	30	28	68	68	5,5	2,0
14	10	20	71	73	3,0	0,5
15	23	10	72	82	3,5	2,0
16	23	20	72	77	4,5	4,0
17	21	28	78	60	1,0	3,5
18	6	6	80	34	3,0	2,0

Discussion

The average percentage of hairs not growing in both groups before the administration of biotin or the placebo was greater than the norm given in the literature. Its value in several cases differed considerably as well (0.5-7 mg). It was observed that the intensity of treated tissues always corresponded to the amount of sebum secretion determined by weight.

Table 3. Statistical Combination of Results

	Group A		Group B	
	Bef. Treatment	Aft. Treatment	Bef. Treatment	Aft. Treatment
Average %age of hairs not growing	29	26	22	16
Average %age of hairs growing properly	63	67	69	79
Average amount of sebum secretion	3.5 mg	2.8 mg	3.1 mg	2.6 mg
Average drop in %age of hairs not growing		3(δ 16)		6(δ 13)
Average increase in %age of hairs growing properly		4(δ 17)		7(δ 19)
Average drop in amount of sebum secretion		0.7 mg(δ 17)		0.5 mg(δ 19)

The average state of hair roots in the group treated with biotin was somewhat worse than in the control group, but sebum secretion was greater.

In both groups, after four weeks of using the preparations, the average state of the hair roots was somewhat improved, but sebum secretion was reduced. In several cases in both groups, its improvement in both the state

of the hair roots and in sebum secretion was very different; in several cases the data number proved worse.

It was observed that there was no correlation between state of the hair roots and amount of sebaceous secretion.

A statistical analysis of the results obtained spoke for the fact that biotin did not have an effect on the state of the hair roots, or on sebum secretion. This hypothesis was verified at a reliability level of 0.05. If this hypothesis is correct, the statistic t can be distributed as Student's t on $n_A + n_B - 2$ degrees of freedom. The calculations are presented in Table 4.

Table 4. For all three values of observed t not in excess of the critical value t

Average difference bef. and aft. use of medium	for X_A	X_B	S_A	S_B	# of deg. freedom $f=(n_A+n_B-2)$	Value of Statistic t^2 (calc.)	Value of Statistic t (read fr. table)
%age of hairs not growing	-3	-6	16	13	46	0.652	2.014
%age of hairs properly growing	+4	+7	17	19	40	0.513	2.000
Sebum secretion	-0.7	-0.5	17	19	44	0.036	2.017

Conclusions

1. No effect of vitamin H was found in administration of large doses for four weeks either on the state of hair roots or on sebum secretion.

2. The telogene number, the percentage of dysplasic and dystrophic hairs, and the amount of sebum secretion were in several cases greatly different and could yield significant fluctuation even in the course of four weeks.

3. There was indicated no correlation between increase in alopecia in women and sebum level.

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Pawlowski-'65
Bib No. 1060

PRZEGLĄD DERMATOLOGICZNY
1965, LII, 265

Andrzej Pawłowski, Wojciech Kostanecki

WPŁYW BIOTYNY NA STAN KORZENI WŁOSÓW I WYDZIELANIE ŁOJU U KOBIET Z ŁYSIENIEM ROZLANYM

Z Kliniki Dermatologicznej AM w Warszawie
Kierownik: prof. dr *S. Jabłońska*

Stosując metodę podwójnie ślepą nie wykazano wpływu biotyny ani na stan korzeni włosów, ani na ilość wydzielanego łoju u kobiet z łysieniem rozlanym.

Kliniczne podobieństwo zmian chorobowych u zwierząt z niedoborem biotyny do łojotokowego zapalenia skóry u ludzi skłoniło wielu badaczy do zastosowania witaminy H w przypadkach schorzeń łojotokowych. Wyniki były różne, często sprzeczne, na ogół niezachęcające *(Petrykiewicz* i wsp.). Z danych piśmiennictwa wynika, że działanie lecznicze biotyny u człowieka jest nieudowodnione *(György, Harris)*.

Opierając się na nieudowodnionym i wątpliwym związku przyczynowym między łojotokiem a łysieniem typu męskiego i rozlanym łysieniem u kobiet, wielu dermatologów stosuje w tych ostatnich przypadkach witaminę H. Mechanizm nadmiernie rozlanej utraty włosów u kobiet jest według *Braun-Falco* spowodowany głównie zwiększeniem się odsetka włosów dysplastycznych i dystroficznych. Natomiast *Kostanecki* i współpracownicy stwierdzili w tych przypadkach wyraźny wzrost liczby telogenowej w kątach czołowych.

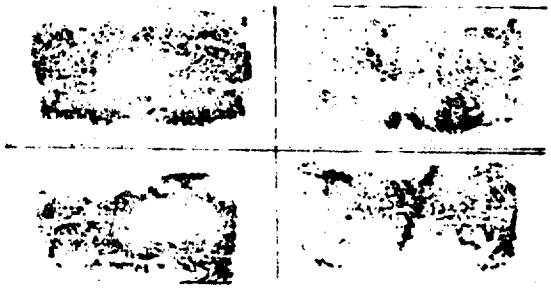
Nadmiernej utracie włosów często towarzyszy łojotok, ale zagadnienie równoległości jego nasilenia do zaburzeń we wzroście włosów jest dyskutowane.

Wartości fizjologiczne liczby telogenowej wykazują duże wahania indywidualne. Ostatnio *Witzel* i *Braun-Falco* obliczyli, że wynosi ona u kobiet 12, niezależnie od wieku i okolicy skóry owłosionej głowy. Dokładnych metod oznaczania wydzielonego łoju nie ma. Wydaje się, że ocena wagowa jest najbardziej miarodajna, aczkolwiek wartości prawidłowe są różnie podawane przez autorów. Średnie ilości wydzielanego łoju na czole wynoszą w mg/cm² w ciągu 3 godzin według *Kligmana* 0,3 — 0,4. Wartości te jednak ulegają wahaniom w kolejnych dniach badania. Podobne wynki uzyskali *Pawłowski* i *Petrykiewicz*, natomiast *Kordas* niemal dwukrotnie wyższe.

Celem pracy była próba przedmiotowej oceny wpływu biotyny na wydzielanie łoju i wzrost włosów w przypadkach rozlanego łysienia u kobiet.

M a t e r i a ł i m e t o d y k a. Materiał obejmuje 46 kobiet w wieku od 17 do 51 lat (średnia wieku 32 lata) z łysieniem rozlanym trwającym od 1 miesiąca od 24 lat.

Grupa 28 osób otrzymywała w ciągu 28 dni witaminę H w dawce dziennej 10 mg. W grupie kontrolnej obejmującej 18 osób podawano w tym samym czasie placebo. Pracę przeprowadzono, stosując metodę podwójnie ślepą.



Ryc. 1. Bibułki wybarwiane parami kwasu osmowego (przypadek 12. grupy B). U góry przy wartości wagowej 6 mg, u dołu 3 mg.

Tabela I

Wyniki badań stanu korzeni włosów i wydzielania łoju w grupie osób, którym podawano biotynę

A.	% włosów nie rosnących		% włosów prawidłowo rosnących		Wydzielanie łoju w mg na 28 cm² w ciągu 3 godz.	
Lp.	przed leczeniem	po leczeniu	przed leczeniem	po leczeniu	przed leczeniem	po leczeniu
1	24	25	—	—	5,5	4,0
2	47	44	41	34	5,0	3,5
3	12	11	86	86	3,0	2,5
4	10	10	86	88	2,0	5,0
5	18	20	74	62	2,0	1,0
6	44	38	50	54	4,5	3,8
7	45	42	43	48	4,5	2,5
8	10	66	90	40	2,0	3,5
9	2	2	58	58	1,0	3,0
10	17	19	65	62	0,5	3,5
11	4	5	—	—	3,5	6,0
12	19	15	79	80	4,0	2,0
13	3	18	—	—	7,0	4,0
14	41	17	51	76	—	—
15	43	39	47	52	4,0	3,0
16	18	16	80	80	3,0	3,0
17	17	20	—	—	4,0	2,0
18	38	25	—	—	5,0	4,5
19	23	16	71	61	4,0	0,5
20	26	24	—	—	5,0	4,0
21	29	66	51	39	3,0	1,0
22	34	30	66	66	1,0	1,0
23	31	25	60	63	4,0	1,0
24	65	42	30	57	—	—
25	12	8	76	55	2,5	3,0
26	55	25	45	75	3,0	1,0
27	37	33	44	65	2,5	4,0
28	14	30	86	66	3,5	1,0

Przed rozpoczęciem i po zakończeniu leczenia określano stan korzeni włosów w okolicy skroniowo-ciemieniowej (metodą *Straussa* i *Pochi* zmodyfikowaną przez *Pawłowskiego* i *Petrykiewicza)* i ilość wydzielanego łoju. Bibułki użyte na początku badania wybarwiano parami kwasu osmowego i porównywano z wynikami uzyskanymi metodą wagową. Stan korzeni włosów przedstawiono, podając odsetek włosów nie rosnących, tzn. łącznie telogenowych i katagenowych oraz odsetek włosów prawidłowo rosnących — anagenowych poza dysplastycznymi i dystroficznymi.

WYNIKI

Wyniki badań zestawiono w 3 tabelach.

Tabela II

Wyniki badań stanu korzeni włosów i wydzielania łoju w grupie osób, którym podawano placebo

B.	% włosów nie rosnących		% włosów prawidłowo rosnących		Wydzielanie łoju w mg na 28 cm² w ciągu 3 godz.	
Lp.	przed leczeniem	po leczeniu	przed leczeniem	po leczeniu	przed leczeniem	po leczeniu
1	22	25	68	66	4,5	1,0
2	32	8	68	91	4,5	4,0
3	15	10	68	78	6,0	3,0
4	14	3	75	95	1,5	1,5
5	7	2	79	95	1,5	1,5
6	57	5	37	93	2,0	1,5
7	25	7	64	92	3,0	4,5
8	16	16	78	74	1,5	4,0
9	23	23	70	70	3,5	3,0
10	17	13	69	83	3,0	2,0
11	5	7	95	93	1,0	1,0
12	53	56	35	41	2,5	6,0
13	30	28	65	66	5,5	2,0
14	10	20	71	73	3,0	0,5
15	23	10	73	82	3,5	2,0
16	23	20	73	77	4,5	4,0
17	21	28	78	60	1,0	3,5
18	6	6	80	34	3,0	2,0

OMÓWIENIE

Średni odsetek włosów nie rosnących w obu grupach przed podawaniem biotyny względnie placebo był większy niż podawanie w piśmiennictwie normy. Wartość ta w poszczególnych przypadkach wahała się bardzo znacznie: od 2 do 65. Odsetek włosów prawidłowo rosnących był w obu grupach niski i ulegał wahaniom od 30 do 95.

Ilości wydzielanego łoju w poszczególnych przypadkach różniły się również znacznie (0,5 — 7 mg). Spostrzeżono, że intensywność wybarwienia bibułek nie zawsze odpowiada określonej wagowo ilości wydzielanego łoju.

Tabela III
Statystyczne zestawienie wyników

	Grupa A		Grupa B	
	przed leczeniem	po leczeniu	przed leczeniem	po leczeniu
Średni % włosów nie rosnących	29	26	22	16
Średni % włosów prawidłowo rosnących	63	67	69	79
Średnia ilość wydzielanego łoju	3,5 mg	2,8 mg	3,1 mg	2,6 mg
Średni spadek % włosów nie rosnących		3 (δ16)		6 (δ13)
Średni wzrost % włosów prawidłowo rosnących		4 (δ17)		7 (δ19)
Średni spadek ilości wydzielanego łoju		0,7 mg (δ17)		0,5 mg (δ19)

Średni stan korzeni włosów w grupie leczonej biotyną był nieco gorszy niż w grupie kontrolnej, a wydzielanie łoju większe.

W obu grupach po 4-tygodniowym stosowaniu preparatów średni stan korzeni włosów nieco poprawił się, a wydzielanie łoju zmniejszyło. W poszczególnych przypadkach obu grup poprawa ta zarówno co do stanu korzeni włosów, jak i wydzielania łoju była bardzo różna, w niektórych dane liczbowe wykazują pogorszenie.

Spostrzeżono, że między stanem korzeni włosów a ilością wydzielanego łoju nie ma korelacji.

Analiza statystyczna otrzymanych wyników przemawia za tym, że biotyna nie wywarła wpływu na stan korzeni włosów, ani na wydzielanie łoju. Hipotezę tę weryfikujemy na poziomie istotności $\alpha = 0{,}05$. Jeśli hipoteza ta jest słuszna, to statystyka „t" ma rozkład t Studenta on $n_A + n_B - 2$ stopniach swobody. Obliczenia przedstawiono w tabeli IV.

Tabela IV
Dla wszystkich 3 wartości zaobserwowane t nie przekracza krytycznej wartości ta

Średnie różnice przed i po stosowaniu środków	dla X_A	X_B	S_A	S_B	Liczba stopni swobody r $(n_A + n_B - 2)$	Wartość statystyki t_α (wyliczona)	Wartość statystyki t_α (odczytana z tablic)
% włosów nierosnących	−3	−6	16	13	46	0,652	2,014
% włosów prawidł. rosnących	+4	+7	17	19	40	0,513	2,000
wydzielanie łoju	−0,7	−0,5	17	19	44	0,036	2,017

WNIOSKI

1. Nie stwierdzono wpływu witaminy H podawanej w dużych dawkach przez 4 tygodnie ani na stan korzeni włosów, ani na wydzielanie łoju.

2. Liczba telogenowa, odsetek włosów dysplastycznych i dystroficznych oraz ilość wydzielanego łoju są w poszczególnych przypadkach nader różne i mogą ulegać znacznym wahaniom już w ciągu 4 tygodni.

3. Nie wykazano korelacji między nasileniem łysienia u kobiet a stopniem łojotoku.

A. Павловски, В. Костанецки

ВЛИЯНИЕ БИОТИНА НА СОСТОЯНИЕ КОРНЕЙ ВОЛОС И НА ВЫДЕЛЕНИЕ САЛА У ЖЕНЩИН С ДУФФУЗНОЙ ПЛЕШИВОСТЬЮ

Содержание

Группе, состоящей из 46 женщин с диффузной плешивостью, применяли биотин по вдвойне слепому методу в течение 4 недель ежедневно по 10 мг (28 случаев) или же успокоительное средство placebo (18 случаев). До и после применения этих средств исследовалось состояние корней волос и определялось количество выделяемого сала. Корреляция между состоянием корней волос и интенсивностью себореи не обнаружена.

A. Pawłowski, W. Kostanecki

EFFECTS OF BIOTIN ON HAIR ROOTS AND SEBUM EXCRETION IN FEMALES WITH DIFFUSE ALOPECIA

Summary

46 females with diffuse hair-loss were given biotin (10 mg daily for 4 weeks — 28 cases) or placebo (18 cases) in double-blind test. Examination of hair roots and quantitative estimations of sebum excretion were performed prior to and after the treatment. No correlation between the appearance of the hair roots and intensity of seborrhoea could be traced.

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Petrelli-1967
Bib No. 1067

Ital. J. Biochem 16(3): 151-155.

RELATIONSHIP BETWEEN BIOTIN SUPPLY AND CARBOHYDRATE METABOLISM

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(Received 15 May 1967)

Biotin is known to take part in many metabolic processes. According to most authors biotin plays a part in carboxylation and decarboxylation reactions; however the mechanism by which the vitamin affects these reactions is mostly unknown even if in some particular cases it has been shown to believe as a coenzyme.

The metabolic alterations due to biotin deficiency are many. Dakshinamurti *et al.* (1962) report that the incorporation of amino acids in liver proteins is reduced in biotin-deficient rats kept on a diet containing 70% glucose as the sole source of carbohydrates. When glucose was replaced by sorbitol or fructose, the incorporation was restored to the normal level. According to the authors, the deficient animal utilizes fructose or sorbitol as sources of energy for its metabolic processes but is unable to utilize glucose. That glucose utilization is decreased in biotin deficiency is confirmed by the shape of tolerance curves: when equal amounts of glucose are administered to normal and biotin-deficient rats, the latter show a transient hyperglycaemia which is much greater than that of control animals.

Furthermore biotin deficiency decreases glycogen synthesis (Dakshinamurti *et al.*, 1962), decreases the oxidation of glucose ^{14}C to CO_2 (Singh *et al.*, 1963) and increases the excretion of ketone bodies (Dakshinamurti *et al.*, 1962) but does not seem to affect the glucose level in circulating blood (Singh *et al.*, 1963). On the other hand Terroine (1956) found a slight hypoglycaemia in rats kept on an avidin diet.

The administration of insulin prevented alterations due to decreased utilization of glucose in deficient animals (Dakshinamurti *et al.*, 1962); according to those authors this finding supports the hypothesis that the biochemical lesion of carbohydrate metabolism is at the level of hexokinase.

A relationship between biotin and carbohydrate metabolism has also been observed in some micro-organisms. Moat *et al.* (1954) found that yeasts grown on a biotin-free medium ferment glucose at a rate which is much slower than yeasts grown on a medium containing optimal amount of the vitamin. The addition of biotin to a culture of *S. cerevisiae* affected positively both the fermentation oxidation rates.

On the basis of what has been said so far, we have studied the effects of administering various amounts of biotin or of biotin plus insulin on the blood sugar level of normal rats.

Methods

Young adult female Wistar rats weighing about 200 g. were used. They were fed Mc Collum diet and were fasted 14 hr. before the experiments.

Blood sugar was determined by the method of Nelson and Somogyi. The blood sugar level was followed for three hours in experiment 1 and for six hours in experiment 2.

Blood was removed from the tail before administering biotin or biotin plus insulin and subsequently at various time intervals as shown for the single experiments.

Biotin was injected endoperitoneally in aqueous solution in single doses of 0.5, 1 and 2 mg per rat immediately after the first blood sample was taken. In experiment 2 the administration of biotin was repeated after 90 min.

0.1 I.U. insulin (insulin-zinc protamine obtained from Lilly and Co) were injected intramuscularly to each rat immediately after taking the first blood sample.

Experiment 1

This experiment was carried out in order to consider the possible effect of various doses of biotin on the glycaemic state of normal rats.

Four groups of 12 rats each were used. Animals of the first, second and third group received respectively 0.5, 1 and 2 mg biotin per rat. Animals of the fourth group were used as controls.

Blood samples were removed before the administration of biotin and 10, 20, 30, 60, 90, 120 and 180 min. after administration.

The graphs reported in fig. 1 show the blood sugar curves of the four experimental groups. It may be seen that the administration of 1 mg biotin per rat led to a well-defined increase in blood sugar which reached a maximum 30 min. after administration and then returned

slowly to normal. An effect of the same magnitude was observed following the injection of 2 mg biotin per rat while the injection of 0.5 mg did not appear to produce marked changes in the blood sugar.

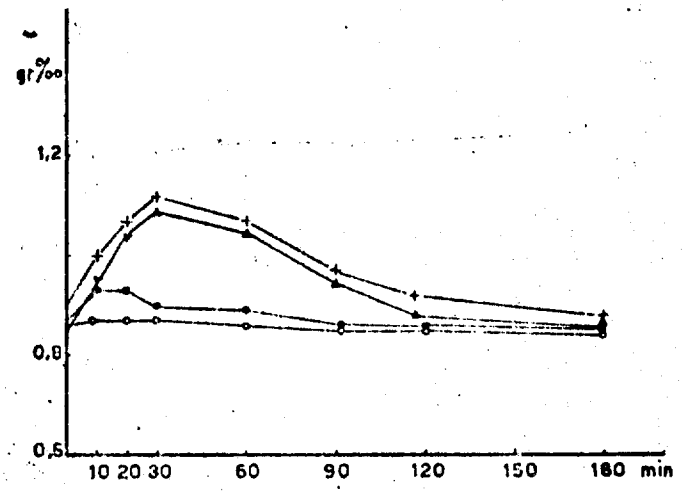

Fig. 1. - Mean values of the blood sugar level of groups of 12 rats treated with biotin and of 12 control rats.

Abscissae: time from the beginning of the experiment.

Ordinates: blood sugar expressed as g./1000 ml.

O = control

● = 0.5 mg. biotin

▲ = 1 mg. biotin

\+ = 2 mg. biotin

Experiment 2

The results of the previous experiment led us to study the course of glycaemia following the simultaneous administration of biotin and insulin to check if the hyperglycaemic action of biotin could antagonize or reduce the hypoglycaemic action of insulin.

Three groups of 12 rats each were used.

The first group was used as controls. Animals of the second and third group were given respectively insulin or insulin plus biotin at the beginning of the experiment.

Blood samples were removed before administration and 30 min., 1, 2, 3, 4, 5 and 6 hr. after.

The results are reported in the graphs of fig. 2 which show that biotin does not affect the hypoglycaemia produced by the administration of insulin.

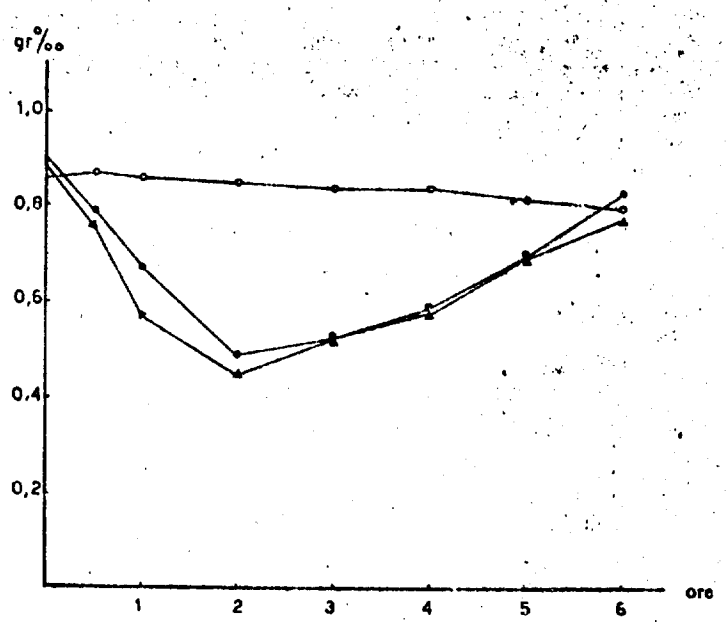

Fig. 2. - Mean values of the blood sugar level of 12 control rats and of 12 rats treated with insulin or with insulin plus biotin.

Abscissae: time from the beginning of the experiment.
Ordinates: blood sugar expressed as g./1000 ml.

○ = control
● = insulin
▲ = insulin plus biotin.

Discussion

It has been found that biotin administration affects the carbohydrate metabolism of normal rats, leading to a rapid increase of the blood sugar level. This increase which quantitatively was not very large, was constantly found in all the animals. The magnitude of the hyperglycaemia was not proportional to the dose of biotin administered.

As regards the relationship between biotin and insulin, biotin under the present experimental conditions did not modify the hypoglycaemic state due to insulin. It may be assumed that the two substances exert

their biochemical effect by mechanisms which are altogether independent. Hence the present data though confirming the existence of a relationship between biotin and glucose metabolism, do not permit to draw conclusions on the nature of this relationship.

Further experiments are being carried out in this laboratory to clarify better the subject.

SUMMARY

The effect of biotin and biotin plus insulin administration on the blood sugar level of normal rats has been studied. The administration of biotin affected carbohydrate metabolism leading to a rapid increase, even if not very large, of the blood sugar level.

At least under the present experimental conditions, biotin did not appear to modify the hypoglycaemic state due to the injection of insulin.

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Pillai-'69
Bio No.-1084

Effect of Biotin on the Fertility of the Yellow Fever Mosquito, *Aedes aegypti* (L.)

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Biotin is an indispensable nutritional factor for growth and metamorphosis in many insects. However, it has been reported that excess of biotin causes loss of reproductive capacity in two species of insects [1, 2]. This has aroused interest in investigating the effect of excess of dietary biotin in the mosquito, *Aedes aegypti* (L.).

Larvae of *A. aegypti* were reared in distilled water by feeding them on brewer's yeast containing 0.00022% biotin (Nutritional Biochemicals Corp.) and blood albumen (10:1). In this rearing medium biotin (D(+)biotin, E. Merck Labor.) was mixed to get the desired concentrations. In each experiment 200 third instar larvae were allowed to grow till pupation in enamel pans with 250 ml distilled water containing various concentrations of biotin supplemented with the usual quantities of the diet. Fifteen treated or normal males were either crossed with the same number of biotin-fed or normal females separately in screened cages. Number of eggs laid for 15 days from the start of the cross was scored and the viability of those eggs was subsequently determined.

In biotin-fed larvae the rate of growth and metamorphosis were identical with the normal ones. Biotin was found to cause high mortality at pupal stage (Table). The diet con-

Table. *Mortality, fecundity and hatchability of A. aegypti fed on biotin in the diet at larval stage*

Conc. in ppm	% mortality			Sex treated	Eggs laid/ female	% sterility
	Larva	Pupa	Adult			
60	2.0	24.9	18.0	Male	103.8	10.4
				Female	101.2	21.4
				Both	51.5	44.6
80	1.0	35.0	30.0	Male	117.0	11.7
				Female	5.0	54.0
				Both	5.0	48.0
120	20.0	80.0	—	—	—	—
Control	0.5	—	7.5	Neither	103.1	5.1

taining 60 ppm biotin showed considerable reduction in the oviposition and induced 44.6% sterility. Sterility increased to 48% with 80 ppm in crosses where both the sexes were treated. Ovarian follicles of preovipositional and post-ovipositional stages of normal and treated females were found to be as follows:

	No. of ovarian follicles/female	
	Normal	Biotin-fed (80ppm)
Preoviposition	48.8 ± 2.5	50.5 ± 3.1
Postoviposition	48.2 ± 5.3	15.3 ± 2.2

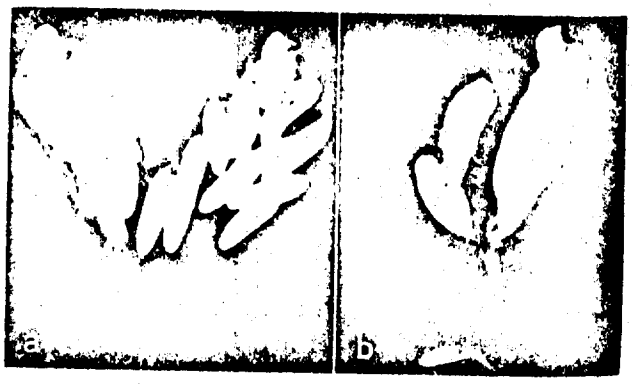

Fig. 1a and b. Ovaries of *A. aegypti*; a) normal — b) biotin-fed. 25 ×

Ovaries of biotin-fed females were identical in size at preovipositional period but at postovipositional period they were considerably reduced (Fig. 1), indicating degeneration of follicles already formed. This fact evidently indicates that biotin interfers with certain metabolic processes of egg maturation. It is interesting to note that the effect of biotin is specific to ovaries, as testes of treated males were identical with that of normal males in size, number and motility of sperms.

We wish to thank Prof. B. K. SESHACHAR for encouragement.

Received December 7, 1968 and January 20, 1959

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Rajalakshmi-'64
Bib No. 1127

Indian J. Biochem. 1(1): 23-25.

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Effect of Biotin on Cholesterol Synthesis*

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Manuscript received 20 *November* 1963

Biotin when administered in excess to rats enhances the level of cholesterol in serum, liver and carcass. This elevation of cholesterol in the tissues is found to be due to an increase in the synthesis of cholesterol in the biotin treated rats. Biotin exerts its action on cholesterol synthesis prior to the formation of mevalonic acid. The mechanism by which biotin accentuates cholesterol synthesis in rats may be by facilitating the utilization of C-5 acids towards cholesterol synthesis. The lipotropic action of inositol consists in the rapid removal of cholesterol from the site of synthesis and not by suppressing the synthesis.

BIOTIN is necessary for the maintenance of growth of probably all living forms. Nevertheless, the exact biological function of this vitamin has never been satisfactorily elucidated. The work of Williams and Fieger and others on microorganisms[1,2] and the studies of McHenry and Patterson on animal nutrition[3] suggested the participation of biotin in lipid metabolism. The role of biotin in fatty acid synthesis has been fairly well recognized in recent years, particularly by the work of Wakil and Gibson[4].

Although the coenzymic role of biotin in fatty acid synthesis has been demonstrated, its role in cholesterol metabolism still remains to be elucidated. A critical survey of literature on this subject reveals that the results obtained by various workers are contradictory[5-7] and permit no clear-cut conclusion. In most of these experiments animals that were rendered deficient by feeding avidin diet were employed and there was always a period of inanition. Recently, Easley *et al.*[8] have

* This paper forms part of the work of Ph.D. Thesis submitted to the Madras University by Miss S. Rajalakshmi in 1962.

reported that rats administered excess biotin exhibited enhanced activity of oxalacetic carboxylase. This suggests the possibility of an increase in the activities of enzymes which are dependent on biotin when the nutritional status of the animal in regard to biotin has been enhanced. One of the potential sources of β-hydroxy-β-methyl glutaryl CoA (HMG-CoA)—the key intermediate in cholesterol synthesis, is in the enzymic carboxylation of C-5 acids such as hydroxy isovaleric acid and β-dimethylacrylic acid. Since this carboxylation reaction is entirely biotin dependent, it was of interest to study the synthesis of cholesterol in rats administered excess biotin. The study of biosynthesis of cholesterol from acetate-1-C^{14} and mevalonic acid-2-C^{14} was, therefore, undertaken and the results are reported in this communication.

Materials and Methods

Young male albino rats weighing 30-40 g. were used in these experiments. The rats were housed in individual cages and given food and water *ad libitum*. The animals were divided into four groups and given the following diets: Group I — basal diet of composition described earlier[9]; group II — basal diet plus 100 μg. of biotin, administered subcutaneously; group III — basal diet plus 100 μg. of biotin and 1 per cent inositol; and group IV — basal diet plus 100 μg. biotin plus 1 per cent inosithin, a phosphatide rich in inositol and isolated from soyabean (gift sample from Lecithin & Co., New York). At the end of eight weeks the animals were killed by decapitation, after which carcass, liver and serum were analysed.

The procedures for the extraction of lipid from carcass, liver and serum, and the estimation of cholesterol, pyridine nucleotides, protein nitrogen and ribonucleic acid were the same as described in an earlier communication[9].

In vivo incorporation of acetate-1-C^{14} and mevalonic acid-2-C^{14} — The rats from groups I, II and III were administered intraperitoneally 20 μC. of acetate-1-C^{14} (Radiochemical Centre, Amersham, UK), 4 hr prior to sacrifice. In a similar manner four rats belonging to groups I and II were given 5 μC. of mevalonic acid-2-C^{14} (Hoffmann La Roche, Basel, Switzerland). Cholesterol was precipitated as digitonide which was purified by methods described earlier[9]. The digitonide was then cleaved by anhydrous pyridine according to the procedure of Schoenheimer and Dam[10]. A portion of it was plated on to a stainless steel planchet and its radioactivity determined in a Geiger tube connected to a scaler (Nuclear Instruments, Chicago)[11]. The values are expressed as counts/min./mg. cholesterol. The values were corrected for both self-absorption and background.

Results

The data on cholesterol concentration of serum, liver and carcass of rats given biotin in excess are summarized in Table 1. The data reveal an elevation of serum and liver cholesterol level, and only a slight increase in the carcass. The inclusion of either 1 per cent inositol or 1 per cent inosithin restored the cholesterol level to normal in serum and liver. However, the rats of group IV exhibited a slight decrease in the carcass cholesterol level as compared to that of control group.

The results on the incorporation of labelled acetate and mevalonic acid are presented in Table 2. Usually, an increase in the level of hepatic cholesterol would decrease the specific activity due to dilution effect. But, the specific activity value for cholesterol synthesis in the rats administered with biotin is higher than the control value in spite of the accumulation of cholesterol in the liver of these rats. Therefore, the increase in the specific activity itself could be taken as an index for enhanced synthesis and is recorded in Table 2. The results indicate a significant enhancement in the incorporation of acetate-1-C into hepatic cholesterol of rats of group II and very little change in that of mevalonic acid-2-C^{14}. Even though the rats whose diet was supplemented with inositol maintained the normal cholesterol levels, yet the specific activity and total counts on acetate administration are high and comparable with those of group II. This would mean that inositol is not able to counteract the stimulatory effect of biotin on cholesterol synthesis, but prevents the accumulation of cholesterol by facilitating the removal from the site of synthesis, i.e. liver in this particular

TABLE 1 — CHOLESTEROL CONCENTRATION IN SERUM, LIVER AND CARCASS OF BIOTIN ADMINISTERED RATS

[Values are given ± SD; No. of rats used in each group was 10]

Group of rats	Supplements to diet	Total cholesterol conc. mg. % wet wt		
		Serum mg./100 ml.	Liver	Carcass
I	Nil	82±4·2	217±10·1	365±10·5
II	Biotin, 100 μg.	120±2·1	482±8·2	400±20·2
III	Biotin, 100 μg. +inositol, 1%	90±3·6	248±2·6	350±8·6
IV	Biotin, 100 μg. +inosithin, 1%	86±1·8	220±1·2	302±4·2

TABLE 2 — INCORPORATION OF ACETATE-1-C^{14} AND MEVALONATE-2-C^{14} INTO HEPATIC CHOLESTEROL OF RATS ADMINISTERED EXCESS BIOTIN

[20 μC. of acetate-1-C^{14} and 5 μC. of mevalonate-2-C^{14} were injected intraperitoneally 4 hr prior to sacrifice. Values are the average of 4 rats and those presented in parentheses represent the percentage increase over control]

Group of rats	Supplement to basal diet	Level of cholesterol mg./liver†	Specific activity* c.p.m./mg.	
			Acetate-1-C^{14}	Mevalonate 2-C^{14}
I	Nil	11·00	28	3656
II	DL-Biotin, 100 μg.	29·00	66 (136)	4046 (11)
III	DL-Biotin, 100 μg. +inositol, 1%	18·00	60 (114)	—

*Values are corrected for self-absorption and background.
†There was no significant change in liver weight and, therefore, cholesterol value per liver was recorded.

instance. The biotin treated rats showed no change in hepatic levels of pyridine nucleotides, proteins, and ribonucleic acids. Hence, the results have not been presented in a separate table.

Discussion

The administration of biotin in excess to rats fed on a purified diet leads to an enhancement in the level of cholesterol in the liver. This elevation in the level of hepatic cholesterol was due to an increase in the synthesis as revealed by the incorporation of labelled acetate and definitely not by mobilization from extra hepatic tissues. If it were to be a mobilization from extra hepatic tissues, the accumulation of cholesterol in the liver would have been accompanied by a decline in the concentration of cholesterol in the serum and carcass. There was, however, an increase in the level of cholesterol in these tissues. The fact that biotin exerts a stimulatory action on the incorporation of acetate-1-C^{14} and not mevalonic acid-2-C^{14} suggests that the action of biotin must be prior to the formation of mevalonic acid.

The only biotin dependent step in the whole series of reactions that lead to the synthesis of cholesterol is the carboxylation of C-5 acids. Bloch *et al.*[12,13] observed that, in *in vivo* experiments, the C-5 acids such as hydroxyisovaleric acid, β-dimethylacrylic acid and β-methyl crotonic acid are better utilized than acetate for cholesterol synthesis. Recently Jacobson and Corley[14] have reported that the incorporation of radioactive β-dimethylacrylic acid into liver cholesterol is markedly reduced in biotin deficiency. The formation of C-5 acids from acetate appears to be possible from the reports of Rabinowitz and Gavin[15-17].

The utilization of these C-5 acids for cholesterol synthesis would be determined by the level and activity of the carboxylating enzyme which is a biotin dependent one. Activation of this carboxylating enzyme as a consequence of biotin administration to rats might explain the accentuation of cholesterol synthesis as observed in the present investigation. Such an activation of this carboxylating enzyme as a consequence of biotin administration is likely in the light of the observation of Easley and coworkers[8].

The possibility of dilution of HMG-CoA pool due to an increase in the catabolism of leucine exists, though no such dilution which will have the effect of lowering the specific activity could be observed. It is, however, possible that the dilution of HMG-CoA pool may not affect the specific activity if the rate of utilization is also considerably increased. Further, the possibility of formation of C-5 acid from acetate is also possible according to Rabinowitz and Gavin[15-17]. Bloch[18] has observed that the incorporation of labelled carbon dioxide into cholesterol, which was very insignificant, was increased markedly by the simultaneous administration of non-isotopic β-hydroxyisovaleric acid.

The lipotropic action of inositol consists not by inhibiting the synthesis of cholesterol but in the mobilization of it from the liver. Inosithin is as effective as inositol in restoring the level of cholesterol in the rats administered biotin. The accumulation of fat and cholesterol in the biotin treated rats was not comparable to the observation recorded by McHenry and Patterson[3], and the dietary and abnormal conditions employed by the latter might have been responsible for the development of acute fatty liver noticed by them.

Summary

1. Administration of biotin in excess to albino rats increases the concentration of cholesterol in serum, carcass and liver.

2. Biotin enhances the level of tissue cholesterol by stimulating the synthesis of cholesterol from acetate as revealed by the incorporation studies with radioactive acetate and mevalonic acid.

3. An additional supplementation of inositol or inosithin is required to maintain the normal level of cholesterol in the tissues of rats treated with biotin. The mechanism by which biotin enhances the sterol synthesis seems to be by facilitating the utilization of C-5 acids towards cholesterol synthesis.

Acknowledgement

The authors are thankful to the Vanaspathy Manufacturers' Association for financial help.

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Effect of Biotin on Certain Physiological Functions.

JOHN L. SCHMIDT AND MAURICE LANDY. (Introduced by W. E. Hambourger.)

From the Research Laboratories of the S. M. A. Corporation, Chagrin Falls, Ohio.

Biotin, the factor curative of egg white injury[1] and a growth-essential for many bacterial species,[2, 3, 4] is now considered an important component of the vitamin B-complex. Up to the present time, a study of the immediate effect of biotin on physiological functions has not been undertaken. The experiments described here demonstrate the effect of biotin on blood pressure, respiration, frog heart, excised uterus and intestinal strip. Since crystalline biotin is available only in limited quantity and because of its high degree of biological activity (1 μg equals 27 vitamin H rat curative units[1]), relatively small amounts were used in carrying out this study.

Because of its low solubility in water, crystalline biotin methyl ester was hydrolyzed and the acid converted into the more soluble sodium salt. To 5 mg of the methyl ester was added 0.775 mg NaOH, after which dilution was made to 10 cc with distilled water. This mixture was heated in a pyrex tube over a free flame until solution took place (about 2 minutes).

Two small cats (2 kg each) were used for studying blood pressure and respiration. The blood pressure was recorded with a mercury manometer connected to a carotid artery, and respiration was recorded with a Marey tambour attached to a by-pass in the tracheal cannula. Rapid intravenous injection of 500 μg of biotin produced no significant change in blood pressure, heart rate and respiration (Fig. 1).

Smooth-muscle studies were made in a 20 cc excised organ bath of all-glass construction. Eight strips of guinea pig uterus, one strip of rabbit uterus and one segment of rabbit intestine were studied suspended in oxygenated Sollmann-Rademaeker's solution at 38.5°C. Concentrations of biotin up to 1 part in 40,000 did not affect the normal rhythmic contractions of these organs (Figs. 2 and 3).

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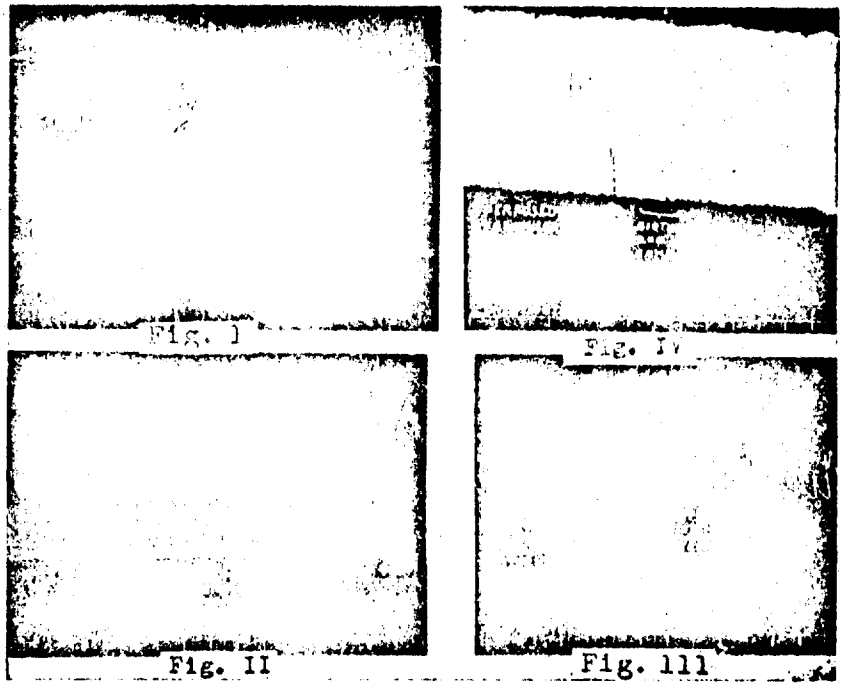

The effect of biotin on the frog heart was studied by perfusing this organ *in situ* through a Greene cannula tied into the vena cava. To each of 3 preparations, 200 μg (0.4 cc) of biotin was introduced through the standpipe of the cannula and washed in by the Ringer perfusate. In no case was there significant alteration in amplitude, rate or rhythm (Fig. 4).

Summary. Biotin failed to produce any changes in the following physiological functions: blood pressure, heart rate and respiration of anesthetized cats following rapid intravenous injection of 250 μg per kilo; excised guinea pig and rabbit uterus and rabbit intestine suspended in concentrations up to 1:40,000; and the frog heart perfused *in situ* with 200 μg of biotin.

Spencer-'64
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Amer. Jour. Physiol. 206(3):653-657. 1964.

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Biotin transport by small intestine of rat, hamster, and other species[1, 2]

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SPENCER, RICHARD P., AND KENNETH R. BRODY. *Biotin transport by small intestine of rat, hamster, and other species.* Am. J. Physiol. 206(3): 653-657. 1964.—At a concentration of 1×10^{-6} M, biotin-H^3 and biotin-C^{14} were transported against a concentration gradient by hamster everted intestinal sacs. Similar transport was shown by the small intestine of the white mouse, Mongolian gerbil, chinchilla, flying squirrel, round-tail squirrel, Malayan black squirrel, and chipmunk (and was maximal in the chipmunk intestine). Transport under these conditions was not shown by the small intestine of the rat, rabbit, guinea pig, ferret, or carp. There does not appear to be a correlation between the need for biotin as a vitamin in a species and the presence of this transport system. Chromatography of the mucosal and serosal solutions did not reveal any radioactive spot other than the original biotin-H^3 or biotin-C^{14}. This evidence, plus the inhibition of transport by avidin, suggests that it is biotin that is being transported, and not a metabolite. The unidirectional flux of biotin (from a side containing the vitamin to a side which did not), was uninfluenced by the addition of avidin to the buffer into which diffusion was occurring. The "apparent K_m" for biotin transport was on the order of 6×10^{-5} M, based on equilibrium measurements. Transport of biotin in the hamster small intestine occurred principally in the first one-third to one-half of the small intestine. Amino acids, urea, biotin-*d*-sulfoxide, as well as biotin-*l*-sulfoxide did not inhibit biotin transport. Biocytin, biotin methyl ester, diaminobiotin, desthiobiotin, thioctic acid, and two other analogues inhibited biotin transport.

intestinal transport Mongolian gerbil chipmunk mouse guinea pig rabbit squirrel chinchilla carp ferret vitamin transport species specificity and biotin transport biotin analogues and transport amino acids and biotin transport

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BECAUSE OF VARIATIONS in the kinds and quantities of vitamins required, it cannot be assumed that the transport of a vitamin by one species will necessarily be identical to its transport by a different species. We wish to point out a distinct example of this, biotin transport. While biotin has been reported not to be transported against a concentration gradient by the small intestine of the rat (9), a finding which we have confirmed, we have noted such transport by the hamster gut in vitro. This appears to be the first reported instance of the accumulative transintestinal transport of a vitamin by the hamster, and it has encouraged us to make a species-to-species comparison of biotin transport.

Received for publication 5 September 1963.
[1] This study was supported by Public Health Service Grants AM 07960 and CA 6519.
[2] A preliminary report of a portion of this work has been published (*Federation Proc.* 22: 227, 1963).

METHODS

Adult golden hamsters (Manor Farms) were allowed laboratory chow and water until the time of sacrifice. The small intestine was removed and everted, and three to six sacs made from the entire length (10). Essentially the same procedures were employed with the small intestine from other species, except that with the rat, rabbit, chinchilla, ferret, and carp, 8–12 or more sacs were made from each small intestine. Each reported result represents determinations on at least two animals (except in the case of studies on one female carp). Sacs were washed in pH 7.4 Krebs-Henseleit buffer (Na^+ 148 mEq/liter, K^+ 15, Cl^- 135, HCO_3^- 26, $H_2PO_4^-$ 2).

When three sacs were prepared from the hamster intestine, the internal (serosal) volume used was 1.0 ml; this was reduced to 0.5 ml when six sacs were made. It was only possible to place 0.5 ml within two or three sacs from the small intestine of the mouse. In all other cases the serosal volume was 1.0 ml and the mucosal volume was always 5.0 ml. For unidirectional flux studies, biotin in buffer was placed on one side of the sac, and buffer on the other side. For studies of transport against a concentration gradient, the initial condition was the identical concentration of biotin in both the mucosal and serosal solutions. The incubating vessels were 25-ml Erlenmeyer flasks which were stoppered after gassing with 95% O_2 + 5% CO_2. Incubation was carried out in an oscillating water bath at 37 C for 1 hr. After incubation, sacs were drained, the mucosal and serosal fluids centrifuged to remove sloughed tissues, and assayed.

Radioactively labeled biotin was employed, and as-

TABLE 1. *Results of species testing for biotin transport*

Species	Intestinal Transport of Biotin (1×10^{-5} M) Against a Concentration Gradient?
Hamster	Yes
White mouse	Yes
Mongolian gerbil	Yes
Flying squirrel	Yes
Malayan black squirrel	Yes
Round squirrel	Yes
Chipmunk	Yes
Rat	No
Rabbit	No
Guinea pig	No
Ferret	No
Carp (female)	No

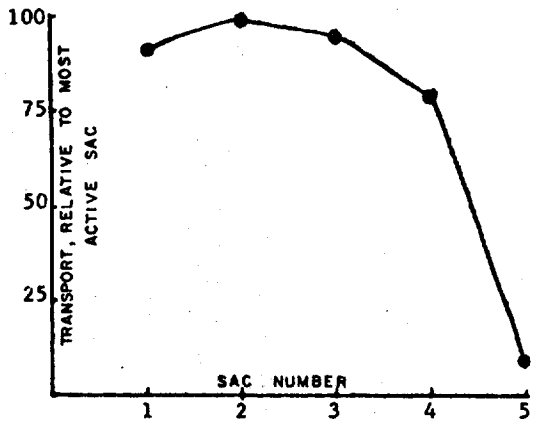


FIG. 1. Spatial distribution of biotin transport by the hamster small intestine in vitro.

sayed by liquid scintillation counting.[3] Initial screening for transport against a concentration gradient in the hamster was performed with all four radioactively labeled biotin preparations with essentially identical results (except that the Nuclear-Chicago preparation was transported less readily as the material stood at refrigerator temperatures; there is some evidence that this may have been due to formation of biotin sulfoxide). Subsequent studies on the concentration dependence of biotin transport, and its behavior in intestinal sacs from other species, were performed using the New England Nuclear Co. preparation of biotin-carbonyl-C^{14}, with occasional checks through use of the tritiated vitamin.

Chromatography of the preparations was carried out in the ascending direction on Whatman no. 1 paper, utilizing butanol:acetic acid:water (4:1:1) as developing solvent. Authentic stable biotin was detected on the chromatograms by its production of a yellow color when sprayed with dilute $KMnO_4$. Peaks of radioactivity were determined by use of a radiochromatogram scanner and recorder. Biotin migrated (R_f = 0.87) as a single spot, as did the fresh radioactively labeled preparations. Radioactively labeled biotin could be eluted from the paper by the Krebs-Henseleit buffer, and this eluted material was transported by intestinal sacs from the hamster. However, if the biotin was allowed to remain on the paper for more than 24 hr, results became erratic, again suggesting oxidation.

A female carp was obtained from the Union City fish hatcheries, through the courtesy of T. Sorensen. The Mongolian gerbils were kindly supplied by Dr. Rupert E. Billingham of the Wistar Institute. Other animals were obtained from commercial suppliers. Sources of some of the compounds employed were as follows: Diaminobiotin was synthesized by the method of Hoffman and co-workers (3). Biotin-*d*-sulfoxide and biotin-*l*-sulfoxide were synthesized according to Melville (7), and checked by both melting points and optical rotation. Biotin methyl ester and biocytin were kindly supplied by Merck Sharp & Dohme Research Laboratories. Samples of 2-carbonyl-3-carboxy-anilide-imid-

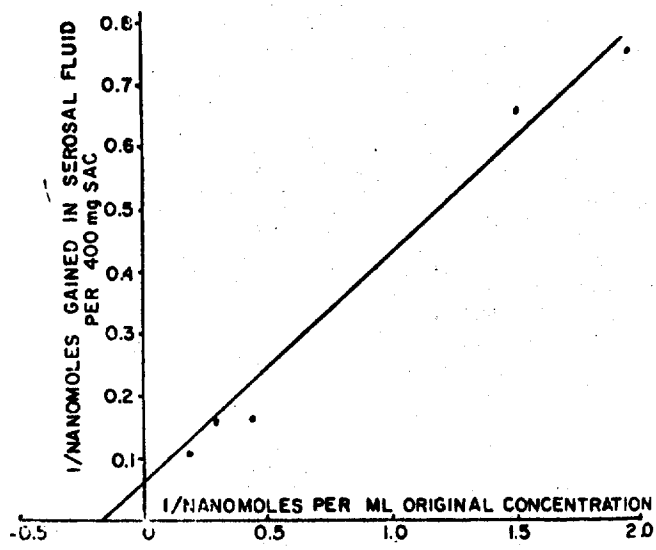


FIG. 2. Lineweaver-Burk type plot of biotin transport by hamster everted intestinal sacs.

TABLE 2

	Diffusion: Into buffer	Diffusion: Into buffer plus avidin
Biotin in mucosal fluid	4.7	4.1
Biotin in serosal fluid	2.0	2.3

"Unidirectional" biotin fluxes were determined by having biotin-carbonyl-C^{14} at a concentration of 5×10^{-5} M on one side of the everted intestinal sacs, and buffer (or buffer plus avidin) on the other side. The avidin concentration was 1 U/ml. Twelve sacs were used for each determination. The standard deviations were about 8% of the mean values reported here. Results are expressed as nanomoles disappearing from the side originally containing the biotin per 400 mg tissue weight.

[3] The sources of the compounds were as follows:

Biotin-carbonyl-C^{14}	New England Nuclear Co.	Sp. act. 3.5 μc/μM
Biotin-carbonyl-C^{14}	Nuclear-Chicago Co.	Sp. act. 2.7 μc/μM
Biotin-carboxyl-C^{14}	Hoffmann-La Roche	Sp. act. 23 μc/μM
Biotin-H^3	Isotopes Specialties Co.	Sp. act. 90 μc/μM

TABLE 3. *Compounds which, at a concentration of 1×10^{-3} M, did not inhibit the transintestinal transport of 1×10^{-5} M biotin*

Biotin analogues
- Thiolutin*
- Aureothricin*
- Biotin-*d*-sulfoxide
- Biotin-*l*-sulfoxide
- Thiophene-2-carboxylic acid

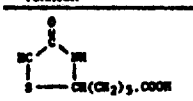
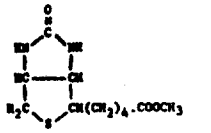
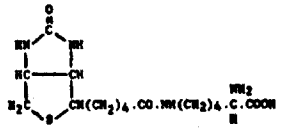
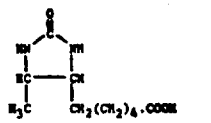
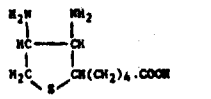
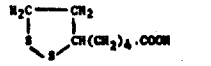
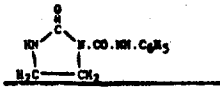
Urea analogues
- Urea
- Thiourea
- Uracil
- Alloxan
- Thymine
- 2-carbonyl-3-carbomethoxy-imidazolidine

Amino acids
- Glycine
- L-Tryptophan
- L-Lysine

* Present at maximum solubility, about 10^{-4} M.

azolidine and 2-carbonyl-3-carbomethoxy-imidazolidine were synthesized by Dr. Howard Schaefer and coworkers at State University of New York at Buffalo, and supplied by these investigators. Aureothricin, actithiazic acid, and Thiolutin were kindly made available by Dr. W. D. Celmer of Chas. Pfizer & Co. D,L-Thioctic acid (California Corp. Biochemical Research) and avidin (Nutritional Biochemical Co., 2.5 U/mg) were used without further purification.

TABLE 4. *Compounds which inhibited biotin transport*

FORMULA	NAME OF COMPOUND USED AT 10^{-3} M	PERCENT INHIBITION OF 10^{-5} M BIOTIN TRANSPORT
	ACTITHIAZIC ACID	100
	BIOTIN METHYL ESTER	100
	BIOCYTIN	100
	DESTHIOBIOTIN	100
	DIAMINOBIOTIN	83
	THIOCTIC ACID	100
	2-CARBONYL-3-CARBOXYANILIDE-IMIDAZOLIDINE	30

RESULTS

At a concentration of 1×10^{-5} M in the pH 7.4 Krebs-Henseleit buffer, biotin transport against a concentration gradient was shown by the hamster small intestine and that from several other rodents (but not by all rodents, and not by the small intestine of the ferret or carp (Table 1)). This qualitative result was unaltered in the rat upon using higher (up to 10^{-3} M) or lower (10^{-6} M) concentrations of biotin. Figure 1 shows that transport against a concentration gradient occurred primarily in the first two-thirds of the hamster intestine. With the chinchilla, two animals showed transport by the first half of the small intestine, but not the last half, and two others showed transport in only the first sac (out of 12). One round-tail squirrel had transport in the first sac (out of 5), while a second round-tail squirrel showed transport in the first two sacs. While the hamster small gut achieved an inside-to-outside concentration ratio of 1.4, in the mouse it was nearly 3, and in the chipmunk intestine it was almost 5.

From the quantity of biotin disappearing from the mucosal and serosal solutions, and assuming that the intestinal sacs were 80% water, it was possible to calculate the concentration of biotin per milliliter of gut water. In all cases, using the hamster, this was a value on the order of the concentration found in the mucosal solution. In other words, transintestinal accumulative transport was not accompanied by significant accumulation within the tissue. Hence, an "apparent Michaelis constant" could not be measured by tissue accumulation, but only by equilibrium data on the net amount transported by the sacs. Experiments were performed at various biotin concentrations, and the reciprocal of the net amount gained in the serosal solution per 400-mg sac plotted as a function of the reciprocal of the concentration (Fig. 2). The points only approximate a straight line, with an "apparent K_m" of 6×10^{-5} M.

Chromatography of mucosal and serosal solutions after transport revealed only a single spot, which corresponded to the biotin standard. Biotin (1×10^{-5} M) transport by the hamster small intestine was completely inhibited by avidin (1 U/ml). The avidin addition, moreover, caused quenching of the biotin-C^{14} or biotin-H^3 in the liquid-scintillation counter. Studies on biotin diffusion from one side of the gut sacs into buffer with avidin on the other side could not be interpreted in terms of biotin gained in the fluid containing the avidin because of the quenching. Hence, such experiments are tabulated in terms of the biotin lost from the original side (Table 2). At a concentration of 5×10^{-5} M, diffusion of biotin across the small intestine into buffer was not augmented by the addition of avidin (1 U/ml) to the buffer.

Several compounds were tested (at 10^{-3} M) for their effect on the transport of 1×10^{-5} M biotin by hamster everted intestinal sacs. A number of biotin analogues, urea analogues, and amino acids were without effect

(Table 3). Seven compounds which bore a structural resemblance to biotin did inhibit biotin transport by the hamster small intestine (Table 4). Efforts were made to determine whether the inhibition was classically competitive or noncompetitive by having the inhibitor at a given concentration and varying the concentration of the substrate. Results were not clear-cut, and are presented below in the discussion.

DISCUSSION

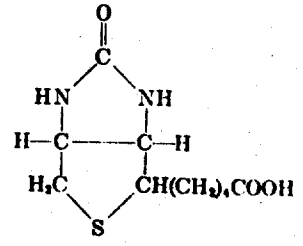
Transport of biotin against a concentration gradient in vitro by some species, but not others, raises problems as to comparative aspects of biologic transport. It would appear that biotin transport has no taxonomic or ecologic significance at present. One could question whether such transport was related to the need for biotin by the species. The answer to this appears to be negative. That is, biotin is required in the diet of the hamster (2), rat (1), rabbit (1), monkey (1), and dog (6), and other species, yet we have been unable to demonstrate the biotin transport system in the intestine of the rat or rabbit. There is of course the possibility that the transport system will be demonstrated under different conditions, but it would then certainly differ as to gross properties from the system studied here. The spatial distribution of the transport system is of note, since it is just opposite to that of vitamin B_{12} (which is maximal in the lower one-third of the small intestine in most species).

The compound undergoing transport appears to be biotin and not a degradation product. The evidence for this is the appearance of but a single spot on chromatographic examination of the final mucosal and serosal solutions of biotin-H^3 or biotin-C^{14}, and the inhibition of transport by avidin. In addition, the product most likely to be formed, biotin-sulfoxide, did not inhibit biotin transport (suggesting that it, itself, was not transported). With the tritiated compound we need not be concerned with possible accumulation of radiolabeled CO_2. In experiments with biotin-C^{14}, to rule out the possibility of $C^{14}O_2$ accumulation in the serosal fluid, we added glacial acetic acid to several of the samples. There was no change in the quantity of radioactivity in the samples (aside from the dilution factor).

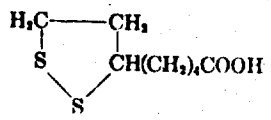
Whether biotin movement represents active transport (against an electrochemical potential gradient) could be approached by short circuiting the membrane and then following transport (to rule out movement of the negatively charged molecule down an electrical gradient). We have made several measurements of the electrical potential difference across the hamster small intestine in vitro, in the absence of glucose. These have revealed values of only 1 millivolt or less (mucosa negative). Hence, the electrical contribution to possible movement is negligible. In addition, we have looked at the behavior of several other negatively charged molecules in the everted gut sacs. At a concentration of 1×10^{-5} M, there was no transport against a concentration gradient of phenylacetic acid-C^{14}, orotic acid-C^{14}, or ascorbic acid-C^{14}. Movement of biotin therefore suggests a specialized system. Failure of three amino acids at high concentrations to interfere with biotin movement perhaps indicates that the amino acid transport system is distinct from that involved in biotin transport.

The seven compounds listed in Table 4 did interfere with biotin transport. Initial studies designed to elucidate the mechanism have not been successful. Some of the reasons, we believe, may be as follows:

a) Thioctic acid. The structural similarity between biotin and thioctic acid prompts thoughts of competitive inhibition. This would seem to have interesting possibilities, since thioctic acid is the cofactor in pyruvate decarboxylation, and biotin is the cofactor in certain carboxylation reactions.



Biotin



Thioctic Acid

However, thioctic acid, at 10^{-4} M, had little effect on the transport of biotin at 10^{-5} M. When the thioctate concentration was 1×10^{-3} M, there was 100% inhibition of biotin transport, which was not overcome by raising the biotin concentration to 10^{-3} M. This suggests that thioctic acid at high concentrations may be altering one or more intestinal mechanisms. It is of interest that Spangenberg and Eakin (8) reported that thioctic (lipoic) acid inhibited the regeneration of *Chlorohydra viridissima*. The gastrodermal cells were sensitive to the compound, and the effect was observed at a concentration of 0.24×10^{-3} M (as compared to the 1×10^{-3} M thioctic acid employed in the present study).

b) Biocytin and biotin methyl ester. The presence of an enzyme which cleaves biocytin (ε-N-biotinyl-L-lysine) to biotin and L-lysine has been recognized for some time, and Wright has summarized some of the literature (11). This enzyme, initially termed biocytinase, was found to be present in human blood. More recently, Koivusalo and co-workers have reported an enzyme, named biotinidase, in bacterial extracts, in rat intestinal homogenates (4), and in the small intestine of the guinea pig and rabbit (5). Biotinidase was capable of cleaving a number of biotin derivatives, including biocytin and biotin methyl ester. It is quite possible that at the high

concentrations employed here (1×10^{-3} M), significant amounts of biocytin and biotin methyl ester were cleaved by the hamster intestine to biotin, thus "swamping" the system with biotin (the radioactive tag would be diluted into a large pool of stable biotin, and a minute amount of transport in the presence of this large quantity could not be detected). We have tried experiments to determine whether transport of radiolabeled biotin could be observed in the presence of 1×10^{-3} M carrier biotin. As suspected, no net gain in the serosal fluid could be shown.

The mode of action of the other biotin analogues in causing inhibition of biotin transport is under investigation, and may offer clues as to the nature of the transport system.

We are grateful to Prof. O. Wiss of Hoffmann-La Roche for making the carboxyl-C^{14} compound available for our use.

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Steigerwald-'61
Bib No. 1299

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On the influence of Biotin upon the intermediate metabolism

It has been known for some years that Biotin, as an effect group of various enzymes in the intermediate metabolism of carbohydrate and fat, has a significance in carboxylation and de-carboxylation processes. Wakil *et al.*[1] have shown that the synthesis of fatty acids is produced from acetate or acetic aldehyde by an enzyme system containing Biotin. Gibson *et al.*[2] isolated from the liver an enzyme system which can synthesize palmitic acid from acetyl coenzyme A. Here too, one of the fractions of this enzyme system contained a considerable amount of protein-bound Biotin. By blocking the protein-bound Biotin the enzyme lost its ability to synthesize fat. The conclusion drawn from this was that Biotin, participates in fat synthesis. Lichstein[3] discovered that the decarboxylation of oxaloacetic acid is Biotin-dependent. Adler[4] established that the carboxylation of itaconic acid takes place only in the presence of Biotin. However, these results are based exclusively upon animal or *in vitro* experiments.

Nothing is as yet known of the influence of Biotin upon the intermediate fat and carbohydrate metabolism in humans. We have attempted, therefore, to test the influence of Biotin upon the intermediate metabolism by the determination of various metabolism by-products of the blood, In addition to determining the total lipids and

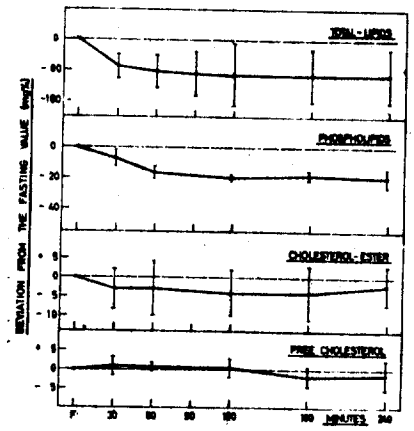

Fig. 1. Mean value curves of the metabolites taken from 15 test persons after infusion of 100 mg Biotin.

various lipid fractions pyruvic, lactic, oxalacetic and ketoglutaric acids (with the aid of Warburg's[5] optical test), were determined as an indicator of the intermediate of carbohydrate metabolism and the citric acid cycle. We further examined the acetone bodies by the method of Engfeldt[6] modified by us, as well as the unesterified fatty acids as metabolites of fat metabolism after Dole[7]. A total of 55 subjects in various age groups with basal metabolism conditions were examined. They received

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either 10 mg Biotin in injection form, or 100 mg in infusion form within a period of 1 h. The blood specimens for the determinations of the various metabolic values were taken before, and at 1/2-h intervals up to 4 h after the injection. The illustrations show the results of the examinations in the form of a mean value curve, *i.e.* the

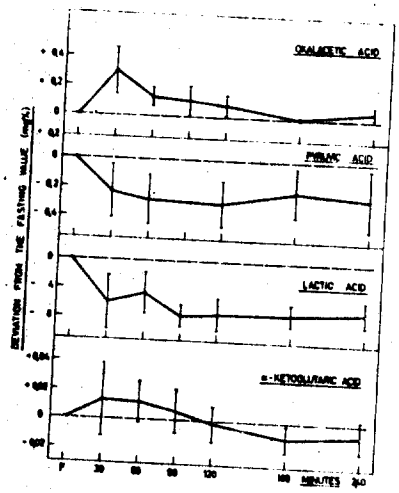

Fig. 2. Same as Fig. 1.

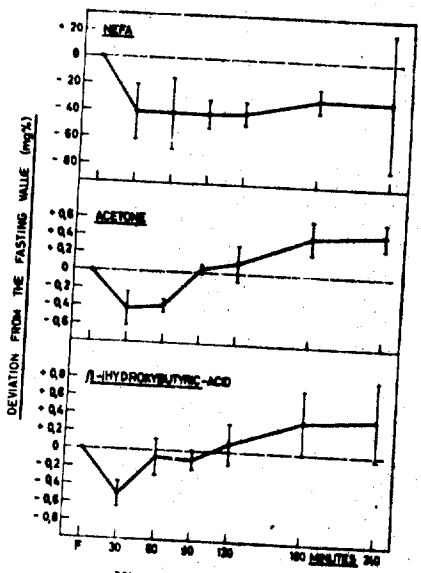

Fig. 3. Same as Fig. 1.

particular deviations from the original value. In Fig. 1, the behavior of the total lipids, phospholipids and cholesterol — both free and esterified — is represented. One can immediately see that the total lipids drop immediately, by 88 mg %. The phospholipids drop only slightly. The free and total cholesterol, however, remain unaffected. Fig. 2 shows the corresponding curves of the oxaloacetic acid, pyruvic acid, lactic acid and α-ketoglutaric acid. The oxaloacetic acid shows an initial rise, whereas pyruvic and lactic acids decline. In contrast, the α-ketoglutaric acid does not experience any significant changes. Fig. 3 shows the behavior of the free, unesterified fatty acids, as well as that of acetone and β-hydroxybutyric acid. Here the drop of the unesterified fatty acid is particularly apparent; although the acetone bodies also initially decline, they rise over their original value after a lapse of approximately 90 min. In addition to the lipids and metabolism byproducts, we also examined the oxaloacetic acid transaminase and the lactic acid dehydrogenase activity in the serum of isolated cases, in order to determine whether or not Biotin exerts an influence upon this enzyme activity. No clear-cut changes, however, could be observed. In order to determine in which way the individual fatty acids were affected in the reduction of the total lipids, gas chromatographic analyses were also carried out. In the course of these analyses, no clear-cut shifts within the fatty acid spectrum during the decrease of the blood lipids could be established. All the fatty acids are affected more or less equally by the reduction. Fig. 4 demonstrates these findings.

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In order to create an impression of the time relationships of the reactions which occur in the various metabolism products as a consequence of the Biotin effect, we prepared a coordinated diagram of the investigation results of one individual case (Fig. 5). Simultaneously with the drop of the total fats and unesterified fatty acids,

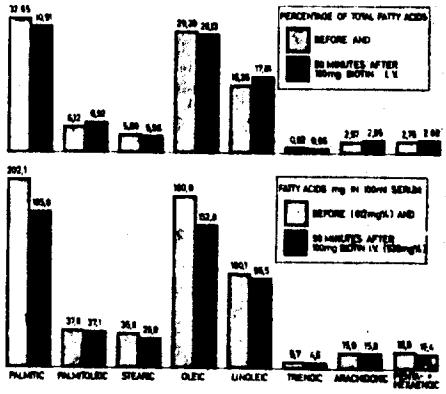

Fig. 4. Effect of Biotin on the fatty acid contents of serum.

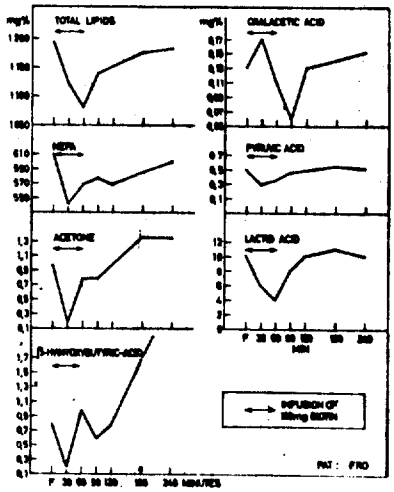

Fig. 5. Behaviour of the metabolic intermediates following infusion of 100 mg Biotin in a single case.

the acetone bodies also initially drop. Oxaloacetic acid shows a rise here, whereas pyruvic acid and lactic acid decline. Hence, two metabolic effects can be observed. The effect upon fat metabolism goes hand-in-hand with a reduction in the unesterified fatty acids and the total lipids, whereby the glycerides are affected above all. The other metabolism effect concerns the end-products of carbohydrate catabolism and is marked by an increase of oxaloacetic acid and a decline of pyruvic and lactic acid.

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The decline of the lipids and of the free fatty acids may have two causes. This decline can be due simply to a wandering off of the unesterified fatty acids and the glycerides from the serum into the depots. The behavior of the metabolism by-products of the serum, however, permits another interpretation. Let us first observe the end-products of glycolysis. It may be presumed here that under the influence of Biotin oxaloacetic acid formation from pyruvic and lactic acid is increased. With this more raw material may be available for the condensation reaction of oxaloacetic acid – acetyl coenzyme A – citric acid, *i.e.* more acetyl components of the end oxidation may possibly be introduced. This actually appears to be the case, since a relatively rapid drop in the acetone bodies takes place after Biotin application. The re-ascent of the acetone bodies, in conjunction with the reduction of the oxaloacetic acid in the serum after a lapse of 60 to 90 min, indicates that immediately after this period the increased production of oxaloacetic acid declines. Correspondingly, not as many acetate components of the end oxidation in the citric acid cycle can now be introduced. The acetone content of the blood again increases and even passes the initial value considerably. The extremely high increase of acetone bodies can easily be assumed to be an expression of the intensified β-oxidation. The equilibrium between oxaloacetic acid and C_2-components thus appears to be shifted in the first phase, in favor of the oxaloacetic acid. This results in the drop of the acetone bodies. In the second phase it is reversed; the C_2-components, which can condense to acetone bodies, now dominate. As has already been mentioned, the rise of the oxaloacetic acid is a possible consequence of the Biotin effect. The behavior of the pyruvic acid speaks for this assumption. The *in vitro* experiments have shown that Biotin exerts an influence upon carboxylation and decarboxylation processes. It may be conjectured that pyruvic acid was carboxylated to oxaloacetic acid.

As has been established by our experiments, Biotin appears in this way to have assumed two points of attack in metabolism activity: it stimulates the production of oxaloacetic acid from pyruvic acid or lactic acid, and secondly, it increases the oxidation of fat. From observations of the lipids and metabolic by-products of the blood, the conclusion may be drawn that Biotin exerts a catabolic metabolism effect.

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(From the 1st Children's Clinic, Prague.)

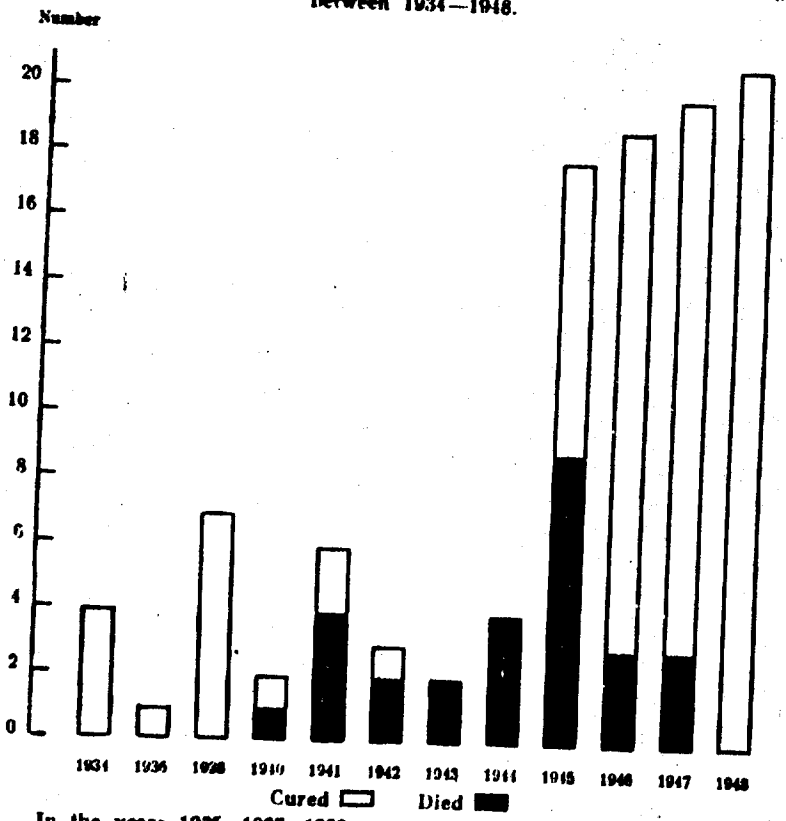
Experimental Experiences with Biotin in Babies.

By J. SVEJCAR and J. HOMOLKA.

In our previous report (1) we showed that the number of cases of erythrodermia desquamativa Leiner rose here in a remarkable manner (Table I) from the end of the second World War, i.e. from 1945. This is assuming we judge the number of cases oc-

TABLE I.

Children suffering from M. Leiner-Moussous at the I. Children's Clinic Prague between 1934—1948.



In the years 1935, 1937, 1939 no case of M.L.-M. at the clinic. From 1946 penicillin therapy, from 1947 penicillin and biotin therapy.

curring in this country according to the number accepted for treatment at the 1st Children's Clinic in Prague. Even if these findings do not give an accurate picture of the incidence of the disease, they enable us to appreciate a rise in its occurrence as, because of the seriousness of the illness, the majority of children are sent to our hospital and accepted. A similar picture was observed after the first World War. The mortality rate of those children, at first very high, fell after the introduction of penicillin therapy, but that therapy did not alter the very long and serious course of the disease, which was materially influenced only by Biotin therapy, which shortens the illness sometimes to a few days. The mortality rate of the disease was also lessened by this therapy, so that in 1948 not one case died. We concluded from this that penicillin acts on secondary infections, especially staphylococcal, which usually cause the death of the affected children, but does not influence the illness itself. The decided action of Biotin leads us to confirm the assumption of *György*, that Biotin is the deciding factor in the etiology of seborrhoic dermatitis in infants, especially in its most severe clinical form, M. Leiner. In view of the fact that the children afflicted by this disease are mostly breastfed infants or at least babies who are nearly wholly breastfed we expressed the opinion that the frequency of the disease in our country is caused by a low content of Biotin in the mother's milk. This lack can be explained by the imperfect nutrition of our women after the War; especially lack of foods rich in Biotin (chocolate, beans, peas, lentils, liver, kidneys).

To confirm the conclusions of our clinical observations, we investigated the Biotin content of the milk of nursing mothers as well as in the urine and blood of children with M. Leiner and of children with other forms of infant dermatitis. During the past 8 months we made 240 analyses of which 30% were estimations of the content of known solutions and of analytically indeterminable findings so that this paper is based on the results of a total of 154 determinations.

Some remarks on the chemistry and physiology of Biotin.

Biotin was first isolated in a pure form by *Kögl* in 1936 from egg yolk. *Du Vigneaud* established the correct structural formula of β-Biotin which is considered to be the natural Biotin. It is 2-keto-3.4-imidazolido-2-tetrahydrothiophen-n-valeric acid.

It has 3 asymmetrical carbon atoms and these give the possibility of 8 optically active forms. The laevo-rotary forms are biologically inactive, the natural Biotin being dextro-rotary. Apart from that there are other forms which have a similar structure and are less active, some inactive. In our investigation the synthetic form made by Hoffmann-La Roche, named d-Biotin, was used.

In the body Biotin is decomposed with loss of sulphur into desthio-Biotin, diaminopelargonic acid and pimelic acid which is also the basic substance for the synthesis of Biotin by some micro-organisms, of course only in the presence of a donator of sulphur such as cystin. Pimelic acid was found during the oxidation of higher fatty acids and fats. Lack of Biotin in rats causes simultaneously lack of folic acid and these two substances are necessary in rats fed on a diet containing succinylsulfathiazol for the formation of pantothenic acid. To cause experimentally a lack of Biotin in animals it is necessary to give disinfectants of the intestinal tract so as to eliminate as far as possible the intestinal flora synthesizing Biotin and other vitamins of the B-complex. For the purpose in hand streptomycin is most suitable as, according to its function, it is a co-ferment such as vitamin B_1, B_2 and B_6 and nicotinamide. The connection of Biotin with avidin contained in the white of eggs, is interesting. This protein has already been produced in the form of crystals. The stability is unusual and is neither disturbed by the proteolytic ferments of the intestinal tract, nor by incubation in the liver, blood, muscles and kidneys, is however destroyed by experimental oxidation with 0.45% of H_2O_2. For details we recommend *Bourquin*'s paper (2).

Our method of determination of Biotin.

The determination of Biotin in minute amounts is only possible by biological methods, either by experiments on rats, on chicken or by observing the growth of micro-organisms in artificial surroundings which contain all the substances necessary for their growth except Biotin. By adding material containing Biotin a growth of the micro-organisms is obtained which is recorded as a turbidity or else we estimate by titration the amount of acids freed from the substance. We used the method of estimating the turbidity which appeared during the growth of a chosen strain of Saccharomyces cerevisiae and have modified the method in some details.

Nutrient fluid (3):

500 c.c. of the solution in redistilled water contain:

glucose	20	gm.	$MgSO_4 \cdot 7H_2O$	0.25	gm.
m inosit	5	mgr.	$CaCl_2 \cdot 2H_2O$	0.25	gm.
β-alanine	0.5	mgr.	H_3BO_3	1	mgr.
vitamin B_1	20 γ		$ZnSO_4 \cdot 7H_2O$	1	mgr.
vitamin B_6	20 γ		$MnCl_2 \cdot 4H_2O$	1	mgr.
l(+)-aspartic acid	100	mgr.	$FeCl_3 \cdot 6H_2O$	0.5	mgr.
$(NH_4)_2SO_4$	3	gm.	$CuSO_4 \cdot 5H_2O$	0.1	mgr.
KH_2PO_4	2	gm.	KJ	0.1	mgr.

the pH of the solution is adjusted by NaOH or hydrochloric acid to 4.5-5.0.

The micro organism used was a selected strain of Saccharomyces cerevisiae, sent to us by Hoffmann-La Roche. The strain was maintained by weekly inoculation on Langeron's oblique agar at a temperature of 26-28° C. The suspension is made by mixing 1 platinum loopful of the culture in 60 c.c. of sterile physiologicel solution (normal saline).

Material: We dilute the *milk* 1 : 100.

Urine is diluted 1 : 20, 1 : 50, 1 : 100.

Blood: 2 c.c. of blood put into 6 c.c. of water and this haemolysed fluid is added to a mixture of 2 c.c. n/10 NaOH and 10 c.c. 0.45% $ZnSO_4 \cdot 7H_2O$ heated untill the first bubbles appear, allowed to cool and filtered into a graduated vessel; the precipitate is washed through quantitatively until we obtain 20 c.c. of the filtrate.

d-Biotin: in ampoules containing 25 γ per 1 c.c., a synthetic product of Hoffmann-La Roche.

Glass: is cleaned by boiling in dilute hydrochloric acid, then distilled water and sterilized by dry heat.

Dilution: in Erlenmeyer's vessels (capacity 50 c.c.) solutions containing in 5 c.c. of water 0.0 0.00005 0.0001 0.0002 0.0003 0.0004 0.0006 0.0008 0.0016 0.0032 γ d-Biotin in redistilled water.

In the same vessels 0.0 0.2 0.4 0.6 0.8 1.0 2.0 3.0 4.0 5.0 c.c. of dilute material and we refill with distilled water up to 5 c.c. With blood, concentrations of 0.2-0.8 are often below the level of sensitivity of the method. It is not practical to prepare a concentration in blood lower than 1/10.

Method: Into all the solutions we add each time 5 c.c. of the

nutrient fluid (its concentration is as said above double than would be necessary for the optimal growth of micro-organisms) and after stirring we remove with clean pipettes twice 2 c.c. from all vessels into test-tubes provided with metal caps. The best size of test-tubes for the optimal growth of micro-organisms is a tube where 2 c.c. of the solution form a column of fluid 1 cm. high. Thereafter sterilization is carried out for 15 minutes at a temperature of 100-105° C. and the solution is allowed to cool and 2 drops of the suspension of Saccharomyces are added to each test-tube. The test-tube is maintained at a temperature of 26-28° in the thermostat. The turbidity is measured in Zeiss' nephelometer in an optically homogenic test-tube 10 / 75 mm. If the turbidities are too strong, the fluid is diluted by 0.0025% chlorthymol.

As far as the results are concerned (we always make 2 tests), we take the average. If too much variation is obtained in the 2 values, these figures are not used. From the values of the blind tests (from vessels without Biotin and without material) we also take the average and put this figure as equal to 100%. The remaining turbidities are transformed into percentages of the blind tests and from these percentages we draw a diagram. We also convince ourselves of the regularity of growth. By comparing equal numbers of known and unknown solutions we are able to determine the concentration of the unknown Biotin solutions. In this case, if we use low or high equal numbers, the results ought to agree and if this is not so, the result should be considered as inexact or discarded. The results are expressed in γ of Biotin contained in 100 c.c. of the original material, that is in γ-percents.

Apart from the examination of blood and urine we examined whole blood which we prepared in the way mentioned above. The lowest dilution of blood which could be used was 1 : 10 and in cases of very low content of Biotin in the blood we reached the lower limit of sensitivity of our method. In spite of that the examination of blood yielded new and valuable information.

As stated in the literature, some fatty acids (4) and lecithin (5) of the plasma influence the growth of Lactobac. arabinosus and Lactobac. casei which can both be used for the estimation of Biotin. In our method, however, Saccharomyces cerevisiae was used; we tried to eliminate those known and some unknown factors from the analysed blood by heating it as mentioned previously. Because after the application of Biotin either by mouth

or intramuscularly the growth always increased, it is unthinkable that the growth of our different micro-organisms was caused by anything different in those cases than Biotin itself and our figures are at least of value for comparison purposes.

The estimation of normal values of Biotin in the milk, urine and blood.

Normal values (Table II) depend on the method used. Therefore we can best compare them with the results of the microbiological laboratories of Hoffmann-La Roche (6), because our

TABLE II.

Normal values of Biotin in children in γ %.

	Mother's milk		Blood	Urine
	3.9		0.04	1.0
	4.0		0.03	0.3
	0.8		0.07	3.0
	2.0		0.06	1.5
	1.5		0.03	1.8
	3.0		0.02	
	0.8	entirely breastfed	0.04	
	1.6			
	1.0			
	1.0	entirely breastfed		
	1.2			
	0.8			
	0.4	entirely breastfed		
	2.0			
	2.3			
	2.5			
Average:	1.8		0.04	1.5

technique is practically the same. In milk, values below 2.0 γ% are considered by them as distinctly low. We found in the milk of mothers whose children did not suffer from any skin disease an average of 1.8 γ% out of 16 values; 4 of them were lower than 1 γ%, always determined in the milk up to 1 month after birth. There we had already found a certain diminution from normal values. According to the findings of the American literature (7) mother's milk contains 0.8 γ% of Biotin and cow's milk 3 γ%. Dried buttermilk made from Eledon contains according to our findings 5 γ% of Biotin and ¾ of dried milk 5.6 γ% of Biotin. It is obvious from this that we obtain higher figures than the above-mentioned authors; relatively, however, they agree well with the

American results. It is stated (8) that the first lot of mother's milk and colostrum are especially poor and contain 0.13-0.38 γ%. A definite level is established between the 2nd and 3rd month. With that our findings agree except that a high level of Biotin in mother's milk may appear also soon after birth. We found on the 9th-14th day after birth in 3 different mothers values of 3.0 3.9 4.0 γ%. The influence of food changes on the content of Biotin in mother's milk is very quick and marked. We gave 2 mothers during 3 days 10 mgr. of Biotinol in tablet form (it is an alcohol from which Biotin is formed by oxidation), and the Biotin level in the milk rose from 1.1 and 1.3 to 22.0 and 40.0 γ%. One mother was given pure Biotin in tablets, 6 mgr. daily for 7 days, and the level in the milk rose from 1.0 to 49.0 and after a week still remained 1.7 γ% of Biotin. The values of Biotin in the urine are not constant. In children without changes of the skin we found an average of 1.5 γ% out of 5 estimations. Messrs. Hoffmann-La Roche say the normal figures for adults to be 1.5-7.0 γ%. Considering that Biotin is eliminated chiefly in the urine (9), an increased amount in the urine points to its removal from the organism. Biotin has not been estimated in the whole blood of children as far as we know. From the literature it is known (6) that the human serum contains 1.0 γ%. We found in prepared whole blood of children without changes of the skin a figure of 0.04 γ% as an average from 7 estimations. The normal figures in the blood which are at our disposal are figures from convalescent children (0.03 γ% in a patient with congenital heart disease, after pneumonia, who died later on in hospital, and in a patient after enterocolitis, 0.02 in a patient with pleuritis, rhinopharyngitis, perihilous infiltration and otitis). It can be assumed that the figures of really perfectly normal children which we never had at our disposal are a little higher.

Investigation of Biotin in children with M. Leiner entirely breastfed (Table III).

The amount of Biotin in the milk of mothers feeding babies afflicted by Leiner's disease is estimated in 10 cases. The average of the amounts found is 0.88 γ% and lower than the average of 1.8 γ% found in the milk of mothers feeding normal babies. In 7 cases the amount was lower than the highest amount in the milk of mothers feeding babies with M. Leiner, i.e. 1.3 γ%, but occurred only in 3 cases of children entirely breastfed and in their

TABLE III.

Investigation of Biotin in children with Leiner's disease, entirely breastfed (in γ %).

Mothers milk	Baby's blood	Baby's Urine	Complication
0.8	—	0.3	—
0.1	—	0.2	—
0.7	0.02	0.4	enterocolitis
1.0	0.03	0.6	—
0.7	0.02	0.5	—
1.1	0.01	0.3	pemphigus, dystrophia
1.0	0.01	0.5	otitis dext.
1.0	0.01	0.2	enterocolitis
1.1	0.01	0.8	praematuritas
1.3	0.03	0.9	otitis bilat.
Average: 0.88	0.017	0.47	

first month. We do not know the further fate of these children, but in children with "allaitement mixte" the intake of Biotin was guaranteed by the cow's milk. In the rest it is possible that the amount of Biotin in the milk increased or that they had additional artificial food later on. On the other hand one has to consider that all the babies sick with Leiner's disease whose mothers had larger amounts of Biotin in their milk were at the same time ill with diarrhoea or had some parenteral infection and even 1 case was a premature baby which certainly influenced the losses or the need of Biotin.

The amount of Biotin found in the blood of children with Leiner's disease is on average lower than in normal children as well as the amounts found in the urine. In 7 cases those figures were followed up during the therapy with Biotin (Table IV). Clinically that therapy was also in these 7 cases fully successful just the same as in the 9 cases described in the previous paper and in 10 cases observed after that time (on the whole in 26 cases cured by Biotin). Biotin was injected at a dose of 0.1 mgr. per day, some mothers were given it by mouth, the amount being 4.0 mgr. a day. Amelioration was observed immediately after the start of the therapy within 2-3 days and a complete cure within 7-14 days. Apart from this therapy penicillin was used to eliminate secondary infection of the skin or elsewhere; vitamin C and vitamin B-complex were given regularly. The above-mentioned dose of Biotin proved to be sufficient not only clinically, but also by its action on the Biotin level in the blood which always

rose during this therapy and on the amount of Biotin excreted by the urine. At the same time the amounts of Biotin in the blood show that amelioration and cure appears only where an increase of the level of the Biotin in the blood is taking place.

Investigations on Biotin in children with Leiner's disease, fed artificially or by "allaitement mixte" (Table V).

There is no doubt that the intake of Biotin is sufficient in the case of those children. The loss of Biotin can only be caused by its elimination from the body (micturition, defecation) or by an increased requirement of it. The therapy was followed up in 5 children and carried out in the same way as in breastfed babies. Clinically the babies became all free of symptoms, but in a longer time, and sometimes with relapses. The amount of Biotin used was on the whole larger than in breastfed babies. It is absolutely understandable that we find in these children always general diseases or diarrhoea with generalized symptoms much more severe than in the first group, because in those diseases lies the cause of the lack of Biotin in the baby. The difference in the cause of the Biotin deficit compared with the breastfed babies explains why here, contrary to the findings in breastfed babies, high values of Biotin in the urine are seen, whilst the values in the blood are normal or even higher. The amount of Biotin excreted in the urine is so high, even at the start of the treatment, that one comes to the opinion that Biotin is being eliminated by an organism which is unable to use it. Only after invading the organism with Biotin and elimination of the unfavourably acting infection the Biotin is being retained. This is indicated by at least a temporary rise of its level in the blood which is followed by the disappearance of the skin symptoms (case No. 9 is characteristic).

Investigation of Biotin in children with other kinds of dermatitis (Table VI).

This group contains 3 cases with seborrhoic dermatitis (cases 13-15), 2 cases of dermatitis clinically identical with M. Leiner which appeared after rehydration with Darrow's fluid (cases 16 and 17), 5 cases of infantile eczema (cases 18-22) and one case of keratodermia ichthyosiformis (case 23).

The first 3 cases were children with "allaitement mixte", only in the first case a low level of Biotin in the blood was found; in the 1st and 3rd case the patient got better without Biotin, in the 2nd with Biotin and quicker than in case 1 and 3. The 2 cases of

dermatitis appearing after rehydration with Darrow's fluid are interesting. The rehydration was carried out because of grave dehydration in a toxic and septic baby. In both patients the Biotin treatment raised the low level of Biotin in the blood, but in the first the level dropped and only improvement was achieved, whereas the second was completely cured. In order to explain the lack of Biotin it is important to know that both children were

TABLE IV.

Morbus Leiner in entirely breastfed babies. Biotin therapy.

Name	Age (months)	Birth weight	Weight on admission and discharge	Duration of illness before admission (weeks)	Stay at hospital (weeks)	Complications
1. M. J. ♂	1 3/4	2.900	3.900 4.300	—	3	otitism. bilat. supp. enterocolitis subac.
2. J. M. ♂	1	3.350	3.600 3.900	3	1	—
3. H. S. ♀	1 1/2	3.400	3.130 3.640	2	3 1/2	dystrophia
4. H. T. ♀	1 1/2	3.000	4.100 4.120	2	3 1/2	otitis m. bilat. — supp.
5. V. S. ♂	1 1/2	3.250	3.150 3.300	2	2	enterocol. subac.
6. V. B. ♀	2	2.550	3.770 4.020	2 1/2	2 1/2	anaemia
7. J. Z. ♂	2	3.250	2.960 3.400	6	4 1/2	otitis. m. bilat. enterocol. subac.

treated with streptomycin per os and the first also with sulfonamides. The cases remind one of the observations of *Brown* (10), where in 3-year-old children who were given sulfonamides for pneumonia erythematous dermatitis of the face developed which was morphologically identical with the changes caused by the ingestion of egg-white. It is questionable whether at the start Darrow's solution containing potassium, an ion of the intracellular metabolism, acted or not.

Therapy	Biotin in γ % Mother's milk	Blood	Urine	Biotin therapy 0.1 mgr. i.m. pro die 4.0 mgr. per os
penicillin	0.7	0.02	0.4	21. 9.
B-complex	0.8	—	0.1	24. 9.
C-vitamin	—	—	5.0	29. 9. after 5 x 0.1
	—	—	—	4. 10. cured
	—	0.05	—	8. 10.
penicillin	0.7	0.02	0.5	9. 11. blood after 1 x 0.1
B_2				urine before Biotin
C-vitamin	0.7	0.04	10.0	16. 11. after additional 5 x 0.1 cured
penicillin	1.1	0.01	0.3	23. 11.
B_1	—	0.04	0.9	1. 12. after 6x 0.1 better within 2 days
C-vitamin				
	0.9	0.04	1.3	7. 12.
				9. 12. cured
penicillin	1.0	0.01	0.5	1. 12.
B_2	1.4	0.06	6.0	7. 12. after 3 x 0.1, after additional 4 x 0.1 within 3 days greatly improved
C-vitamin				
	49.0	0.28	77.0	14. 12. worse, temperature, mother after 40 mgr. per os
	1.7	0.08	15.0	21. 12. again after 7 x 0.1 cured
penicillin	1.0	0.01	0.2	4. 1.
B_1, B_2	—	0.05	3.7	11. 1. after 6 x 0.1
C-vitamin				13. 1. cured
penicillin	1.1	0.01	0.8	4. 1.
B-complex	22.0	0.9	80.0	11. 1. after 3 x 0.1 and 3 x 4.0 cured, mother after 10 mgr. Biotinol per os
C-vitamin				
blood-transf. 3 x 70 c.c.				
penicillin	1.3	0.03	0.9	21. 12.
streptomycin	—	0.02	7.5	4. 1. after 6 x 0.1 within 5 days some improvement
sulfasux.				
blood-transf. 3 x 60 c.c.	40.0	0.8	80.0	11. 1. after additional 3 x 0.1 and 3 x 4.0 mother after 10 mgr. Biotinol per os
B-complex				15. 1. cured

TABLE V.

Morbus Leiner in children with "allaitement mixte", or artificially fed babies, Biotin-therapy.

Name	Age (months)	Birth weight	Weight on admission and discharge	Duration of illness before admission (weeks)	Stay at hospital (weeks)	Complications
8. A. D. ♀	1¹/₆	2.800	3.020 3.860	1	7	otitis m. e. dext. supp., enterocol. subac.
9. F. K. ♂	1¹/₂	3.000	3.240 3.700	2	7	enterocol. subac. dystrophia
10. B. H. ♀	2	3.500	3.640 4.300	4	3	otitis m. l. sin. dystrophia
11. P. D. ♂	2¹/₆	2.700	3.920 4.340	3	3¹/₂	otitis catarrh.
	3⁵/₆	2.700	4.600 4.800	0	2	gastroenteritis
12. P. K. ♂	2⁵/₆	3.000	5.170 5.100	4	5	from 29. 12. enteritis ac., dehydration

TABLE VI.

Other skin lesions.

13. J. F. ♀	2	3.000	3.380 3.840	1	1¹/₂	dystrophia enteritis ac. derm. seborrhoica
14. J. T. ♀	2¹/₆	2.700	3.720 4.040	4	6	pyodermia dyspepsia tox. derm. seborrhoica

Therapy	Biotin in γ % Mother's milk	Blood	Urine	Biotin therapy 0.1 mgr. i.m. p. day 20. mgr. per os
penicillin sulfaguanid. A, B, C, D vit. blood-transf. 90 c.c.	3.5	—	5.3	29. 9.
	—	0.05	—	15. 10. after 6 x 0.1 within 10 days the condition of the skin remains unchanged
	—	0.1	—	22. 10. after 5 x 0.1 within 3 days, skin improved
	—	0.03	7.6	9. 11. cured
penicillin A, C, D-vit.	1.2	0.06	48.0	29. 9.
	—	0.04	—	15. 10. after 6 x 0.1 in 10 days M. Leiner still present
	—	0.12	—	22. 10. after additional 5 x 0.1 in 3 days, improved
	—	0.04	—	2. 11. worse
	—	0.06	—	9. 11. after some more 6 x 0.1
				12. 11. cured
penicillin B_1, C-vit.	—	0.06	13.5	15. 10. after 6 x 0.1, then 2 x 0.1
	—	0.08	—	22. 10.
	—	—	—	26. 10. cured
penicillin B_2-vit. C-vit.	—	0.09	3.3	15. 10. after 3 x 0.1
	—	0.06	—	22. 10. after additional 4 x 0.1 after 3 days
				27. 10. cured
sulfaguanidin B_1, C-vit. blood-transf. 80 c.c.	—	0.02	7.0	1. 12. gastroenteritis, intertrigo of neck and desquamation of face
penicillin streptom. B_1, C-vit. blood-transf. 3 x 80 c.c.	—	0.03	1.6	21. 12.
	—	—	—	29. 12. 6 x 0.1 improved
	—	0.17	69.0	11. 1. after additional 4 x 0.1 and 3 x 2.0 continued 4 x 2.0
				16. 1. cured
penicillin sulfasuxid. C, B-complex	0.3	0.01	—	alimentement mixte, without Biotin, skin improved
penicillin streptomycin sulfadiazin A, B_1, B_2, C, D-vitamin transf. of plasma 15 x	—	0.06	6.3	8. 6. after 2 x 0.1, then 4 x 0.1 16. 6. cured, artificially fed.

Name	Age (months)	Birth weight	Weight on admission and discharge	Duration of illness before admission (weeks)	Stay at hospital (weeks)	Complications
15. J. H. ♀	1	2.150	2.550 3.820	3	8	pyodermia otitis bilat. anthrotomia,derm. seborrhoica
16. P. P. ♂	1¾	2.650	2.570 3.260	1	6	otitis bilat. dehydration dermatitis post sol. Darrow
17. S. J. ♂	¾	1.600	1.400 3.520	3	15½	praematuritas, atrophia pemphigus otitis dext. dehydrat. dermat. p. sol. Darrow
18. H. V. ♀	1	3.000	3.060 3.750	2	4	folliculit.,intertrigo otitis m. l. sin. eczema infant.
19. S. V. ♂	3	3.700	5.560 5.770	8	2	bronchopneum. l. dext. crusta lactea
20. J. K. ♂	6	2.700	6.020 6.360	1	2½	asthma bronchiale pleurit. mediast. eczema of the face
21. J. L. ♂	5½	3.100	4.090 5.060	22	10½	dyspepsia tox. pyoderma, eczema infant. anammest. ecz. seborrh.
22. A. P. ♂	3	3.700	5.540 5.420	12	7	otitis med. l. dext., eczema allerg.
23. J. M. ♂	2¾	3.100	2.950 4.150	2	8	atrophia, keratodermia ichthyosiform.

Therapy	Biotin in γ %: Mother's milk	Blood	Urine	Biotin therapy 0 1-0.5-5.0 mgr. i.m.
penicillin streptomycin sulfasuxid A, B_1, B_2, C, D vitamin	0.7	0.06	6.5	21. 9. allaitement mixte without Biotin
	0.6	—	5.0	24. 9. skin nearly cured
penicillin streptomycin A, B, complex C, D-vitamin pernemyl blood-transf. 6 x	—	0.03	10.0	15. 10. artificially fed
	—	0.09	—	22. 10. after 1 x 0.1,then 5 x 0.1
	—	0.03	—	2. 11. improved, not completely cured
penicillin streptomycin sulphadiazin A, B-complex C, D-vitamin blood-transf. 5 x	—	0.02	—	9. 11. artificially fed.
	—	0.06	14.5	7. 12. after 6 x 0.1
	—	0.04	16.5	14. 12. cured
penicillin sulphadiazin A, B-complex C, D-vitamin	—	0.03	—	2. 11. allaitement mixte
	—	—	9.0	16. 11. without Biotin
	—	0.03	—	23. 11. discharged, improved
penicillin sulphadiazin B, C-vitamin	0.7	—	—	23. 11. entirely breastfed, the last fortnight breastfed + Eledon without Biotin
	0.7	0.01	—	1. 12. not improved
penicillin sulphadiazin B_1, C-vitamin	—	0.07	7.2	14. 12. artificially fed without Biotin, cured
penicillin streptomycin A, B-complex C, D-vitamin blood-transf. 2 x 70 c.c.	—	0.01	—	23. 11. artificially fed
	—	0.02	—	1. 12. after 1 x 0.2
	—	0.02	11.0	7. 12. after additional 5 x 0.2 within a day, much improved
	—	0.04	15.0	14. 12. after more 5 x 0.2 very good condition
	—	—	—	
	—	0.13	29.0	21. 12. after more 1 x 0.2, 4 x 0.5, 1 x 1.0
	—	0.02	16.0	7. 1. after more 5 x 5.0 in 13 days eczema of the body disappeared, only a small eczema behind the ear remained
penicillin scephylan B-complex C-vitamin	1.0	—	4.0	12. 5. after 9 x 0.1, allaitement mixte, mostly however breastfed, discharged, not improved
penicillin streptomycin A, B-complex C, D-vitamin	2.0	0.05	—	2. 11. entirely breastfed
	2.3	—	—	7. 12. without Biotin
				20. 12. cured

Cases 18-28 were allergic eczemas of children. Case 21 had a seborrhoic component at the beginning. There, low figures for the Biotin in the blood and high values in the urine were found. The Biotin therapy in patient No. 21 temporarily raised the level of Biotin in the blood considerably; there also the herpetiform eczema on the trunk disappeared and only the moist eczema around the ear remained. The success there was obvious. In case No. 22 no amelioration was seen after Biotin, in the rest Biotin was not given—their details are incomplete for judgment.

Discussion.

The action of Biotin on the mortality of babies with erythrodermia desquamativa Leiner, its quick action on the disappearance of skin lesions and the low levels of Biotin in the blood and urine of breastfed babies permit the conclusion that the cause of this disease is a lack of Biotin. The further life of these children is dependent often on secondary infections, as shown by the lowering of the mortality rate after the introduction of penicillin therapy. These infections may occur by lowering the resistance of those babies through the lack of Biotin, but they can also cause that lack or at least aggravate it as we can conclude from the observation in artificially fed children. In breastfed children this lack is caused, as proved by the results of the investigations of Biotin in the milk of nursing mothers, in the first place by a lack of Biotin in their milk. From the figures found in the mother's milk of children with M. Leiner as well as of normal children we can conclude that the amount of Biotin in the milk of nursing mothers is on the whole lower in this country as a result of the post-war nutrition. Our figures are lower than those found elsewhere. Erythrodermia desquamativa Leiner appears to be wholly due to the lack of Biotin, while its general picture and malignity is aggravated by secondary, particularly staphylococcal, infections. This view cannot be accepted in children with "allaitement mixte" or in artificially fed babies where the intake of Biotin is sufficiently covered by its amount in cow's milk. However, it can be seen that the action of Biotin is clinically not as obvious as in breastfed babies. The relative amounts of Biotin in the blood and urine are much more complicated and not as stable as in breastfed children. To explain these relationships, the assumption is not sufficient that the synthesis of Biotin is disturbed by a change

of the intestinal bacterial flora synthesizing Biotin; this has been proved so far in animals only. The action of penicillin and sulfonamides on that flora also explains little. The excretion of Biotin in the urine points to the fact that the Biotin which is taken in sufficient amounts is not retained and used by the body tissues. It is difficult to decide whether toxic, infective influences or also a lack of other vitamins are responsible. For the first theory we have the evidence that the illness improves after elimination of the toxic, infective influences. With regard to the second possibility we have only the evidence that in experiments with rats a lack of Biotin also causes a lack of folic acid and that the rats who take sulfonamides need both these substances for the synthesis of pantothenic acid. Thus it could be explained that Biotin can be of help even in some cases where the lack of it was not the cause of the primary disease.

Summary.

1. The levels of Biotin in the blood and urine were estimated by a biological method in normal children. The average was 0.04 γ% in the blood and 1.5 γ% in the urine. In the milk of mothers feeding normal children 1.8 γ% was found. This amount, found normally in the milk of nursing mothers, is lower in Czechoslovakia than in other countries.

2. In entirely breastfed children with M. Leiner the corresponding levels were estimated. They were on the whole 56% lower than in the normal cases. In 7 cases where the levels were controlled during the therapy with Biotin a continuous rise took place during the treatment. As in cases previously observed the Biotin therapy was followed by a quick improvement, sometimes with complete cure of the disease.

3. In 5 children with "allaitement mixte" or artificially fed, who had erythrodermia desquamativa Leiner, the levels of Biotin in the blood were normal and in the urine high. Biotin therapy was also here successful, but more Biotin and more time was needed. These children had always a general disease as well.

4. The levels of Biotin in the blood and urine in 11 cases of other kinds of dermatitis were determined by analysis. In those cases with low levels in the blood the disease, if it was of the seborrhoic variety, got better with the intake of cow's milk or during Biotin therapy.

5. The authors conclude that erythrodermia desquamativa Leiner in breastfed children is caused by a lack of Biotin in the milk of the nursing mothers and is sometimes aggravated by secondary infection. In artificially fed babies the lack of Biotin is caused by general toxic, infective influences which increase the requirement of the vitamin and inhibit the possibility of its use in the body tissues.

Zusammenfassung.

1. Der Biotingehalt in Blut und Urin wurde mit einer biologischen Methode bei Normalkindern gemessen. Der Durchschnitt betrug 0,04 γ% im Blut und 1,5 γ% im Urin. In der Milch der Mütter normaler Kinder wurden 1,8 γ% gefunden. Dieser Gehalt ist in der Tschechoslowakei tiefer als in anderen Ländern.

2. In reinen Brustkindern mit Morbus Leiner wurden um 56% tiefere Werte gefunden. In 7 Fällen wurde der Anstieg unter Biotintherapie kontrolliert. Wie in früheren Fällen folgte eine rasche Besserung, bisweilen eine völlige Heilung.

3. Bei 5 Kindern mit Zwiemilch-Ernährung oder künstlicher Ernährung mit Erythrodermie Leiner war der Biotingehalt im Blut normal, im Urin hoch. Auch hier war die Biotinbehandlung erfolgreich, sie erforderte aber größere Dosen und längere Zeit. Diese Kinder hatten alle eine Allgemeinerkrankung.

4. Biotin in Blut und Urin wurde in 11 Fällen anderer Dermatitis bestimmt. Bei Fällen mit niedrigen Biotingehalten verlief die Krankheit, wenn sie vom seborrhoiden Typus war, mit der Aufnahme von Kuhmilch oder unter Biotintherapie leichter.

5. Die Autoren folgern, daß der Morbus Leiner bei Brustkindern durch Biotinmangel der Milch verursacht ist und zuweilen durch sekundäre Infekte verschlimmert wird. Bei künstlich genährten Kindern wird Biotinmangel durch toxische und infektiöse Einflüsse bewirkt, die einen erhöhten Biotinbedarf durch Schädigung der Nutzung in den Geweben bewirken.

Résumé.

1° Les taux de biotine sanguins et urinaires ont été établis par la méthode biologique chez des enfants normaux. Ils sont de 0,04 γ% dans le sang et de 1,5 γ% dans l'urine. Dans le lait maternel de femme allaitant des enfants normaux, on trouva un taux de 1,8 γ%. Ce taux, considéré normal dans le lait de mères allaitant, est plus bas en Tchécoslovaquie que dans d'autres contrées.

2° On a également établi les taux correspondants chez des enfants exclusivement nourris au sein et atteints de la maladie de Leiner. Dans l'ensemble, ces taux étaient de 56 % plus bas que dans les cas normaux. Pour 7 cas dans lesquels les taux furent contrôlés pendant le traitement à la biotine, on observa une augmentation continue au cours du traitement. Chez les patients jusqu'alors en observation, le traitement au moyen de la biotine fut suivi d'une rapide amélioration, parfois d'une guérison complète de la maladie.

3° 5 enfants recevant un allaitement mixte ou nourris artificiellement, atteints d'érythrodermie desquamative de Leiner, présentaient un taux de biotine normal dans le sang, mais plus élevé dans l'urine. Le traitement à la biotine a, par conséquent, été couronné de succès dans ces cas, mais il fallut administrer davantage de biotine et ce, pendant une période plus longue. Les enfants en question avaient toujours en même temps une maladie quelconque.

4° Les taux de biotine dans le sang et dans l'urine de 11 cas atteints d'autres espèces de dermatites furent déterminés par analyse. Dans ces cas à taux bas dans le sang, la maladie, si elle était d'aspect séborrhéique, fut améliorée lorsqu'on administra du lait de vache ou de la biotine.

5° Les auteurs concluent que l'érythrodermie desquamative de Leiner chez les enfants nourris au sein est causée par une carence en biotine dans le lait maternel et qu'elle est parfois aggravée par une affection secondaire. Chez les enfants nourris artificiellement, la carence en biotine est causée par des influences toxiques générales et infectieuses, qui augmentent le besoin en cette vitamine et inhibent sa mise en valeur dans les tissus du corps.

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Tuszynska-'69
Bib No. 1403

Acta Physiol. Polon. 20: 102-115. 1969

ACTA PHYSIOLOGICA POLONICA
Vol. XX, No. 1, 1969

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INDUCTION OF EXPERIMENTAL ABIOTINOSIS IN RATS AND TRIALS OF ELIMINATION ITS SYMPTOMS

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TUSZYŃSKA S.: *Induction of Experimental Abiotinosis in Rats and Trials of Elimination its Symptoms.* Acta Physiol. Pol. 1 (20) : 125—140, 1969. — In order to determine the conditions of abiotinosis development and its symptoms regression, the degree of biotin penetration into the tissues and their saturation with this vitamin were studied. Experiments were performed on 120 healthy rats, animals with various pathologic conditions and after cure with pharmacodynamic doses of biotin. Biotin was assayed in the liver, kidneys and blood by the microbiologic method. Urinary excretion and skin saturation with biotin were studied. Oral administration of biotin to the animals increased the vitamin content in the liver and kidneys, causing regression of the disease symptoms. Presence of vitamin H was observed also in the skin appendages, and correlation between its levels in this tissue and skin denudation and hair regrowth were found.

In normal conditions, practically no symptoms of biotin deficiency in humans or animals have been detected. In medicine, however, skin troubles in humans have been observed, similar to the symptoms occurring in experimental abiotinosis in animals.

Experimental abiotinosis in animals may be induced in several ways. The most frequently applied one is a diet containing raw hen-egg albumin, which contains avidin — a protein inhibiting biotin activity, by forming with it a stable complex [15, 20, 27, 29, 37]. As avidin unit was adopted the amount capable of binding 1 μg of biotin, whereas 1 mg of avidin can bind 8—9 μg of biotin [11, 21, 30, 41]. Avidin, constituting 0.05 percent of total hen-egg albumin, binds all the biotin contained in the egg-white. Its effect in the human and animal organism is similar. Growth inhibition, disappearance of reserves and above all changes in the skin and its appendages take place, leading finally to skin denudation [26, 27, 40].

The phenomenon of natural abiotinosis is relatively rare in humans, because nutrition conditions and bacterial synthesis of biotin prevent its development, where raw egg-white consumption is moderate. Biotin de-

ficiency may occur in humans in result of a restricted diet as regards this vitamin (eggs, liver, meat, milk), and also in result of insufficient absorption of H vitamin by the intestinal mucosa, or its blocking by functional insufficiency of the liver [37].

Administration of biotin gradually reduces all characteristic symptoms of experimental abiotinosis in animals, but to date there is no precise biological and still less clinical evaluation of the therapeutical effect of that vitamin, both as regards its dosage and the form of its administration [14, 22, 24, 25].

It is known that tissues contain biotin as a permanent component bound with the cell protein, whereas it is excreted in the free state. The process of biotin absorption by the tissues has not so far been precisely elucidated, and the degree of saturation of the organism with biotin in conditions of symptomatic abiotinosis as compared with that of cured individuals has not been studied.

The experiments described in the present paper are an attempt at establishing the conditions of abiotinosis development and return to a normal state by investigation of the degree of saturation of some more important tissues and systemic fluids with biotin in rats, subjected to artificial abiotinosis and cured by pharmacodynamic doses of biotin, as compared with animals, whose requirements of that vitamin were entirely satisfied. Since vitamin H deficiency induces changes in skin and hair more drastic than the lack of other vitamins, and the reasons for that phenomenon have not as yet been elucidated, it was decided to investigate simultaneously other tissues and hair for the presence of biotin, analyzing incidental changes of the vitamin H level in that material, in relation to the degree of saturation of the organism with biotin.

METHODS

The investigations were performed with male rats of the Wistar strain, weighing at the outset 45—55 g, bred at the Institute of Drugs.

The rats were placed in cages, each containing 5 animals, matched according to weight. The biological experiments which tended to elicit a sever state of abiotinosis in the rats, and subsequently to achieve a total cure of its effects, with simultaneous observations of quantitative changes of biotin in the tissues investigated, comprised four experiments in similar conditions, carried out at three-month intervals on 120 rats.

At the outset breeding conditions were elaborated, the diet composition, preventive doses of biotin administered to the controls, and therapeutic doses given to the experimental animals, as well as the procedure applied in quantitative determination of biotin in the material analyzed.

The animals were divided into 3 basic groups: one subjected to abiotinosis, and two control groups. The former group was divided into two subgroups: A-not medicated and H-treated with biotin.

All the animals were fed a synthetic sugar diet, prepared on the basis of latest literature data, supplemented by vitamins and L-cystine [29, 32, 33].

A state of abiotinosis was elicited in the animals by adding to their diet hen-egg crystalline albumin, in the amount of 23 percent of the calorific value of the sugar diet.

In the group of controls, hen-egg albumin was substituted in control I — with casein, and in control II — with an abiotinic diet, supplemented by prophylactic oral administration of biotin doses, in an amount of 25 μg per rat daily. After inducing a severe state of abiotinosis in this group of rats, treatment of the animals in one subgroup was started by feeding them every second day therapeutic doses of biotin, in an amount of 500 μg per rat orally in aqueous solution by means of a probe.

Rats, in control group I, received simultaneously the same doses of biotin as were given therapeutically to the abiotinic rats (Control IH).

The period of induced abiotinosis lasted 6 weeks, and the treatment 2 weeks. The total therapeutic amount of biotin during that period was 4 mg per rat. Each experiment lasted 8 weeks.

In the course of the experiment the weight of the animals was checked, and their appearance and behavior observed, as well as changes of the skin and its appendages. Analytical investigations of the biotin level in tissues and systemic fluids were performed on the material collected after 6 weeks of experiment in all groups of animals, i. e. after eliciting an acute state of biotin insufficiency, as well as after a further two weeks, that is after termination of the treatment of abiotinic rats of subgroup H

Biotin content was determined in fresh liver and kidney homogenates, in blood drawn from the carotid vein and in skin apendages depilated together, with the root part and bulb. The 24-hr biotin excretion with urine was also investigated.

In the above material biotin was determined quantitatively, according to our own modification [18], by the biological method, using the test strain *Lactobacillus arabinosus* 17 5, which reacts to vitamin concentration as low as 0.05 m μg in 10 ml of fluid medium.

In liver, kidney and blood the biotin level was determined after liberating it from the protein bonds by enzymatic digestion with papain „Merck", according to literature data [1, 2, 6].

To release biotin from the hair, two-hour acid hydrolysis was applied with the use of 4 N H_2SO_4, in an autoclave under 1 atm. pressure. The biotin in urine was determined after brief heating at 118°C with citrate-phosphate buffer [23, 38]. Moreover, after remarking in the heated urine of abiotinic rats an abundant proteinic precipitate, not observed in the urine of the treated and control groups, the urine of all rats in all groups was examined for total protein content by the biuret method according to *Gleiss* and *Hinsberg* [13].

RESULTS

The results of biotin determination in tissues and urine and the body weight increment of rats were statistically analyzed with the use for calculation of all numerical data obtained from four experiments. Statistical analysis was performed by means of the Fischer-Snedecor, and

the Student's "t" test. The significance of the differences in biotin content was evaluated in the investigated material between groups of animals after 6 weeks of experiments, after a further two weeks, as well as within the same group of animals after 6 and 8 weeks of experiments.

A. Weight dynamics and somatic changes

In all the experiments with the group of rats submitted to abiotinosis a severe pathological state was obtained after 6 weeks. All the animals manifested symptoms characteristic for abiotinosis (Fig. 1), like: growth

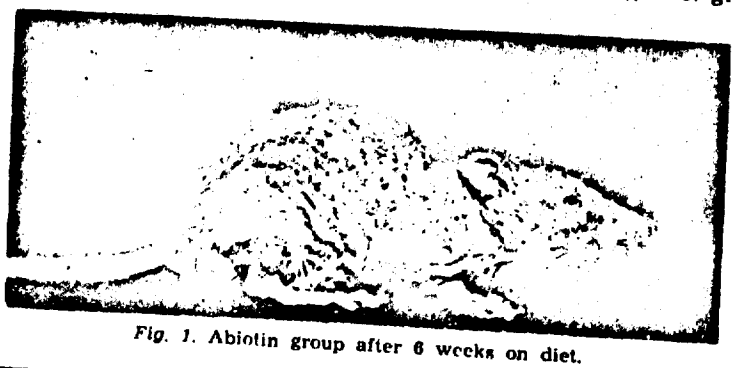

Fig. 1. Abiotin group after 6 weeks on diet.

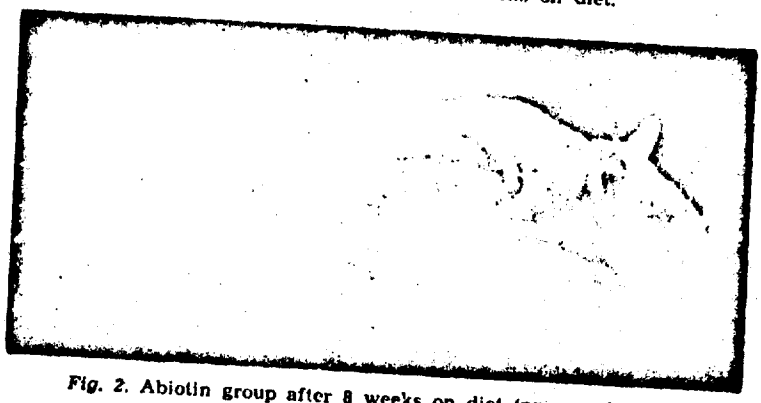

Fig. 2. Abiotin group after 8 weeks on diet (preagonal state).

inhibition, disappearance of adipose tissue, extensive bald patches on back, nape and head, remaining hair lustreless and scarce, congestion and folds in the skin, brown, greasy dendruff, purulent conjunctivitis, characteristic circles around the eyes — „spectacles" and a kangaroo-like

Fig. 3. Abiotin group treated with vitamin H.

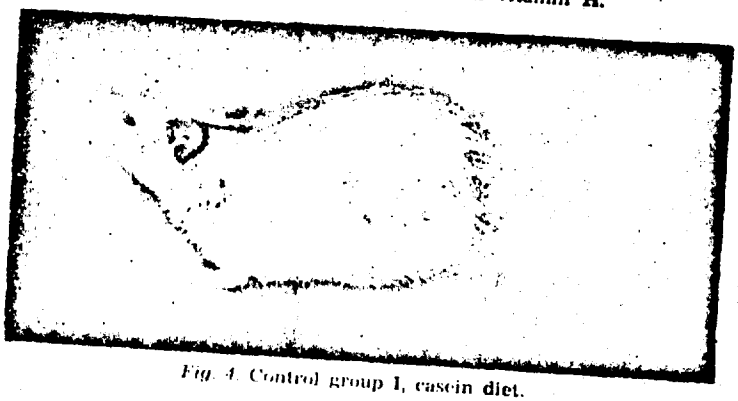

Fig. 4. Control group I, casein diet.

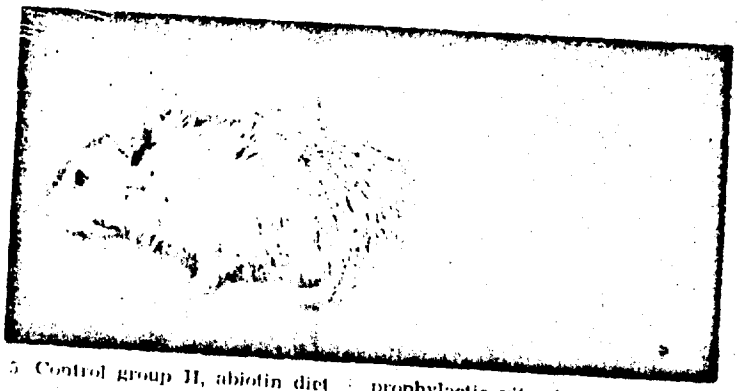

Fig. 5. Control group II, abiotin diet + prophylactic vitamin H administration.

posture. No lack of appetite, however, was noted in these rats, as compared with controls.

The abiotinic diet, applied to these animals for a further two weeks greatly increased the aforementioned symptoms (Fig. 2), bringing the

exhausted organism to an agonal state. The animals almost entirely lost their hair with the exception of the extremities and abdominal side of the body. Mortality attained at that time almost 50 percent. In thus became necessary to interrupt the experiments, as otherwise a shortage of experimental material for analytical studies would have followed in that group of animals. Simultaneously, in the subgroup of treated rats, already 24 hours after administering, of 500 μg of biotin per rat, not only

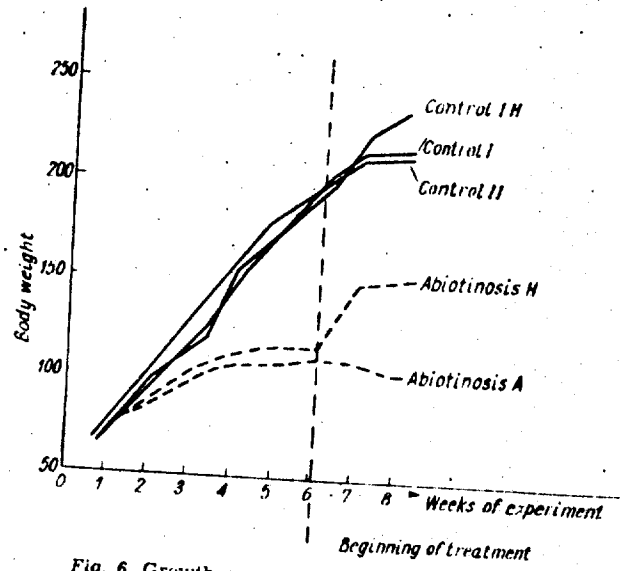

Fig. 6. Growth curves of the experimental rats.

an increase in weight, but a visible regrowth of hair and improvement of the skin state could be observed.

After two weeks of treatment, i.e. after a total dose of 4 mg per animal of biotin, the symptoms of abiotinosis entirely disappeared (Fig. 3), the newly grown hair looked healthy, though it was slightly shorter and not so thick (downless), as compared with that of controls.

The animals of control I (casein diet) and of control II (raw albumin diet) and prophylactic biotin did not manifest any symptoms of biotin insufficiency (Figs. 4 and 5). Merely in the 8th week of the experiment, in the animals of control II, a certain greasiness and viscosity of the hair could be noticed, however, without any inclination to depilation.

The curves of growth of the investigated rats are shown in Fig. 6. In the group of rats subjected to abiotinosis a marked inhibition of growth

may be observed, and during the last two weeks of the experiment, the animals manifested a constant loss of body weight .

In the group of abiotinized rats undergoing treatment, an significant increase in body weight could be observed already after the first oral dose of 500 μg of biotin. At the same time, the weight of the animals in both control groups increased normally during the whole experiment, and

Tabele 1. Mean increment of body weight of the rats in the last two weeks of the experiment

Group of animals	Control I (casein)	Control II (raw egg albumin + vit. H prophylactically)	Abiotinosis	
			untreated	treated,
g per rat	+ 19·1 (216·7)	+ 18·0 (212·5)	—6·3 (107·7)	+58·2 (177·5)

+ weight increment
— weight loss

The figures in paranthesis denote the final weight of the rats (after 8 weeks of the experiment)

the excess of biotin introduced into their organisms (control I) did not induce in its final effect a significant weight increment. Table 1 gives the mean weight increment of rats in the course of the last two weeks of experiment, i.e. during the period of treatment of the abiotinized animals.

B. Analitical studies

Microscopic examination of tissues and systemic fluids demonstrated that the hydrolyzates of liver, kidney, blood and hair, as well as 24-hr urine of rats, fed an abiotinic diet present a darker colour (reddish-brown), as compared with the hydrolyzates of the same tissues originating from animals cured by biotin and from controls.

The livers of abiotinized animals were hypertrophied and showed fatty degeneration as compared with those of the remaining groups of rats.

Table 2 presents the results of quantitative determination of biotin in the examined tissues and urine, expressed as arithmetical means of the values obtained from all the experiments.

The biotin level in the liver showed after a 6-week treatment inducing abiotinosis, a considerable drop. The amount of biotin in the liver of abiotinic rats was by 70—80 percent lower than that in the liver of animals with no H vitamin deficiency. Similarly, in the kidneys the level of vitamin H amounted to 60 percent of normal.

The concentration of vitamin H in the blood of abiotinic rats was lower by about 75 percent than that found in the blood of healthy animals, fed a casein diet (control I). On the other hand, no significant difference could be established as compared with the blood biotin level in rats of control II.

Table 2. Biotin content in the experimental rats after 6 weeks of the experiment (before beginning therapy), after 8 weeks of the experiment (after termination of therapy)

Experimental material / Group of animals	Blood (μg/ml)		Liver and kidneys (μg/g)		Hair (μg/g)		24-hr Urine (mg/rat)	
	after 6 weeks	after 8 weeks	after 6 weeks	after 8 weeks	after 6 weeks	after 8 weeks	after 6 weeks	after 8 weeks
Control I (casein)	0·46	0·08	1·29	2·32 (2·17)	4·08	2·24	12·65	6·48
Control II (raw egg albumin + vitamin H prophylactically)	0·12	0·01	1·97	1·69	1·40	1·20	1·90	1·73
Abiotinotis untreated	0·08	0·03	0·46	0·49 (0·92)	1·72	0·62	0·88	0·38
Abiotinosis H treated	—	0·24	—	2·63 (1·70)	—	1·81	—	64·00

Each figure is the arithmetic mean from 4 experiments.
The figures in parantheses denote contents of biotin in the kidneys.

At the same time the amount of biotin excreted in 24 hr urine by abiotinic rats was more than ten times smaller than the quantity excreted by rats of control I. The animals from control II excreted relatively small amounts of this vitamin in relation to control I.

Investigation of the material collected from the same groups of animals after a further two weeks of experiment did not demonstrate a significant drop of abiotinosis level in the tissues of rats, brought to a lethal state of abiotinosis. The biotin content dropped only in the 24-hr urine of these rats to 0.38 μg per rat. The corresponding animals cured of abiotinosis after a two week treatment exhibited a rise of the biotin level in the liver and kidneys as well as an almost 10-fold increase of the vitamin concentration in the blood and also a twentyfold increased excretion with urine. Investigation of skin appendages showed that in rats fed 6 week an abiotinic diet, the amount of biotin detected in the depilated hair was by about 70 percent lower than the level of this vitamin in the hair of control rats with satisfied biotin requirement.

Similar investigations of skin appendages still remaining in the skin of rats brought to lethal abiotinosis demonstrated a further drop in the level of biotin in this material, in respect to the state noted 2 weeks before. On the other hand, the hair of cured rats contained three times more biotin as compared with its level in the skin appendages of untreated animals, and its quantity differed significantly from the level detected in the hair of healthy animals.

Albumin in the 24-hr urine of rats revealed that animals brought to a point of lethal abiotinosis excrete an important amount of albumin in urine (50 mg in 24-hr urine of one rat), while in the urine of controls and cured animals only traces could be detected.

DISCUSSION

A. Somatic changes

An increased amount of raw albumin in the diet in the form of crystalline egg albumin up to 2 percent of the total caloritic value of the sugar diet shortened by about 4 weeks the period necessary for inducing abiotinosis is rats as compared with the time reported by other authors [20, 27, 28, 29].

The addition to the abiotinic diet, besides vitamin soluble in fats, of the whole complex of group B vitamins in a twice larger amount than that commonly applied and the addition of ascorbic acid, as well as L-cystine favored the induction of abiotinosis. The above mentioned substances did not inhibit the process of abiotinosis but protecter the animals from secondary abiotinosis. At the same time a protective role of vitamin C during the severe pathological state of the animals was noted, thus reducing their mortality in the final phase of the experiment.

This is also confirmed by the studies of *Terroine* [29, 32, 33, 34]. The introduction into the experiment of the additional control group II, allowed to establish: 1) that the symptoms of abiotinosis in rats were induced exclusively by the specific action of avidine contained in the raw albumin of hen egg, 2) the prophylactic action of biotin administered orally in 25 ug doses daily per rat, with a diet containing 1 mg of avidine.

The comparison not attempted up till now of the pharmaco-dynamic effect of biotin doses on an organism, with satisfied requirement of thus vitamin, and on an organism deprived of it, proved to be still another means of checking in investigations the physiological role of biotin in the system.

The body weight increments in the investigated rat groups witness to the high effect of biotin on food assimilation in rats. The weight of ani-

mals deprived of biotin supply in respect to that of rats with satisfied biotin demand shows a difference already in the first week of the diet applied, as a result of rapid exhaustion of biotin reserves in the young organism. The results obtained confirm the conclusions of *Pachelska* [20], with the only difference that the author observed a loss of apetite in abiotinic rats as compared with the control group, which was not observed in the present experiments.

Literature data referring to the therapeutic doses and the way of treating abiotinosis are highly divergent. The authors apply biotin therapeutically as an addition to the diet or parenterally, at various time intervals, in doses varying from 10 to 500 ug per rat daily. Biotin was seldom administered orally, considering its slow absorption through the intestinal walls and penetration to the circulation [1, 3, 15, 31, 33, 34]. Orally administered biotin, according to the method used in the present study was sufficient to relieve in two weeks all pathological symptoms.

The general results obtained during treatment were in accordance with those of other authors, who applied biotin in hight doses as injections [8, 33].

B. Analytic studies

The use of papain for digesting the enzymatic tissues in order to extract from them biotin for microbiological determination proved effective and confirmed the opinion of *Baker* and *Sobotka* [2, 23]. This method of extraction allows to obtain the highest yield. The darker colour of the tissues and urine of abiotinized rats as compared with those of controls, or biotin-treated animals is probably connected with an abnormal metabolism of porphyrin compounds, which may lead to accumulation of these compounds, in the liver kidneys and blood, and increase of their excretion to urine, as it happens in the case of nicotinic and pantothenic acid in rats [4, 35]. Fatty degeneration of the liver, kidneys and testicles of abiotinized rats confirms the role of biotin in fat metabolism [10, 36].

So far only *Terroine* [28] compared the changes in biotin concentration in the liver, muscles and brain of abiotinic rats with those of normal ones. The absolute values of vitamin H in the liver, obtained by that author were lower than those recorded in the present study. This was probably due to the difference in the course of hydrolysis of the investigated tissue. As a rule the decrease in biotin reserve in the liver of rats brought to a state of lethal abiotinosis was in agreement with the data of that author.

The level of biotin in kidneys of normally fed rats, reported by *Bialecki* et al. [5] was slightly lower as compared with the level found in

the present study, what may be the result as well of the method of feeding the animals and of a different technique of liberating biotin from the tissues.

The lowered blood and urine level of biotin of control II rats, approximating the concentration of that compound in the blood and urine of abiotinic rats may indicate a rapid penetration of vitamin H from the blood of control II rats to the tissues, in order to replenish the biotin reserve and also maximal utilization of the vitamin by the organism, when prophylactically administered to prevent the noxious action of avidin in the diet.

In the present study the level of free and bound biotin in whole blood was determined, which makes it difficult to compare the results obtained with other data from the Polish literature [5]. Neither could be found in the literature precise data concerning the excretion of biotin in urine [9]. Neither could data be found on the determination of biotin in skin appendages, solely *Gounelle* et al. investigated quantitative changes of riboflavin, nicotinic acid and pyridoxine in the cut hair of pellagrous humans. In the present paper the examination of depilated skin appendages of rats revealed the presence in them of biotin in comparatively large amounts, approximating the concentration of the vitamin in liver and kidneys, this being probably connected with its specifically dermatropic action in the organism.

A drop in the biotin level was found in the hair of abiotinized rats: this was probably due to a general exhaustion of the biotin reserve in the organism, and consequently, to its lowered supply to the hair papilla and further to the bulb. The two times lower biotin content in control II hair, as compared with that of the control I rats is doubtless connected with a decrease of the vitamin content in their blood. This could lead consequently to a general deterioration of the hair state in the final phase of the experiment as has been discussed in part A of this chapter.

The treatment of rats, submitted to a 6-week abiotinic diet induced, simultaneously with abolishment of external symptoms of severe abiotinosis a fivefold increase of the biotin level in the liver, compensating the vitamin H reserve in that tissue to normal, and raising the level of biotin in the kidneys by 85 percent in respect to the normal level.

The administration of pharmacodynamic doses of biotin to rats subjected to therapy increased its concentration in the blood as compared with the state prior to treatment, and also increased the excretion of this vitamin in urine [7]. The corresponding feeding of control I rats with analogous doses of biotin as those used in treatment of abiotinic animals revealed a relatively slight and statistically insignificant increase of the liver biotin level; on the other hand, the concentration of vitamin H in

the blood and its excretion in urine greatly increased. Examination of the hair in this control group did not reveal a significant increase of the biotin amount in the skin appendages. There are no studies in the literature relating to the changes in biotin concentration taking place in the organism under the therapeutic action of this vitamin as compared with the pathological cases.

Additional results of urine analysis for total albumin, obtained in the present study are identical with those of *Terroine* [28, 33, 34], who in the paper on the role of biotin in protein metabolism established an enhanced excretion of protein nitrogen in rats, submitted to experimental abiotinosis. This would indicate disturbances in protein metabolism induced by biotin deficiency in the living organism.

CONCLUSIONS

1. A state of severe experimental abiotinosis in young rats may be induced already after 6 weeks of feeding them a sugar diet containing 23 percent of dried hen-egg albumin.

2. Biotin, administered prophylactically, in 25 μg oral daily doses with an biotinic diet, containing 1 mg of avidin, prevented the symptoms of avitaminosis in rats.

3. Biotin, given therapeuticelly *per os*, every second day in doses of 500 μg per rat, induced in the course of two weeks a receding of all symptoms of experimental abiotinosis so that the animals returned to their normal appearance.

4. The exhaustion of about 3/4 of the biotin reserve in the liver and about one half of it in the kidneys, with a fivefold lowering of its concentration in the blood, induced a severe pathological state in the rats.

5. Rats, brought to a state of lethal abiotinosis can not utilize any more the remaining biotin in liver and kidneys in their metabolism, but excrete it in urine in trace amounts.

6. The lack of biotin supply, in a diet containing raw hen-egg albumin, elicits a markedly reduced food uptake already in the first week of the diet.

7. A complete recession of experimental abiotinotic symptoms in rats appears after replenishing the liver biotin reserve in 50 and that in the kidneys in 80 percent and compensation of its concentration in the blood to the level found in healthy animals.

8. Similarly as it happens in the tissues, biotin is also found in the skin appendages of rats, and its amount is related to the state of saturation of the organism with this vitamin. After a long period of deficiency the drop of biotin in the hair of the animals, follows decrease of its level

in the liver and blood, and treatment with biotin brings a return to the normal level.

9. Administration of large doses of biotin orally to the organism of rats, whose biotin requirement is satisfield, doses not induce a significant increase in weight of the animals nor does it increase significantly the biotin concentration in their liver, kidneys and skin appendages.

10. The excretion with urine of excessive amounts of albumin during experimental abiotinosis in rats and the receding of this pathological symptom in cured rats confirms the role of biotin in protein metabolism of the system, and may also witness to the abnormal reabsorption in the renal tubules in avitaminotic states.

11. The exhaustion of biotin reserves in the liver and kidneys, the observation of the growth and course of depilation permit the conclusion that the organism of rats submitted to experimental abiotinosis utilizes in the first place the reserves of biotin in the liver and kidneys, and then draws them from the dermal tissue. This is the reason of insufficient supply of vitamin H to the hair bulbs as the first symptom of abiotinosis, followed by a loss of weight and next denudation of the skin.

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Waibel-'69
Bib No. 1434

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Poultry Sci. 48: 1979-1985. 1969.

Effect of Supplementary Biotin on Performance of Turkeys Fed Corn-Soybean Meal Type Diets[1]

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(Received for publication June 9, 1969)

THAT biotin is an essential vitamin for the chicken hen has been known for over a quarter of a century (Cravens *et al.*, 1942, 1944). Further work in the same laboratory (Couch *et al.*, 1948a, 1949) demonstrated that the dietary requirement of the hen for biotin was modified by variable intestinal synthesis. Couch *et al.* (1948b) showed that the biotin content of the egg white or yolk was highly responsive to the amount of biotin in the diet and upon apparent intestinal synthesis. Waibel *et al.* (1952) found that dietary penicillin resulted in increased deposition of biotin and folic acid in egg yolk.

The symptoms of biotin deficiency in young chickens and turkeys were described by Patrick *et al.* (1942) and include dermatitis, perosis, and retarded growth. They estimated the daily biotin requirement for poults as 5 mcg. during the first four weeks; this level was about two and one half times the level required for chicks. Robblee and Clandinin (1953) found that addition of biotin to practical starter diets containing wheat products improved growth and decreased the incidence of broken feathers, dermatitis, perosis, and diarrhea. Slinger and Pepper (1954) observed good growth responses and decreased diarrhea when biotin was added to a practical diet containing wheat; further, the addition of a dietary antibiotic spared the requirement for biotin. Wagstaff *et al.* (1961) found biotin deficiency signs when chicks were fed a diet containing 75% hulless barley (with purified proteins) and that wheat, hulless barley, and barley in the diet resulted in severe biotin deficiency whereas corn, milo, or oats fostered lesser responses to biotin. These workers determined that the biotin in barley was about 35% available.

Reports have been obtained recently (Misner, 1967; Johnson, 1967) indicating that field cases of dermatitis and perosis in turkeys were corrected by supplementary biotin. Specific information was not given about the diets used; however, it is assumed that they were of the corn-soybean meal type.

The objectives of this study were to examine the influence of biotin added to simplified corn-soybean diets on performance of three strains of large type turkey breeders and to study the activity of supplementary biotin on progeny of these stocks. Also examined was the influence of selected drug treatments on biotin adequacy.

[1] Published as Paper 6959, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota. Supported in part by a grant from Hoffman-LaRoche, Nutley, N. J.

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TABLE 1.—*Composition of breeder diet in experiment 1 and poult diet in experiment 2*[1]

Ingredient	Breeding Exp. 1	Starting Exp. 2
	%	%
Corn, ground yellow	76.84	60.28
Soybean meal, solvent (44% protein)	16	—
Soybean meal, dehulled (48½% protein)	—	30
Fish meal, herring (70% protein)	—	5
DL-methionine (98%)	—	0.08
L-lysine (50%)	—	0.14
Dicalcium phosphate	2.25	2.4
Calcium carbonate	4.25	1.5
Salt	0.5	0.3
Trace mineral mixture	0.1[2]	0.03[2]
Vitamin mixture	0.033[4]	.27[5]
Choline chloride (70% solution)	0.032	—

[1] Starting and growing diets in experiment 1 were reported by Krista *et al.* (1969) as diet series 3.

[2] Trace mineral mixture ("Z-2 Delamix"®): 6% manganese, 0.12% iodine, 2% iron, 0.2% copper, 2% zinc, 0.2% cobalt, 25% calcium. Limestone Products Corporation, Newton, N. J.

[3] Trace mineral premix (mg. per kg. of diet): $MnSO_4$ (27% Mn) 222, ZnO (72% Zn) 78, ethylene diamine dihydroiodide (80.4% I) 0.625.

[4] Vitamin mixture provides the following in mg. per kg. of diet: vitamin A (250,000 I.U./g.) 32, vitamin AD_3 (250,000 I.U. each of vitamins A and D per g.) 8, vitamin E acetate (275 I.U./g.) 72, vitamin K (35 mg. MSBC/g.) 86, riboflavin 8, *d*-calcium pantothenate 20, niacin 30, vitamin B_{12} (0.1% trituration on mannitol) 20, thiamine·HCl 4, pyridoxine·HCl 4, folic acid 2, and sucrose 46.

[5] Vitamin mixture provides the following in mg. per kg. of diet: vitamin A (30,000 I.U./g.) 258.5, vitamin D_3 (30,000 I.U./g.) 68, vitamin E acetate (275 I.U./g.) 78.5, vitamin K (35 mg. MSBC/g.) 71.1, riboflavin (35 mg./g.) 173, *d*-calcium pantothenate (70 mg./g.) 147.8, niacin (50%) 150, and choline chloride (50%) 1750.

METHODS

Experiment 1. Three strains of Broad White turkeys, each consisting of low and high blood pressure lines, were housed in 24 pens (2.7 m.×4.3 m., wood shavings litter) in a pole type building, and divided into two treatment groups of 12 pens each. One group was fed the breeder diet shown in Table 1 and the other group was fed the same diet plus 300 mcg. biotin per kg. Other experimental variables were represented equally. Records included egg production, fertility, hatchability of fertile eggs, body weight, feed efficiency, and progeny performance. Data for breeders represented a 16 week period of egg production.

Progeny of the last three hatches, all from eggs laid after the hens were in production for 10 weeks, were divided equally according to variables and fed the starting and growing diets reported as diet series 3 in the paper by Krista *et al.* (1969), without and with 100 mcg. biotin per kg. In addition to recording body weights at 1, 6, 11, and 18 weeks of age, turkeys were scored at the first three ages for foot dermatitis, leg weakness, and feathering. The poults were housed in batteries with raised wire mesh floors during the first three weeks and were transferred to floor pens containing wood shavings litter at three weeks of age. When employed, statistical treatment utilized multiple classification analyses of variance.

Diets and eggs were analyzed for biotin by the microbiological assay procedure of Wright and Skeggs (1944).[6] Averaging all analyses, the unsupplemented and supplemented starting diets contained 292 and 420 mcg. per kg. and the breeder diets contained 144 and 413 mcg. per kg., respectively.

Experiment 2. Broad White male turkeys were utilized in this separate growth study of three weeks duration. The poults were housed in batteries containing raised wire-mesh floors. Details of the treatments are given in table footnotes.

The diet (Table 1) contained 23.7% protein and 2,955 kcal. of metabolizable energy per kg. By calculation the biotin content was 145 mcg. per kg. Analyses of the unsupplemented and supplemented diets indicated respective values of 193 and 325 mcg biotin per kg., 4.9 and 6.1 mg. vitamin B_6 per kg., and 0.283 and 1.20

[6] Appreciation is expressed to Dr. H. Wilgus and staff at Hoffmann-LaRoche for performing the vitamin analyses on coded samples.

mg. of folic acid per kg. No explanation is apparent for the high level of biotin found in the basal diet by microbiological assay.

RESULTS AND DISCUSSION

Breeder study. Data for egg production, fertility, and hatchability of fertile eggs are shown in Table 2. The main effects for biotin indicated small, non-significant differences in these observations.

Regarding egg production, the analysis of variance considering strains, blood pressures, and presence of biotin showed that none of the treatments or their interactions were significant at the 5% level. The main effect of blood pressure showed significance at the 10% level wherein the high blood pressure birds laid at a somewhat higher rate than the lower blood pressure birds. The interaction of strain ×blood pressure×biotin level was barely significant at the 10% level. No other treatments or interactions yielded F values of any magnitude.

Regarding fertility, none of the treatments or their interactions approached significance. There was a highly significant effect in hatchability dealing with strain of turkeys; no other main effect or interaction showed significance. While the presence of biotin indicated a 3% average improvement in hatchability, the variation was such that an F value of 1.29 was obtained while 4.13 was required for significance at the 5% level.

Birds receiving the basal and biotin supplemented diets lost 0.05 and gained 0.06 kilograms, respectively, from the time lights were turned on until the end of the egg production season. Values for efficiency of feed utilization were 0.50 and 0.51 kg. of feed per egg, respectively.

The biotin content of eggs produced at the middle and at the end of the 16 week experimental period is shown in Table 3. Considering the entire flock, the presence of 300 mcg. of biotin per kg. of diet resulted in about a 10% increase in egg yolk biotin. It is not known why this increase was so modest unless, under these conditions, a saturation point may have been reached. Data with chicken hens (Couch *et al.*, 1948b; Waibel *et al.*, 1952) indicated biotin levels in the same range or somewhat higher than those found in the present study. Couch *et al.* (1948b) observed that the biotin content of the yolk could be reduced to 0.028 mcg. per gm. of yolk. Couch *et al.* (1948a) found that eggs with 0.150 mcg. per gm. supported normal embryonic development. The diet in the present study was formulated to contain as little biotin as possible with a corn-soybean meal type diet (105 mcg. per kg. of diet) by setting the protein level at 14% to keep the level of soybean meal low. The hens were maintained on wood shavings litter and therefore had access to both

TABLE 2.—*Effect of supplementary biotin[1] on performance of turkey breeders[2]*

Treatment			Egg production	Fertility	Hatchability of fertile eggs
Strain	Blood pressure	Presence of biotin[1]			
			%	%	%
A	High	−	59.1	61.2	72.2
A	High	+	50.9	57.2	65.4
A	Low	−	53.8	63.2	75.1
A	Low	+	55.0	58.7	72.1
B	High	−	54.5	58.1	60.5
B	High	+	56.7	60.6	60.5
B	Low	−	52.0	50.5	52.9
B	Low	+	51.8	59.0	66.8
C	High	−	56.7	52.6	62.0
C	High	+	62.3	62.4	70.4
C	Low	−	56.0	58.7	60.0
C	Low	+	51.1	62.4	68.2
Main Effects:					
A			54.7	60.2	71.8
B			53.8	57.1	60.4
C			56.5	58.9	65.3
	High		56.7	58.7	65.5
	Low		53.3	58.8	66.0
		−	55.4	57.4	64.2
		+	54.6	60.1	67.2

[1] Level of supplementary biotin in breeding ration was 300 mcg./kg.
[2] Length of egg production period was 16 weeks

TABLE 3.—*Biotin content of egg yolk*

Nature of stock	Added biotin	7 Weeks[1]		15 Weeks[1]		Average
		No. eggs	Yolk biotin	No. eggs	Yolk biotin	
			mcg./gm.		mcg./gm.	
Entire flock	−	12	.31	14	.31	.31
	+	12	.34	14	.34	.34
Low blood pressure	−	6	.34	7	.32	.33
	+	6	.39	7	.32	.36
High blood pressure	−	6	.28	7	.30	.29
	+	6	.30	7	.35	.32
Strain A	−	4	.27	6	.25	.26
	+	4	.36	6	.31	.34
Strain B	−	4	.34	4	.38	.36
	+	4	.28	4	.36	.32
Strain C	−	4	.35	4	.33	.34
	+	4	.38	4	.36	.37

[1] Average time after commencement of egg production.

litter and fecal droppings. No antibiotic was present in the diet.

Progeny. The body weight gain and condition scores of male and female poults from this breeder study are presented in Table 4. Supplemental biotin in the breeder diet did not improve progeny growth rate or condition scores in any instance. These results might be expected when considering the high biotin content of egg yolks from hens on non-supplemented diets.

The poult diet was not designed to be low in biotin but was the usual turkey diet employed in the aortic rupture-blood pressure studies. The diet contained a greater amount of biotin than estimated as the requirement and the 100 mcg. of biotin added per kg. represented an amount above the requirement.

Poults receiving the added biotin showed no weight difference at 1 week of age. However, at 3, 6, and 11 weeks of age a significant reduction in body weight was associated with added biotin. Although statistical analysis was not conducted on the 18 week data, the differences were still evident.

Poults receiving added biotin showed a greater incidence of hock disorder at 3 and 6 weeks of age; however, by 11 weeks of age a very excellent recovery had been made so that all treatments were similar. Males which received added biotin showed a greater number of toe callouses at 3 weeks of age while females fed added biotin had a lesser incidence at all ages.

The effects of added dietary biotin on condition of foot pads were inconsistent: biotin added to the diet resulted in somewhat roughened pads in males at 3 and 11 weeks of age. Regarding condition of feathers, all differences were of a small magnitude.

It was surprising to obtain poorer performance due to biotin addition in the starting diet. Analyses for biotin in the starting diets indicated that the additional biotin was in the proper diet and further analyses of the diets for protein, manganese, and vitamin A suggested that the diets were mixed correctly. It is not the

TABLE 4.—*Influence of added biotin[1] in breeder and growing diets on performance during growing period*

Measurement (weeks)	Added biotin	Males[2]				Females[3]			
	Breeder:	−	−	+	+	−	−	+	+
	Growing:	−	+	−	+	−	+	−	+
Body weight (kg.)									
1		0.122	0.120	0.121	0.121	0.116	0.113	0.113	0.115
3		0.470	0.386	0.471	0.391	0.493	0.364	0.410	0.367
6		1.47	1.36	1.49	1.36	1.22	1.14	1.23	1.16
11		3.97	3.80	4.00	3.78	3.10	3.02	3.13	3.04
18		7.36	6.98	7.29	6.95	5.42	5.28	5.46	5.28
Condition of hocks, score[4]									
3		1.74	2.82	1.74	2.57	1.28	2.29	1.48	2.23
6		2.02	2.47	2.02	2.40	1.19	1.44	1.27	1.51
11		2.23	2.17	2.20	2.33	1.04	1.04	1.08	1.09
Condition of toes, score[5]									
3		1.52	2.13	1.64	2.34	2.13	1.77	1.93	1.64
6		1.25	1.42	1.34	1.39	1.70	1.37	1.52	1.28
11		1.20	1.42	1.46	1.43	1.42	1.24	1.28	1.18
Condition of pads, score[6]									
3		1.30	1.59	1.19	1.63	1.48	1.44	1.32	1.39
6		1.88	1.86	1.72	1.90	2.44	2.26	2.27	2.12
11		2.06	2.20	1.95	2.15	2.49	2.55	2.21	2.39
Condition of feathers, score[7]									
3		1.57	1.55	1.64	1.63	1.58	1.62	1.56	1.63
6		1.43	1.57	1.42	1.54	1.35	1.45	1.52	1.47
11		1.80	1.99	1.83	1.94	1.56	1.65	1.61	1.70

[1] Levels of added biotin in breeding and starting diets were 300 and 100 mcg./kg. respectively.
[2] The number of males at 1, 3, 6, 11 and 18 weeks was 695, 695, 686, 649 and 605, respectively.
[3] The number of females at 1, 3, 6, 11 and 18 weeks was 679, 679, 674, 656 and 637, respectively.
[4] Condition of hocks scored as follows: 1, normal; 2, questionable enlargement; 3, definite enlargement; 4, enlarged and flattened or twisted; 5, slipped tendon. The more severely affected leg served as basis for score.
[5] Toes were scored as follows: number of phalanges with fissures were added together with an added point if blood was present.
[6] Foot pads scores: 1, normal; 2, rough; 3, calloused; 4, calloused and bloody.
[7] Scoring of flight feathers: 1, normal; 2, barbs missing and/or shaft broken; 3, feathers missing; 4, combination of 2 and 3.

intention of this report to conclude that additional biotin detracted from performance; rather the data are included for comparison in event other workers find similar results.

Experiment 2. The separate poult study in Table 5 was established to determine whether selected dietary drugs can suppress intestinal synthesis or adversely affect utilization of certain vitamins with turkey poults fed corn-soybean meal type diets and thereby cause a deficiency condition and need for supplementation.

The analysis of variance indicated that the drug treatments were mainly responsible for growth responses observed and that B-vitamin supplementation had no effect. The addition of zinc bacitracin and streptomycin to the ration significantly ($P<0.05$) increased poult growth over the control (when treatments with and without vitamin supplementation were combined).

Under the conditions of this experiment, the high level feeding of the various drugs did not depress growth, produce evidence of dermatitis, or other symptoms associated with biotin deficiency. Merely using this selection of drugs does not rule out the possibility of other drugs, drug

TABLE 5.—*Influence of selected drug treatments on performance of turkeys fed natural diet without and with additional B-vitamins*[1]

Treatment[2]	Body weight at three weeks	Feed / gain	Mortality
	gm.		%
No additional B-vitamins			
Control	453 ab[3]	1.74 bcd[3]	0.0
Bacitracin	514 c	1.49 a	0.0
Streptomycin	495 bc	1.52 ab	0.0
Amprolium	449 ab	1.62 ab	0.0
Antibiotic mixture	481 abc	1.53 ab	0.0
Antibiotic-vitamin injection	452 ab	1.92 d	33.3
With additional B-vitamins[4]			
Control	439 a	1.67 abc	4.1
Bacitracin	490 bc	1.51 ab	0.0
Streptomycin	506 c	1.56 ab	4.1
Amprolium	449 ab	1.68 abc	0.0
Antibiotic mixture	472 abc	1.56 ab	0.0
Antibiotic-vitamin injection	434 a	1.89 cd	33.3

[1] Broad White male turkeys started on experiment at one day of age; each treatment contained three replicate groups of 8 poults each.

[2] Details of treatments (shown are dietary levels, except injection treatment):

Bacitracin was zinc bacitracin (pharmaceutical grade) 0.11%, streptomycin was dihydro streptomycin sulfate (crystalline) 0.10%, and amprolium 0.075%, supplied by Amprol® (Merck and Co.) a 25% premix with corn gluten feed and soybean oil.

Antibiotic mixture: sulfaquinoxaline (0.035%) supplied by a 40% premix (Merck) on wheat standard middlings, soybean oil, and soy lecithin; furazolidone (200 gm./ton) supplied by NF-180® (Hess and Clark) in an 11% premix on corn meal; and chlortetracycline·HCl (200 gm./ton) supplied as Aureomycin® (American Cyanamid) at a concentration of 22 gm./kg. on degerminated corn meal and soybean meal.

Antibiotic-vitamin injection at day of age: each 0.2 cc. of aqueous antibiotic solution was injected subcutaneously in posterior of each poult's neck and provided neomycin sulfate 3.08 mg., streptomycin sulfate 19.4 mg., procaine penicillin G 4,600 units, thiamine·HCl 0.14 mg., riboflavin 0.028 mg., niacin 1.4 mg., pyridoxine·HCl 0.05 mg., and calcium pantothenate 0.05 mg.

[3] Means with different letters are significantly different at $P<0.05$ (Duncan's multiple range test, see Steel and Torrie, 1960).

[4] The following additional B-vitamins were included (mg./kg. diet): biotin 0.225, vitamin B_{12} 0.003, folic acid 0.9, pyridoxine·HCl 3.0, and thiamine·HCl 2.0.

combinations, or environmental stresses affecting synthesis or utilization of biotin to the point where deficiency might occur. By calculation, those diets which did not include the B-vitamin supplement contained 145 mcg. biotin per kg., which is considerably lower than the 225 mcg. per kg. requirement suggested by Scott (1968). (However, as described in the methods section, a somewhat higher biotin level was obtained in these diets by analysis.)

Mortality caused by antibiotic injection occurred in all replicates within 12 hours after injection. This deleterious effect was not observed in a previous experiment; no explanation is apparent in this instance.

SUMMARY

A study was conducted with 288 turkey breeder hens and 1,374 progeny to determine the influence of supplementary biotin at both ages. Addition of 300 mcg. biotin/kg. to a corn-soybean type breeder diet containing 105 mcg. of biotin per kg. by calculation did not improve hen or progeny performance. Addition of biotin to the poult diet did not result in improved weight gains, condition of feathering, or reduced incidence of leg deformities.

In another experiment, various high level drug treatments did not induce a deficiency of biotin, folic acid, vitamin B_{12}, thiamine or pyridoxine with a simplified diet containing corn, soybean meal, 5% fish meal, minerals, and vitamins. Positive growth responses were produced by zinc bacitracin and streptomycin.

ACKNOWLEDGEMENT

The following firms generously provided materials for this study: Abbott Laboratories, No. Chicago, Ill.; American Cyanamid Co., Princeton, N. J.; Commercial Solvents, Corp., Terre Haute, Ind.; Distillation Products Industries, Rochester, N. Y.; E. I. du Pont de Nemours and Co., Western Springs, Ill.; Hoffmann-LaRoche, Inc., Nutley, N. J.;

Merck and Co., Rahway, N. J.; Nopco Chemical Co., Newark, N. J.; and Van Hoven Co., Inc., So. St. Paul, Minnesota.

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Weirich-'60
Bib No.

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69. E. G. Weirich - Freiburg i. Brsg.: Investigations of the Onychogenic Effect of Biotin. With 3 figures.

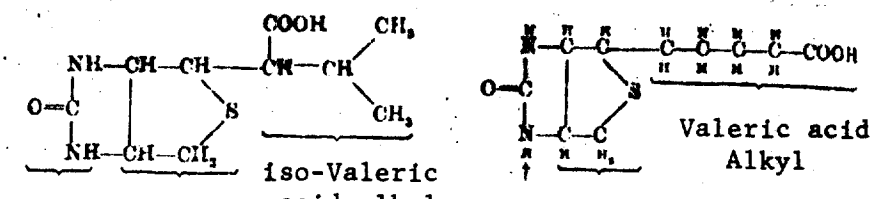
In the realm of our investigations on the therapeutic possibility of influencing the growth and formation of nails we examined, among other things, Biotin for its onychogenic effect.

Because of its complicated history of discovery - mainly in the foreign literature on this field - biotin, which belongs to the vitamin B complex, is known equivalently under various names:

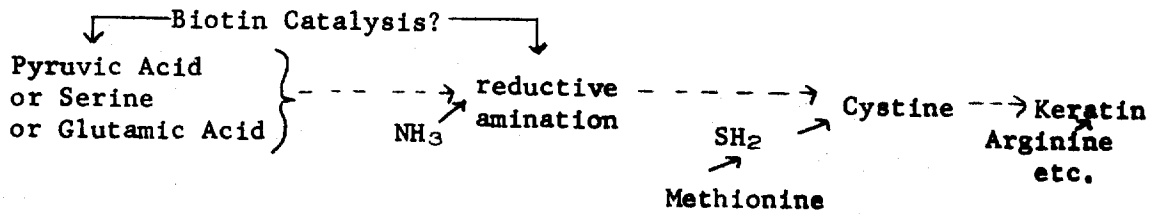
Table 1. Biotin Synonyms and Authors in Chronological Order

1. Bios II	nach Lucas (1924)
2. Faktor X	nach Boas (1927)
3. Vitamin H	nach György (1931)
4. Coenzym R	nach Allison, Hoover und Burk (1933)
5. Bios II b	nach Miller (1934)
6. Anti-egg-white-injury-factor	nach Leason und Parsons (1934)
7. Biotin	nach Kögl (1935)
8. Faktor W	nach Elvehjem (1936)
9. Vitamin B_w	nach Lunde und Kringstad (1940)
10. Skin-factor	nach Marshall (1939)
11. Alpha/beta-Biotin	nach Kögl (1943)

With the help of complicated decomposition reactions, its chemical composition was first explained in 1942 by du Vigneaud, Hofmann and Melville and confirmed in 1943 by Kögl, who, at the same time, pointed out the existence of two (in the valerianyl side chains) structure - isomers of biotin (alpha- and beta-biotin). Harris and co-workers first succeeded in 1943 to synthesize biotin (above its first stages "Desthiobiotin").

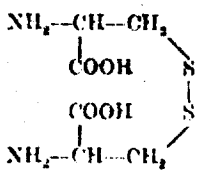


α-Biotin ($C_{10}H_{16}O_3N_2S$) β-Biotin ($C_{10}H_{16}O_3N_2S$)

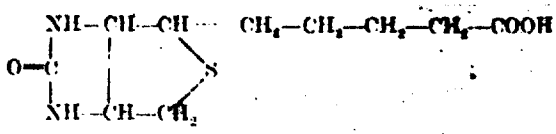


Hypothetical Role of Biotin in Keratin Synthesis

Cystine



β-Biotin



Structural Similarity of Cystine and Biotin (without side chains)

The spatial constitution of the biotin molecule was explained partially by x-ray analysis (Harris).

The clinical effects of biotin, already described by numerous authors, shall not be entered into here (see excellent presentations by Leipold, Geimer, Stepp-Kühnau-Schroeder, etc.). Fundamentally characteristic of biotin is a promoting or stabilizing effect on physiological cell formation and regeneration in the skin and mucous membranes as well as in red bone marrow (erythropoiesis); further, a regulatory effect on lipoid metabolism. Hence, its general biological value as a growth factor ("growth hormone") for numerous plants (which explains the high biotin content of colloidal humin material pointed out by Schmid) for yeast, fungus and bacteria, its significance as an essential metabolite for almost all animal types and its strikingly high concentrations in embryonic tissue and in tumors (Kögl, v. Hasselt West and Wogland, Rhoads and Abels, Oppelt et al., Burk and Winzler, Geimer, Gerloff, etc.).

Because of the especially marked, continual regeneration activity of skin, hair and nails, a functional influence of biotin on these tissues or their germanitive structures can theoretically be assumed, in analogy to the abundance of biotin of the meristematic plant tissues (root tips, coleoptile tips).

The influence, especially on the growth of nails, can be derived from the indicated metabolic functions of biotin and can be explained by a catalysis of keratinsynthesis. The metabolic functions of biotin are given below in a gross outline:

Table 2. Indicated Metabolic Functions of Biotin

A. Elementary Functions

1. Effect as Transcarboxylase

Decarboxylation, carboxylation and transfer of CO_2 groups by alternate opening and closing of the urea bridge in the biotin molecule (Burk and Winzler, Lardy et al., Gerloff etc. Proof by isotope analysis, among others: C^{14} atoms in CO_2 groups) (keratin synthesis!)

2. Effect as Transaminase

Deamination, amination and transfer of NH_2 groups; mechanism still unexplained (Lichstein, Harris, et al., György etc.) (keratin synthesis!)

B. Resultive Functions

1. Effectiveness as a catalyst in the Citric Acid Cycle by activation of oxaloacetic acid formation from pyruvic acid + CO_2. (Lardy et al., Gresham et al., Shive and Rogers, Stores et al.)

2. Promotion of Pyruvic Acid Metabolism (Sydenstricker et al., Gerloff etc. Biotin lowers the oxidation value of pyruvic acid metabolism in the rat liver).

3. Promotion of Pantothenic Acid Metabolism (Schoop, Gerloff etc. As with pantothenic acid, biotin can provoke a graying of the hair or shedding of the body hair of young mink).

C. Correlative Functions

1. Stimulation of fat synthesis, increase of liver cholesterol (Gavin and McHenry).

2. Depression of serum-cholesterol level (Sydenstricker et al.).

3. Control of the lipoid content of the skin (Kirk, Geimer, Abderhalden, Berger, Leipold, Kuhn, Ferreira-Marques).

4. Stimulation of erythropoiesis (Sydenstricker et al., Schmid etc.).

5. Reduction of the bile pigments (Sydenstricker et al.).

Keratin is especially rich in sulfur, which is conditional through the high cystine content of keratin polypeptides; next to cystine, arginine is found in keratin in greater amounts than all others.

Hence, keratinsynthesis, which is responsible for nail formation, must precede a cystine or cysteine synthesis, because cysteine is known to belong to the essential amino acids, which originate exclusive of food. The cysteine synthesis - cysteine easily goes to cystine (double molecule) and can likewise dissociate again from this - proceeds according to Sturm and others in a simple reversal of the physiological decomposition path.

The role of biotin as a catalyst of amination processes and as activator of the utilization of pyruvic acid justifies adopting a corresponding catalytic influence in cysteine synthesis.

The investigations of Leclero also speak for this connection. He obtained an acceleration of nail growth through pantothenic acid; pantothenic acid possibly plays a role of an intermediate metabolite dependent on biotin.

The indicated scheme of keratin synthesis as the decisive basic process of onychogeny finds its confirmation through the findings of:

a) Brown and Klauder: Lowering of the sulfur content of onychodystrophic nails (normally about 3%); increase again and normalization after administering cysteine-rich wool hydrolyzate. Similar findings were obtained earlier by Zuntz, Matoni and others. We are, incidentally, reminded of the "Humagsolan".

b) Knobloch: Demonstrated the effect of methonine (S-donor!) and of cysteine in furthering nail growth by exact measurements (after eight-week medication, about 13% increase of nail growth). - Similar results had been found earlier by Jeghers (cystine), Sidi (cysteine and methionine) and others.

c) Hornemann: Increase of nail growth and normalization of nail structure by gelatin (which preponderantly contains cysteine, glutamic acid, and glycocoll) or especially by glutathione (tripeptide from glutamic acid, cysteine and glycocoll). Similar results had been found earlier by, among others, Leyd-Tyson.

Whether there might exist a direct reaction relation between biotin and cysteine or cystine - perhaps over reversible rearrangement processes or something similar (see the partial structural similarity of cystine and biotin in formula 3) - is unsettled. The findings of Eakins (pimelic acid and cysteine increase biotin synthesis by aspergillus niger) point in this direction, however.

In order to establish the possible influence of biotin on nail growth, we carried out studies of the onychogenic effects of high doses of biotin on various groups of people. Use was made of the onychometric procedures described in detail earlier [Methods see Derm. Wschr. 131 (1955) 219: - Arch. klin. exp. Derm. 204 (1957) 236: - Z. Haut u. Geschl. - Kr. 18 (1957) 32: - Zbl. Haut u. Geschl. - Kr. 99 (1957) 1].

An especially impressive result was seen in a group of five patients with onychodystrophy. All patients were between 18 and 48 years old, for all every fingernail was attacked, all had already tried for a long time (up to several years) every imaginable means and had been treated without result by doctors of various specialties and by lay medical practitioners. In all cases, the nail plates were greatly undeveloped, opaque, overly thick and unevenly arched, had bad lateral folds, were full of ridges, brittle and evinced nail bed hyperkeratosis. The causes of onychodystrophy were: in two cases, an earlier, completed severe nail mykosis (fungus now in the native

preparation and in the culture negative), in one case an earlier completed polyneuritis, in one case a chronic chemotoxic damage (laboratory worker), and in one case idiopathic nature, or cryptogenetic. - All patients of this group were housewives or performed regular house work in addition to their usual occupation.

After the usual preliminary control phase (determination of the primary onychodiagram and of nail growth) of 40 days, these five patients received daily, for a 50-day uninterrupted medication phase, two tablets of biotin, 20 mg each, three times a day for a total of 120 mg per day. This dose had been fixed by us as probably effective in a series of preliminary experiments.

The following describes the result of this high dose of biotin medication:

In order to follow the effectiveness of biotin, the nail index (nail characteristic line) as a measure of the nail plate size, the lunula index (lunula characteristic line) as a measure of the development of the lunula, as well as naturally the nail growth, i.e., the daily rate of growth in length of the nail plate, were most important. - The characteristic line of the finger (finger quotient) and the curvature characteristic line (curvature quotient) are omitted from the research onycho diagrams since the finger quotient naturally does not change and the curvature quotient could not be determined exactly because of the appreciable primary deformation of the nail plate.

The positive effect of biotin on the daily rate of nail growth is still clearer in the following presentation. Figure 3 gives the behavior of the biotin effectiveness on nail growth as well as the behavior of the growth prior to (40 days) and after (80 days) the biotin medication; the values for the post phase are no longer calculated from the average and mean values of all five patients but rather from those of three patients. One patient withdrew during the post phase and with another (the case of idiophathic onychodystrophy), the value of nail growth reverted almost immediately after the removal of the biotin from a practically normalized value to the original pathological lower state.

As is immediately evident from the onychodiagrams and behavior curves, biotin medication lead to:

1. A significant acceleration of growth of length of the nails, that is to an increase of the daily rate of about 10% (in overall average).

2. A general renormalization of the nail index, which had previously been lowered as a consequence of the dystrophic deformation and a recovery of the normal form and size of the nail plate, and

3. An almost complete normalization of the lunula index.

With all patients, the previously rudimentary nail plates returned to their natural form, lost their brittleness, were again flat and bright, of normal coloring and firmness; they grew again symmetrically over the nail beds, now free from hyperkeratosis, had to be cut more often, etc. - In three cases, this success lasted even after the discontinuation of the biotin, as previously mentioned.

For one comparison group of five healthy women, we could establish an acceleration of the nail growth by biotin of, one the average, 4% and with a collection of eight healthy men a growth acceleration, on the average, of 6%; singly, for these groups, the acceleration values for different finger nails lay within the physiological fluctuation range of the daily rate of growth.

In the meantime, Roth has done further comparative experiments at our clinic on the onchyogenic effects of biotin and shown, among other things, that with biotin the daily rate of nail growth of a group of healthy people (10 men and 10 women) increased on the average 5.1% (with psoriasis on the contrary e.g., only about 0.2% and with endogenic excematoid only about 0.5%).

Hence, the influence of biotin on the onychogeny must be considered certain. For therapy of onychosis or onychopathy, there is regretfully the difficulty that biotin must be administered in very large doses, and this first of all is a truly expensive affair.

The magnitude of the natural and therapeutic biotin concentrations is given in the following informative survey:

Table 3

Magnitude of Natural and Therapeutic Biotin Concentrations

A rat-unit of biotin = that daily dose which brings relief from the biotin deficiency syndrome in rats, after 4 weeks of administration (György)

28,000 rat-units = 1 mg biotin

Biotin content of the yolk of a duck-egg: Kögl obtained 1.1 mg of biotin from 250.0 kg of dried duck-egg yolk

Biotin content of a human liver: about 0.1 mg-%

Biotin content of human neoplasm: about 0.05 - 0.5 mg-%

Curative biotin dose for rats: 0.036 γ/dose (0.0000357 mg/dose)

Therapeutic (curative) biotin dose for man according to the classical indication (dermatitis seborrhoides etc.): 10.0 mg/dose = 28,000 rat units

(Continuation of Table 3)

Our therapeutic biotin dose (for onychodystrophy): 120.0 mg/dose = 3.6 million rat-units

We could not establish any harmful side effects of the biotin medication with our large dosage.

More or less incidentally, generally first from spontaneous expression of the people in the experiment or the patients, we observed other secondary phenomena: increased fat deposits with increase of body weight (we reproduced this phenomenon in one case, after an intermediate anti-fat cure, without any difficulty), improvement of appetite, disappearance of chronic "skin uncleanliness", recession of sebum secretion and dandruff formation, lessening of feelings of thirst (in two cases!) etc. Although we did a (simple) trial experiment in many cases to clarify the form of biotin effectiveness and we obtained positive results, i.e., the placebo did not show these effects (general comment of the subjects: "This time it has not done as much good" - this occurred after about 2 to 3 weeks), we make no attempt at the hypothetical significance of our observations.

Nail Growth and Behavior during Onychodystrophy/Without Biotin

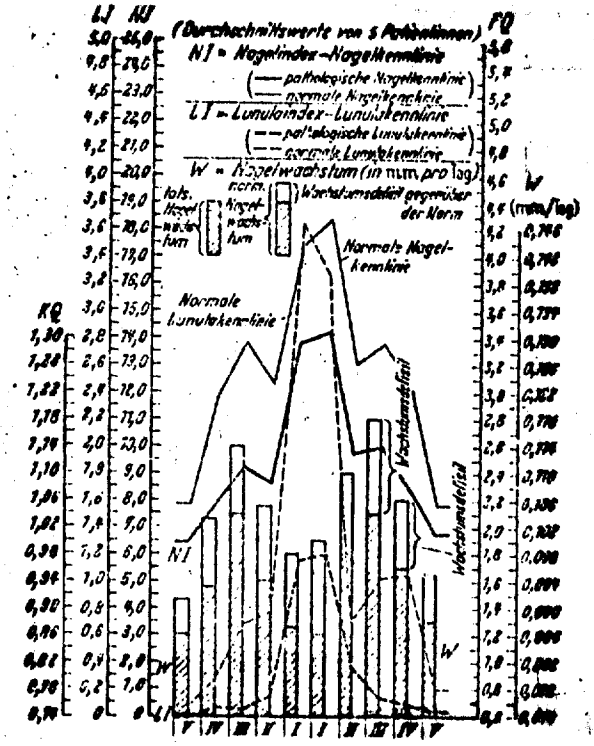


Fig. 1

Note: In numbers, translate comma as decimal point.

Translations:

LJ = Lunula Index

NJ = Nail Index

FQ = Finger Index

KQ = Curvature Quotient

W(mm/Tag) = Growth (mm/day)

Durchschnittswerte von 5 Patientinnen = Average values from 5 patients

Nagelindex-Nagelkennlinie = Nail Index-Nail Characteristic Curve

pathologische Nagelkennlinie = pathological nail curve

normale Nagelkennlinie = normal nail curve

Lunulaindex-Lunulakennlinie = Lunula Index-Lunula Curve

Nagelwachstum (in mm pro Tag) = nail growth (in mm/day)

tats. Nagelwachstum = actual nail growth

Wachstumdefizit gegenüber der Norm = Growth deficit compared to normal

Nail Growth and Behavior for Onychodystrophy/After 50 Days of Biotin Therapy (120 mg/dose)

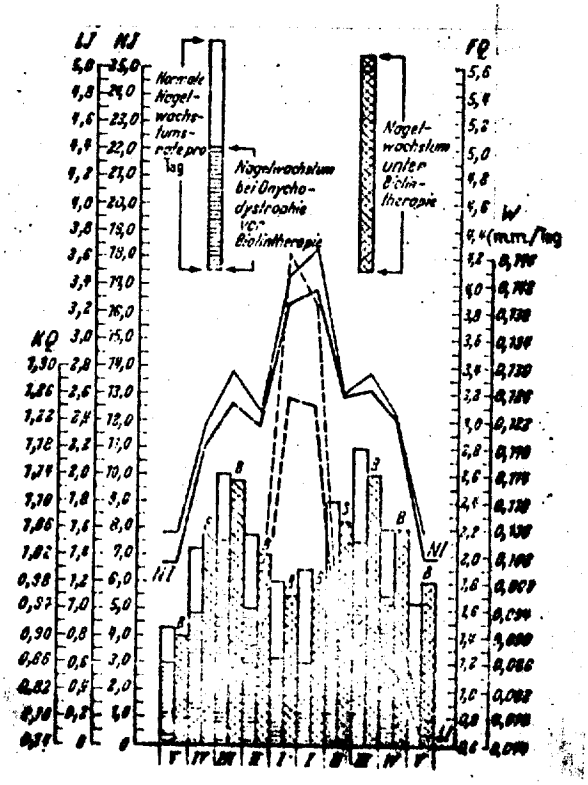


Fig. 2

Note: In numbers, translate comma as decimal point.

Translations:

LJ = Lunula Index

NJ = Nail Index

FQ = Finger Index

KQ = Curvature Quotient

W(mm/Tag) = Growth (mm/day)

Normale Nagelwachstumsrate pro Tag = Normal nail growth-rate per day

Nagelwachstum bei Onychodystrophie vor Biotintherapie = Nail growth for onychodystrophy prior to biotin therapy

Nagelwachstum unter Biotintherapie = Nail growth under biotin therapy

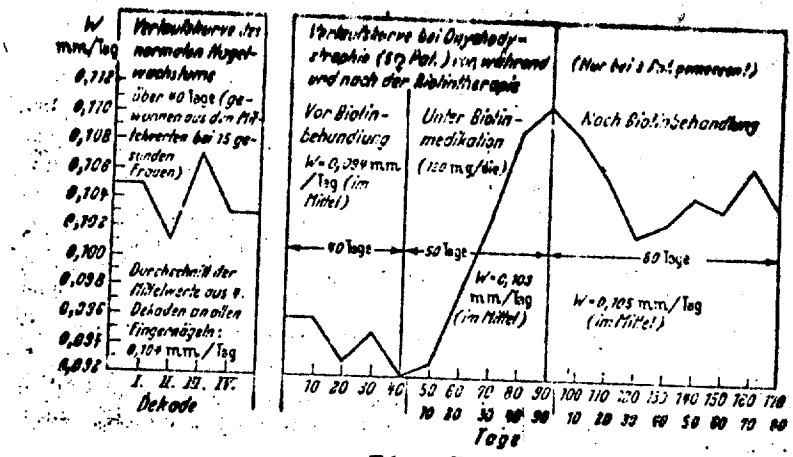


Fig. 3

Note: In numbers, translate comma as decimal point.

Translations:

W(mm/Tag) = Growth (mm/day)

Verlaufskurve des normalen Nagelwachstums über 40 Tage (gewonnen aus den Mittelwerten bei 15 gesunden Frauen) = Curve of normal nail growth over 40 days (obtained from the mean values of 15 healthy women)

Durchschnitt der Mittelwerte aus 4 Dekaden an allen Fingernägeln: 0,104 mm/Tag = Average of the mean values from the 4 decades for all finger nails: 0.104 mm/day

Dekade = decade

Verlaufskurve bei Onychodystrophie (5 Pat.) vor, wahrend und nach der Biotintherapie = Curve for Onychodystrophy (5 Patients) before, during and after biotin therapy

Vor Biotinbehundlung = before biotin treatment

(im Mittel) = (on the average)

Unter Biotinmedikation = Under biotin medication

(Nur bei 3 Pat. gemessen!) = Measure only for 3 patients

Nach Biotinbehandlung = After biotin treatment

Weirich-'60
Bib No.

Arch. Klin. Exp. Derm. 211:381-389. 1960.

69. E. G. Weirich-Freiburg i. Brsg.: Untersuchungen über die onychogene Wirkung des Biotins. Mit 3 Textabbildungen.

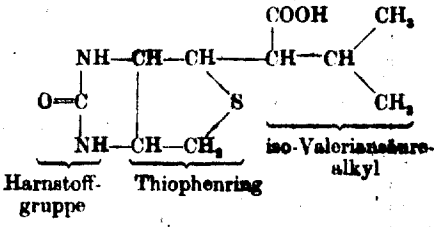
Im Rahmen unserer Untersuchungen über die therapeutische Beeinflußbarkeit des Wachstums und der Gestaltung der Nägel prüften wir u. a. auch *Biotin* auf seine onychogene Wirkung.

Das zum Vitamin-B-Komplex gehörige Biotin ist entsprechend seiner verwickelten Entdeckungsgeschichte — vor allem in der ausländischen Fachliteratur — noch unter verschiedenen anderen Bezeichnungen bekannt.

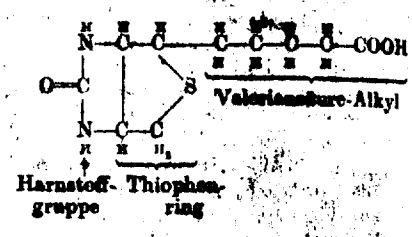
Tabelle 1. *Biotin-Synonyma und Autoren in chronologischer Ordnung*

1. Bios II	nach Lucas (1924)
2. Faktor X	nach Boas (1927)
3. Vitamin H	nach György (1931)
4. Coenzym R	nach Allison, Hoover und Burk (1933)
5. Bios II b	nach Miller (1934)
6. Anti-egg-white-injury-factor	nach Lease und Parsons (1934)
7. Biotin	nach Kögl (1935)
8. Faktor W	nach Elvehjem (1936)
9. Vitamin B_w	nach Lunde und Krimostad (1940)
10. Skin-factor	nach Marshall (1939)
11. Alpha/beta-Biotin	nach Kögl (1943)

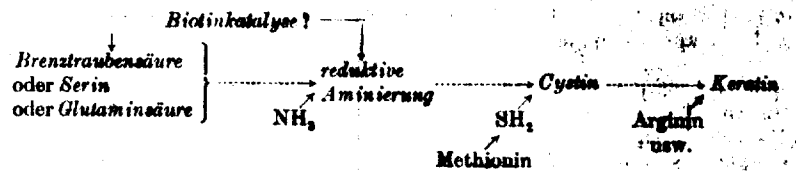
Seine chemische Konstitution wurde mit Hilfe komplizierter Abbaureaktionen durch du Vigneaud, Hofmann und Melville erst 1942 aufgeklärt, durch Kögl — der gleichzeitig die Existenz von zwei (in der Valerianylseitenkette) strukturisomeren Biotinen (alpha- und beta-Biotin) nachwies — 1943 bestätigt. 1943 gelang Harris u. Mitarb. auch erstmals die Synthese von Biotin (über dessen Vorstufe „Desthiobiotin").



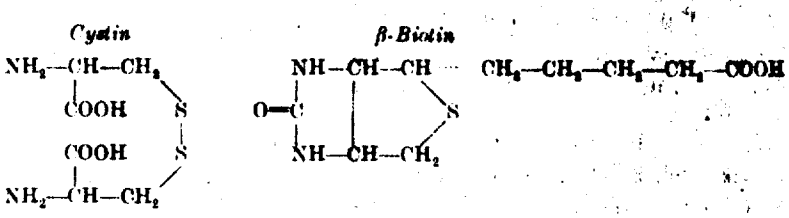
α-Biotin ($C_{10}H_{16}O_3N_2S$)



β-Biotin ($C_{10}H_{16}O_3N_2S$)



Hypothetische Rolle des Biotins bei der Keratinsynthese



Strukturähnlichkeit von Cystin und Biotin (ohne Seitenkette)

Die Raumkonstitution des Biotinmoleküls wurde teilweise röntgenanalytisch aufgeklärt (HARRIS).

Auf die von bereits zahlreichen Autoren beschriebenen klinischen Wirkungen des Biotins soll hier nicht eingegangen werden (ausführliche Darstellungen s. bei LEIPOLD, GEIMER, STEPP-KÜHNAU-SCHROEDER usw.). Grundsätzlich ist für Biotin ein fördernder bzw. stabilisierender Effekt auf die physiologische Zellbildung und Regeneration in Haut und Schleimhäuten sowie im roten Knochenmark (Erythropoese), ferner eine regulierende Wirkung auf den Lipoidstoffwechsel, charakteristisch. Dem entspricht seine allgemeine biologische Wertigkeit als Wachstumsfaktor („Wuchshormon") für zahlreiche Pflanzen (woraus sich der, von SCHMID nachgewiesene, hohe Biotingehalt kolloidaler Huminstoffe erklärt), für Hefen, Pilze und Bakterien, seine Bedeutung als essentieller Metabolit für fast alle Tierarten und seine auffallend hohe Konzentration in embryonalen Geweben und in Tumoren (KÖGL, v. HASSELT, WEBER und WOOLLEY, RHOADS und ABELS, OPPELT et al., BURK und WINZLER, GEIMER, GERLOFF usw.).

Wegen der besonders ausgeprägten, kontinuierlichen Regenerationsaktivität der Haut, der Haare und der Nägel kann theoretisch — auch in Analogie zum Biotinreichtum gerade der meristematischen Pflanzengewebe (Wurzelspitzen, Koleoptilspitzen) — ein funktionaler Einfluß des Biotins auf diese Gewebe bzw. ihre Germanativstrukturen angenommen werden.

Der *Einfluß speziell auf das Nagelwachstum* läßt sich aus den nachgewiesenen metabolischen Funktionen des Biotins ableiten und über eine *Katalyse der Keratinsynthese* erklären. Nachstehend die metabolischen Funktionen des Biotins in groben Umrissen:

Tabelle 2. *Nachgewiesene metabolische Funktionen des Biotins*

A. Elementarfunktionen

1. Wirkung als *Transcarboxylase*
   Decarboxylierung, Carboxylierung und Übertragung von CO_2-Gruppen durch alternierende Öffnung und Schließung der Harnstoffbrücke im Biotinmolekül. (BURK und WINZLER, LARDY et al., GERLOFF usw., Nachweis u. a. durch Isotopenanalyse: Einbau von C^{14}-Atomen in CO_2-Gruppen) (Keratinsynthese!)
2. Wirkung als *Transaminase*
   Desaminierung, Aminierung und Übertragung von NH_2-Gruppen; Mechanismus noch ungeklärt.
   (LICHSTEIN, HARRIS et al., GYÖRGY usw.) (Keratinsynthese!)

B. Resultative Funktionen

1. Katalysatorwirkung im *Citronensäurezyklus*
   durch Aktivierung der Oxalessigsäurebildung aus Brenztraubensäure + CO_2. (LARDY et al., GREENHAM et al., SHIVE und ROGERS, STOKES et al.)
2. Förderung des *Brenztraubensäurestoffwechsels*
   (SYDENSTRICKER et al., GERLOFF usw. Bei Biotinmangel sinken die Oxydationswerte des Brenztraubensäurestoffwechsels der Rattenleber.)

3. Förderung des *Pantothensäurestoffwechsels* (Schoop, Gerlóff usw. Durch Biotinkarenz beim und durch Pantothensäurekarenz bei jungen Nerzen eine Graufärbung der Haare bzw. ein Ausfall der Deckhaare provoziert werden.).

C. Korrelative Funktionen

1. Stimulierung der Fettsynthese, Erhöhung des Leberfettgehalts (Gavin und McHenry).
2. Senkung des Serum-Cholesterinspiegels (Sydenstricker et al.).
3. Steuerung des Lipoidgehalts der Haut (Kirk, Gehrer, Abderhalden, Berger, Leipold, Kuhn, Ferreira-Marques).
4. Stimulierung der Erythropoese (Sydenstricker et al., Schmid usw.).
5. Verminderung der Gallenfarbstoffe (Sydenstricker et al.).

Keratin ist besonders reich (4 bis 5%) an *Schwefel*, was durch den hohen *Cystingehalt* des Keratinpolypeptids bedingt ist; neben Cystin findet sich im Keratin vor allem noch Arginin in größerer Menge.

Demnach muß also der für die Nagelbildung verantwortlichen Keratinsynthese eine *Cystin- bzw. Cysteinsynthese* vorangehen, da ja *Cystein* bekanntlich zu den essentiellen Aminosäuren — welche ausschließlich der Nahrung entstammen — gehört. Die Cysteinsynthese — Cystein lagert sich leicht zu Cystin(Doppelmolekül) zusammen und kann ebenso aus diesem wieder dissoziieren — verläuft nach Sydenstricker u. a. in einfacher Umkehr des physiologischen Abbauweges.

Die Rolle des Biotins als Katalysator von Aminierungsprozessen und als Aktivator der Brenztraubensäureverwertung berechtigt zu der Annahme eines entsprechenden katalytischen Einflusses auf die Cysteinsynthese.

Für diesen Zusammenhang sprechen auch die Untersuchungen von Lucksch, der durch *Pantothensäure* eine Beschleunigung des Nagelwachstums erhielt; Pantothensäure spielt möglicherweise die Rolle eines — von Biotin abhängigen — Zwischenmetaboliten.

Das gezeigte Schema der Keratinsynthese als entscheidendem Grundprozeß der Onychogenie findet seine Bestätigung durch die Befunde von:

a) Brown und Klauder: Erniedrigung des (normaliter um 3% betragenden) Schwefelgehalts onychodystrophischer Nägel; Wiederanstieg und Normalisierung nach Verabreichung *cysteinreicher* Wollhydrolysate. Ähnliches fanden schon früher Zuntz, Matoni u. a. An das „Humagsolan" sei beiläufig erinnert.

b) Knobloch: Beweis der nagelwachstumsfördernden Wirkung des *Methonins* (S-Donator!) und des *Cysteins* durch exakte Messungen (nach 8wöchiger Medikation etwa 13%ige Steigerung des Nagelwachstums). — Ähnliches fanden schon früher Jeghers (Cystin), Sidi (Cystein und Methionin) u. a.

c) Hornemann: Steigerung des Nagelwachstums und Normalisierung der Nagelstruktur durch *Gelatine* (welche vorwiegend *Cystein, Glutamin-*

mure und Glykokoll enthält) bzw. speziell auch durch *Glutathion* (Tripeptid aus Glutaminsäure, Cystein und Glykokoll). Ähnliches fand schon früher u. a. LEYD-TYSON.

Ob zwischen Biotin und Cystein bzw. Cystin möglicherweise eine unmittelbare Reaktionsbeziehung besteht — etwa über reversible Umlagerungsvorgänge oder Ähnliches (s. die teilweise Strukturähnlichkeit von Cystin und Biotin in Formel 3) — ist ungeklärt. Die Befunde von EAKINS (Pimelinsäure und Cystein steigern die Biotinsynthese bei Aspergillus niger) deuten jedoch in diese Richtung.

Um den möglichen *Einfluß des Biotins auf das Nagelwachstum* festzustellen, führten wir mit Hilfe der schon früher eingehend beschriebenen onychometrischen Verfahren [Methodik s. Derm. Wschr. 131, 219 (1955): — Arch. klin. exp. Derm. 204, 286 (1957): — Z. Haut u. Geschl.-Kr. 18, 32 (1957): — Zbl. Haut u. Geschl.-Kr. 99, 1 (1957)] bei verschiedenen Personenkollektiven Untersuchungen über den onychogenen Effekt hochdosierter Biotingaben durch.

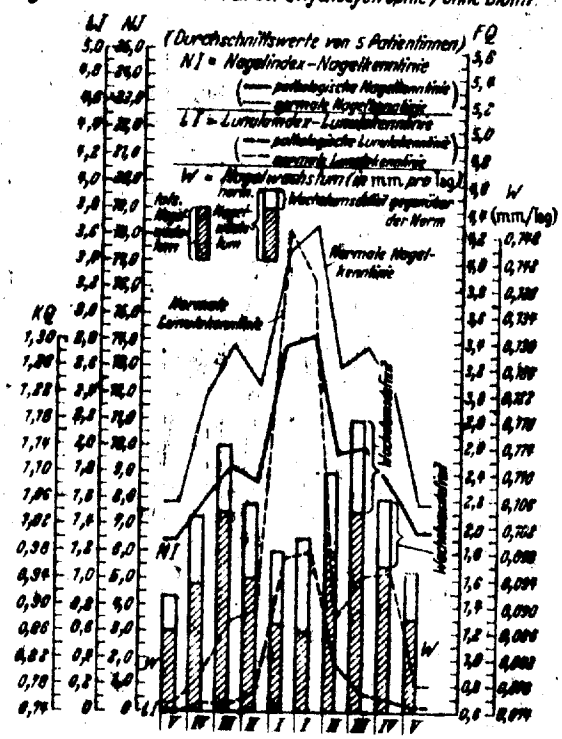


Abb. 1

Ein besonders eindrucksvolles Resultat sahen wir bei einer Gruppe von fünf Patientinnen mit *Onychodystrophie*. Alle Patientinnen waren zwischen 18 und 48 Jahre alt, bei allen waren sämtliche Fingernägel befallen, alle hatten bereits lange Zeit (bis zu mehreren Jahren) jedes nur erdenkliche Mittel versucht und sich ergebnislos bei Ärzten verschiedenster Fachrichtung und bei Heilpraktikern behandeln lassen. In allen Fällen waren die Nagelplatten stark rudimentiert, opak, überstark und unregelmäßig gewölbt, schlecht im seitlichen Falz eingefügt, rissig, spröde und von Nagelbetthyperkeratosen unterlagert. Die Ursache der

Onychodystrophie war in zwei Fällen eine früher durchgemachte schwere Nagelmykose (Pilze jetzt im Nativpräparat und in der Kultur negativ), in einem Fall eine früher durchgemachte Polyneuritis, in einem Fall eine chronische chemotoxische Schädigung (Laborantin) und in einem Fall idiopathischer Natur bzw. kryptogen. — Sämtliche Patientinnen dieser Gruppe waren Hautfrauen oder vernichtete [illegible] sonstigen Befund.

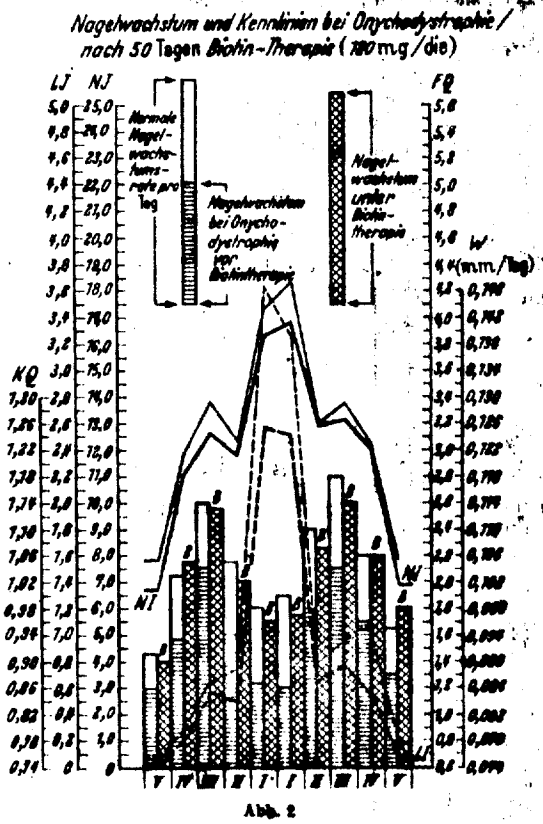


Abb. 2

Diese fünf Patientinnen erhielten — nach der ähnlichen vorherigen Kontrollphase (Ermittlung des primären Onychodiagramms und Bestimmung des Nagelwachstums) von 40 Tagen — während einer ununterbrochenen Medikationsphase von 50 Tagen dreimal 2 Tabletten Biotin zu 30 mg = 180 mg täglich. Diese Dosis war von uns an einer Versuchsreihe als wahrscheinlich wirksam festgelegt worden.

Das Resultat dieser hochdosierten Biotinmedikation zeigt die folgende Darstellung.

Für die Verfolgung der Biotinwirkung war vor allem der *Nagelindex* (Nagelkennlinie) als Maß für die Nagelplattengröße und der *Lunulaindex* (Lunulakennlinie) als Maß für die Ausbildung der Lunula, sowie natürlich das *Nagelwachstum*, d. h. die Tagesrate des Längenwachstums der Nagelplatte, wichtig. — Die *Fingerkennlinie* (Fingerquotient) und die *Krümmungskennlinie* (Krümmungsquotient) sind in den Untersuchungsonychodiagrammen weggelassen, da sich der Fingerquotient naturgemäß nicht änderte und der Krümmungsquotient wegen der erheblichen

primären Deformierung der Nagelplatten nicht exakt bestimmt werden konnte.

Auf den folgenden Darstellungen wird der positive Effekt des Biotins auf die Tagesrate des Nagelwachstums noch deutlicher. Abb. 3 gibt die Verlaufskurve der Biotinwirkung auf das Nagelwachstum sowie auch die Verlaufskurve des Wachstums *vor* (40 Tage) und *nach* (80 Tage) der Biotinmedikation wieder; die Werte der Nachphase sind nicht mehr errechnet aus den Durchschnitts- und Mittelwerten aller fünf Patientinnen, sondern nur noch aus denjenigen von drei Patientinnen. Eine Patientin entzog sich während der Nachphase unserer Kontrolle und bei einer

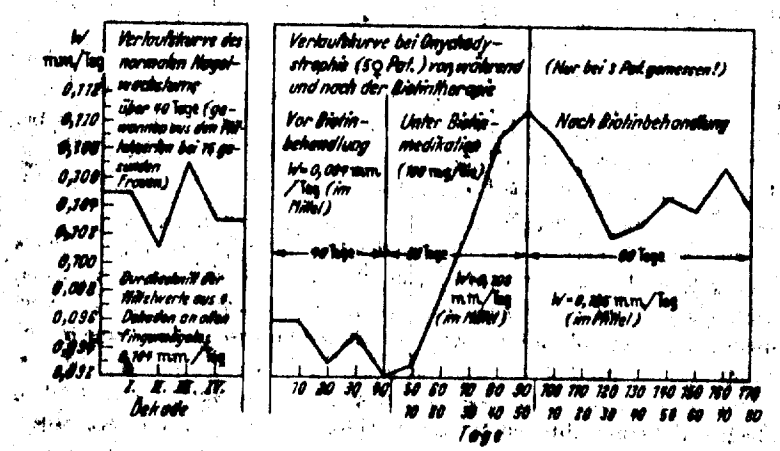


Abb. 3

weiteren Patientin (Fall der idiopathischen Onychodystrophie) gingen fast unmittelbar nach dem Absetzen des Biotins die — zuvor praktisch normalisierten — Nagelwachstumswerte wieder auf den ursprünglichen, pathologisch erniedrigten, Stand zurück.

Wie aus den Onychodiagrammen und der Verlaufskurve ohne weiteres ersichtlich, kam es unter der Biotinmedikation:

1. zu einer signifikanten Beschleunigung des Nagellängenwachstums, nämlich zu einer Erhöhung der Tagesrate um etwa 10% (im groben Durchschnitt),

2. zu einer allgemeinen Renormalisierung des — infolge der dystrophischen Deformationen — vorher pathologisch erniedrigten Nagelindex, somit also zu einer Wiederherstellung der normalen Form und Größe der Nagelplatten und

3. zu einer nahezu vollständigen Normalisierung des Lunulaindex.

Bei sämtlichen Patientinnen gewannen die vorher rudimentären Nagelplatten ihre natürliche Form zurück, verloren ihre Sprödigkeit,

wurden wieder glatt und glänzend, von normaler Färbung und Festigkeit; sie wuchsen nun wieder gleichmäßig über das — hyperkeratosenfreie — Nagelbett vor, mußten wieder öfter beschnitten werden usw. — In drei Fällen hielt dieser Erfolg — wie bereits erwähnt — wahrscheinlich auch nach dem Absetzen des Biotins noch an.

Bei einer *Vergleichsgruppe von fünf gesunden Frauen* konnten wir unter Biotin eine *Beschleunigung des Nagelwachstums* von durchschnittlich 4% und bei einem *Kollektiv von acht gesunden Männern eine Wachstumsbeschleunigung* von durchschnittlich 6% feststellen; vereinzelt lagen bei diesen Vergleichsgruppen die Beschleunigungswerte an verschiedenen Fingernägeln eben noch innerhalb der physiologischen Schwankungsbreite der Wachstumstagesrate.

Inzwischen hat Roth an unserer Klinik weitere vergleichende Untersuchungen über den onchyogenen Biotineffekt angestellt und dabei u. a. nachgewiesen, daß unter Biotin die Tagesrate des Nagelwachstums bei einem *Kollektiv Gesunder* (10 *Männer* + 10 *Frauen*) um durchschnittlich 5,1% anstieg (bei Psoriasis hingegen z. B. nur um 0,2% und bei endogenem Ekzematoid nur um 0,5%).

Somit muß tatsächlich ein Einfluß des Biotins auf die Onychogenese als sicher gelten. Für die Therapie von Onychosen bzw. Onychopathien ergibt sich leider die Schwierigkeit, daß Biotin in sehr hohen Dosen verabreicht werden muß und dies vorerst eine recht kostspielige Angelegenheit ist.

Die Größenordnung der natürlichen und der therapeutischen Biotinkonzentrationen ergibt sich aus der nachfolgenden orientierenden Übersicht:

Tabelle 3

Größenordnung der natürlichen und der therapeutischen Biotinkonzentrationen

Eine Ratteneinheit Biotin = diejenige Tagesdosis, welche nach 4wöchiger Verabreichung das Biotinmangelsyndrom der Ratte zum Verschwinden bringt (Grösser)

28000 *Ratteneinheiten = 1 mg Biotin*

Biotingehalt des Enteneigelbs: Kögl gewann aus 250,0 kg getrocknetem Enteneigelbe *1,1 mg Biotin*

Biotingehalt der Menschenleber: etwa *0,1 mg-%*

Biotingehalt menschlicher Neoplasmen: etwa *0,05—0,5 mg-%*

Kurative Biotindosis bei Ratten: 0,036 γ/die (0,0000387 mg/die)

Therapeutische (kurative) Biotindosis beim Menschen nach der klassischen Initiation (Dermatitis seborrhoides usw.): *10,0 mg/die = 280000 Ratteneinheiten*

Unsere therapeutische Biotindosis (bei Onychodystrophien): *120,0 mg/die = 3,6 Mill. Ratteneinheiten*

Schädliche Nebenwirkungen der Biotinmedikation konnten wir auch bei unseren hoher Dosierung nicht feststellen.

Mehr oder weniger beiläufig, gewöhnlich erst auf spontane Äußerung der Versuchspersonen bzw. der Patientinnen hin, beobachteten wir an sonstigen Nebenerscheinungen: Verstärkten Fettansatz mit Zunahme des Körpergewichts (dieses Phänomen reproduzierten wir in einem Falle, nach einer zwischengeschalteten Entfettungskur, sogar ohne Schwierigkeiten), Verbesserung des Appetits, Verschwinden chronischer „Hautunreinigkeiten", Rückgang von Talgsekretion und Kopfschuppenbildung, Verminderung des Durstgefühls (in zwei Fällen) usw. Obwohl wir in verschiedenen Fällen einen (einfachen) Blindversuch zur Klärung derartiger Biotinwirkungen einschoben und dabei positive Resultate erhielten, d. h. das Placebo diese Wirkungen nicht zeigte (üblicher Kommentar der Versuchspersonen: „Dieses Mal hat es nicht mehr so gut gewirkt" — dies bezog sich auf einen Zeitraum von gewöhnlich etwa 2 bis 3 Wochen), möchten wir vorerst keine hypothetische Deutung unserer Beobachtungen versuchen.

Welch-'43
Bib No. 1461

J. Nutr. 25:555-570. 1943.

THE ROLE OF "FOLIC ACID" AND BIOTIN IN THE NUTRITION OF THE RAT

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(Received for publication January 19, 1943)

The recognition of the importance of thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, inositol, p-aminobenzoic acid and choline as essential components of the diet has made it possible to rear rats on highly purified rations at a growth rate approximating that obtained on good commercial foods (Jukes, '40; Unna et al., '41; Richardson et al., '41; Martin, '42). Evidence is now accumulating, however, that purified rations containing these added factors are adequate, not because they include all exogenous substances required for growth, but because the synthetic activity of certain intestinal bacteria also supplies essential factors. Such factors are normally provided by a diet composed of relatively crude components.

When certain sulfonamides are incorporated into highly purified diets, typical signs of dietary deficiency are produced (Black et al., '41; Mackenzie et al., '41; Daft, Ashburn and Sebrell, '42; Daft, Ashburn, Spicer and Sebrell, '42; Welch, '42). For this purpose sulfonamides which are poorly absorbed from the intestinal tract have proved particularly useful. Black, McKibbin and Elvehjem ('41) employed sulfaguanidine (sulfanilylguanidine) at levels of from 0.5 to 2.0% in a purified diet fed to rats and noted that the growth rate of the animals was markedly reduced. The addi-

tion of p-aminobenzoic acid to the diet was only partially effective in preventing the decrease in rate of growth caused by the sulfonamide. On the other hand, these workers found a liver extract to be completely effective in counteracting the effect of the drug. Another sulfonamide, succinylsulfathiazole (2-(N^4-succinyl-sulfanilamido)-thiazole), which is less toxic than sulfaguanidine (Welch et al., '42) and which is absorbed to an even lesser degree from the alimentary tract was found by Welch ('42) to produce a similar effect on the growth of the rat when added to a highly purified ration to the extent of 1.0 to 2.0%. Although the addition of liver extract to a diet containing succinylsulfathiazole was effective in maintaining a normal growth rate, p-aminobenzoic acid was reported as failing to exert its anticipated influence on the action of the sulfonamide.

The extremely low toxicity of succinylsulfathiazole, the very poor absorption of the drug from the alimentary tract, and the pronounced effect of the compound on certain intestinal bacteria, suggested that this drug might be useful in the detection of those factors normally synthesized by the bacteria of the intestinal tract. The work herein reported describes the identification of two factors which must be supplied to the rat to permit good growth and development when succinylsulfathiazole is incorporated in a synthetic ration adequate in the well-recognized dietary essentials for this species. During the progress of this work Black et al. ('42) and Nielsen and Elvehjem ('42) reported that the growth inhibition produced by succinylsulfathiazole can be largely overcome by dietary supplements of "folic acid" concentrate [1] and biotin. Under somewhat different conditions additional evidence has been accumulated in this laboratory which points to the essentiality of "folic acid" and biotin as dietary constituents when succinylsulfathiazole is incorporated in highly purified diets.

[1] The term "folic acid" refers to concentrates which furnish an essential growth factor for *L. casei*. Although the activity of the concentrates in rats is attributed to their "folic acid" content, other substances are present, since the materials used contained only a small proportion of actual "folic acid."

EXPERIMENTAL

Experiments directed toward the identification of the factors which are required by the rat, apparently synthesized by intestinal bacteria, and present in liver extract, have involved the testing of many substances of known structure and the assay of fractions derived from various crude sources of active material.

During the progress of the study the basal diets have been modified from time to time as indicated in table 1. Rations

TABLE 1

Composition of basal diets employed in feeding experiments.

	S–1	S–4–A	S–4–B	S–7
	gm.	gm.	gm.	gm.
Casein (SMA or Labco)	18.0	18.0	18.0	18.0
Hydrogenated cottonseed oil (Primex)	...	...	...	10.0
Corn oil	2.0	2.0	2.0	2.0
Sucrose	76.0	76.0	76.0	59.9
Salts[1]	4.0	4.0	4.0	4.0
Cellu flour	...	...	...	4.0
A, D and E concentrate[2]	*	*	*	0.08
Choline chloride	0.2	0.1	0.1	0.1
	mg.	mg.	mg.	mg.
Thiamine hydrochloride	3.0	3.0	30.0	0.2
Riboflavin	3.0	3.0	30.0	0.4
Pyridoxine hydrochloride	3.0	3.0	30.0	0.2
Nicotinic acid	2.5	2.5	25.0	4.0
Calcium pantothenate	2.0	2.0	20.0	4.4
p-Aminobenzoic acid	...	...	...	4.0
Inositol	...	...	...	8.0

[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 milliliter, 7 gm.; α-tocopherol, 2 gm.; corn oil, 41 gm.

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

containing succinylsulfathiazole were prepared by substituting it for an equivalent amount of sucrose. The animals used in the growth studies were male black and piebald weanling rats.[3] Each animal was caged individually over wide-mesh screening. Food and water were supplied ad libitum.

[3] Sunny Hill strain.

Succinylsulfathiazole, when incorporated to the extent of 5 or 10% in a complete diet of natural materials,[3] caused no demonstrable effect on the rate of growth of rats, as compared with the growth rate of animals maintained on the same diets without drug (table 2). The animals appeared normal in every way and no evidence of pathological changes was demonstrable histologically. However, much smaller amounts of succinylsulfathiazole (1 or 2%) were effective in producing marked inhibition of growth when added to purified rations which were otherwise adequate in their content of the hitherto recognized essential dietary factors. The drug appeared to have little if any effect on the growth rate during the first or second week of feeding; evidence of the

TABLE 2

Effect of succinylsulfathiazole, when added to a complete commercial diet,[1] on the growth rate of rats.

LEVEL OF ADDED SUCCINYL-SULFATHIAZOLE	NUMBER OF ANIMALS	ORIGINAL WEIGHT [2]	DURATION OF EXPERIMENT	GAIN IN WEIGHT	
				Total [2]	Per week
		gm.	*days*	*gm.*	*gm.*
None	10	113 ± 4.1	33	98 ± 6.4	14.9
5%	10	115 ± 4.4	33	106 ± 3.8	16.1
None	7	75 ± 8.8	44	152 ± 10.6	24.2
10%	8	77 ± 4.5	44	167 ± 6.7	26.5

[1] Wayne brand.

[2] These weights are expressed as averages, together with the standard error: $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

inhibition of growth was apparent during the third and fourth week and was marked thereafter (table 3). Other effects on the animals were seen frequently but irregularly. These included alopecia, usually restricted to a definite pattern, spectacled eyes and pan-ophthalmitis, porphyrin-caked whiskers, and achromotrichia.

It was reported (Black et al., '41) that p-aminobenzoic acid (PABA) is partially effective in combatting the growth-inhibiting effect of sulfaguanidine. When succinylsulfathiazole, at a level of 1%, was employed as the inhibitor of growth, the administration of PABA also appeared to have only a partial effect in suppressing the action of the sulfonamide

[3] Either Wayne brand or Purina brand.

(table 4). Methionine, which is known to have some antisulfonamide action (Kohn and Harris, '41), was ineffective in promoting growth when added to the basal diet (succinylsulfathiazole, 1%) to the extent of 0.6%; inositol was without effect when fed at a level of 0.25%. Biotin, at a level of 20 μg. per cent, appeared to have a slight stimulating effect on growth. A yeast concentrate and a liver extract, each at a level of 1%, partially antagonized the effect of the sulfonamide, while an extract of rice polishings, at the same level, was possibly more active. The effect of proteose-peptone [4]

TABLE 3

Effect of succinylsulfathiazole, when added to a synthetic ration,[1] on the growth rate of rats during a period of 6 weeks.

LEVEL OF ADDED SUCCINYL-SULFATHIAZOLE	NUMBER OF ANIMALS	AVERAGE WEEKLY GAIN IN WEIGHT DURING			TOTAL GAIN IN WEIGHT [2]
		0–2 wk.	2–4 wk.	4–6 wk.	
%		gm.	gm.	gm.	gm.
0	20(0) [3]	23.0	15.0	11.7	99.5 ± 4.3
1	19(1)	22.4	11.4	3.9	75.5 ± 2.8
2	10(0)	21.8	5.5	0	53.3 ± 2.1
5	7(3)	17.9	7.7	— 3.6	44.1 ± 4.5

[1] Diet S-1 was employed.

[2] These weights are expressed as averages, together with the standard error.

[3] Figures in parentheses indicate the number of additional animals which failed to survive the 6-week test period; data on these animals are not included in the growth summary.

(5%) was very striking; not only was the action of the drug abolished, but the gain produced during a 43-day period clearly exceeded that noted for the basal diet without the drug. That this effect was not due to the additional protein derivatives made available to the animals was shown by the absence of the effect when an equivalent amount of casein was fed.

Before initiating fractionation procedures to characterize the new factor or factors, there were tested several compounds which have been implicated in one way or another as possible essential dietary constituents or growth factors for various species of animals or bacteria. The following compounds or substances were found to be inactive in promoting growth,

[4] Difco brand.

TABLE 4

Effect of various supplements on growth of rats fed a purified diet containing succinylsulfathiazole.

SUPPLEMENT	LEVEL OF SUCCINYL-SULFATHIAZOLE	DIET USED	NUMBER OF ANIMALS	DURATION OF EXPERIMENT	GAIN IN WEIGHT	
					Total [1]	Per week
	%			*days*	*gm.*	*gm.*
None	0	S–1	20	43	99.5 ± 4.3	16.2
None	1	S–1	19	43	75.5 ± 2.8	12.3
PABA (0.2%)	1	S–1	9	43	87.6 ± 5.1	14.3
dl-Methionine (0.6%)	1	S–1	8	37	61.4 ± 2.7	11.6
Inositol (0.25%)	1	S–1	10	43	77.6 ± 3.6	12.6
Biotin concentrate (20 μg. %)	1	S–1	9	43	82.6 ± 1.8	13.4
Yeast concentrate (1%)	1	S–1	10	43	85.9 ± 7.4	14.0
Rice polish concentrate (1%)	1	S–1	9	34	81.2 ± 4.7	16.7
Liver extract (1%)	1	S–1	9	43	87.7 ± 2.6	14.3
Proteose peptone (5%)	1	S–1	10	43	122.3 ± 6.9	19.9
None	0	S–4A	9	42	103.0	17.2
None	2	S–4A	7	42	46.1	7.7
Proteose peptone (1%)	0	S–4A	4	42	118.5	19.7
Proteose peptone (5%)	0	S–4A	5	42	144.0	24.0
Casein (5%)	0	S–4A	4	42	105.3	17.6
Proteose peptone (1%)	2	S–4A	5	42	65.0	10.8
Proteose peptone (5%)	5	S–4A	5	42	76.0	12.7
Calcium pantothenate (18 mg. %)	0	S–4A	5	42	101.6	16.9
Calcium pantothenate (18 mg. %)	2	S–4A	3	42	33.3	5.6
None	0	S–4B	9	42	92.7	15.4
None	2	S–4B	7	42	30.6	5.1
Inositol (0.25%) + PABA (0.25%)	0	S–4B	5	42	101.2	16.9
Inositol (0.25%) + PABA (0.25%)	2	S–4B	4	42	59.5	9.9
Inositol (0.25%) + PABA (0.25%) + biotin (20 μg. %)	0	S–4B	5	42	127.8	21.3
Inositol (0.25%) + PABA (0.25%) + biotin (20 μg. %)	2	S–4B	5	42	93.8	15.6

[1] Some of these weights are expressed as averages, together with the standard error.

when tested alone, in synthetic rations containing succinylsulfathiazole: glutamine, indole-3-acetic acid, anthranilic acid, chondroitin sulfuric acid, gum arabic, calcium gluconate, and adenine.

In a survey of various natural products which were effective in promoting growth in a synthetic diet containing succinylsulfathiazole, powdered grass [5] appeared to have unusual activity. Since it was conceivable that the remarkable effect of proteose-peptone in promoting growth was due to its content of sulfonamide inhibitors (Lockwood and Lynch, '40), and to its ability to promote bacterial growth, rather than to any unusual content of essential dietary factors, a study of the effective factor or factors present in powdered grass was undertaken.

Heat stability

Fifty grams of powdered grass were mixed with 500 ml. of water and autoclaved at 15 pounds pressure for 1 hour. The mixture was filtered and the filter cake repeatedly washed with water. The combined filtrates were evaporated to dryness in a current of air on the steam bath. The activity was readily extracted by water and there appeared to be no heat destruction of the active materials. Extraction of powdered grass with water in a Waring blendor gave a less satisfactory yield of the active fraction.

Adsorption characteristics

The combined filtrates (from the extraction by autoclaving of 50 gm. of powdered grass with 500 ml. of water) were brought to pH 2 and stirred with 20 gm. of Darco G-60 charcoal for 30 minutes; this process was repeated with a 10 gm. portion of charcoal for 15 minutes. The filtrates were neutralized with sodium hydroxide and evaporated to dryness in a current of air on a steam bath. The effect of this extract on the growth rate of rats, on a diet containing 2% succinylsulfathiazole, indicated that the factor or factors involved were completely adsorbed on charcoal (table 5).

[5] Cerophyl.

Elution with barium hydroxide

The combined charcoal residues from the adsorption of powdered grass extract were stirred with four successive 250 ml. portions of saturated aqueous barium hydroxide. The combined eluates were neutralized with sulfuric acid and the filtrate evaporated to dryness. Barium hydroxide was ineffective as an eluting agent for the active material (table 5).

The failure to be eluted from charcoal by saturated barium hydroxide was taken as evidence of the dissimilarity of the factor or factors in question and the so-called G. P. 1 factor of Woolley ('42), which is readily eluted from charcoal by barium hydroxide. Microbiological assay indicated that "folic acid" was not eluted from charcoal by saturated barium hydroxide under the conditions employed.

Precipitation with lead acetate

The combined filtrates from the extraction by autoclaving of 50 gm. of powdered grass with 500 ml. of water were treated with an excess of saturated aqueous lead acetate solution. Excess lead was removed from the filtrate with hydrogen sulfide. The first lead precipitate was decomposed by trituration with excess sulfuric acid, and the lead sulfate removed by filtration. Both fractions were evaporated to dryness and fed as indicated in table 5. Activity was present in both portions and indicated the possible existence of two growth-promoting factors. Microbiological assay showed that 90% of the "folic acid" in powdered grass was present in the lead precipitated fraction.

Ether extraction

Fifty grams of powdered grass were extracted with anhydrous ether in a Soxhlet extractor until a colorless extract was obtained. The ether was evaporated and the unextracted residue was allowed to dry in the air. All the activity appeared to be retained in the unextracted portion (table 5).

TABLE 5

The effect of various 'Cerophyl' fractions on the growth of rats fed a diet containing 2% succinylsulfathiazole.[1]

SUPPLEMENT[2]	LEVEL OF SUCCINYL-SULFA-THIAZOLE	NUMBER OF ANIMALS	DURATION OF EXPERI-MENT	AVERAGE GAIN IN WEIGHT	
				Total	Per week
	%		*days*	*gm.*	*gm.*
None	0	5	28	78	19.5
None	2	4	28	55	13.8
'Cerophyl'	0	6	28	96	24.0
'Cerophyl'	2	6	28	96	24.0
Room temperature extract of 'Cerophyl'	2	7	28	82	20.5
Autoclaved extract of 'Cerophyl'	2	6	28	81	20.2
Room temperature residue of 'Cerophyl'	2	6	28	94	23.5
Autoclaved residue of 'Cerophyl'	2	6	28	67	16.8
Room temperature extract and residue combined	2	5	28	90	22.5
Autoclaved extract and residue combined	2	5	28	82	20.5
None	0	5	42	131	21.9
None	2	5	42	77	12.8
'Cerophyl'	0	5	42	154	25.7
'Cerophyl'	2	5	42	121	20.2
'Cerophyl' extract	2	5	42	131	21.8
'Cerophyl' extract adsorbed at pH 2 with norite	2	8	42	81	13.5
$Ba(OH)_2$ eluate from charcoal after adsorption at pH 2	2	9	42	85	14.2
None	0	3	42	133	22.2
None	2	3	42	66	11.0
'Cerophyl'	0	5	42	149	24.8
'Cerophyl'	2	5	42	142	23.7
Lead acetate filtrate	2	5	42	96	16.1
Lead acetate precipitate	2	6	42	88	14.7
Alcoholic extract of 'Cerophyl'	2	6	42	83	13.8
Alcoholic residue of 'Cerophyl'	2	6	42	133	22.2
Ether extract of 'Cerophyl'	2	5	42	69	11.5
Ether residue of 'Cerophyl'	2	4	42	113	19.0

[1] Diet S–7 was employed.

[2] All supplements were tested at a level equivalent to 5% 'Cerophyl'.

Alcohol extraction

Fifty grams of powdered grass were percolated with absolute alcohol until a colorless extract was obtained. The alcohol was evaporated on the steam bath and the unextracted residue dried in the air. All the activity appeared to be retained in the unextracted portion (table 5).

The occurrence of marked "anti-succinylsulfathiazole" activity in dried grass, the ease of adsorption of activity on charcoal, the failure to be eluted with barium hydroxide, the precipitation by lead acetate, and the insolubility of the material in alcohol and ether, all pointed to the possibility of one of the factors being "folic acid." At this stage of the investigation a personal communication from Dr. C. A. Elvehjem indicated that he had found "folic acid" remarkably effective in promoting growth in a ration containing succinylsulfathiazole. Biotin was found by him to accentuate the growth-promoting effect of "folic acid."

A concentrate of "folic acid" (200,000 Snell-Peterson units per gram) and either a biotin concentrate (SMA no. 200) or crystalline biotin were administered orally by stomach tube daily to rats which had reached a plateau in their growth rate on a synthetic ration containing succinylsulfathiazole (2%). In our hands "folic acid" and biotin, when administered singly, each has some activity in overcoming the growth depression caused by succinylsulfathiazole; however, when these substances are administered together a striking stimulation of growth results (table 6).

In animals fed over several weeks on purified diets containing added succinylsulfathiazole occasional spontaneous bleeding has occurred; sometimes the animals succumbed to an acute blood loss but usually they survived. The prothrombin times have been determined occasionally on experimental animals. In one series of determinations results were obtained as indicated in table 7. Animals receiving only the basal ration containing succinylsulfathiazole had a definite prolongation of the prothrombin time. Supplements of either

TABLE 6

The effect of feeding "folic acid" and biotin to rats fed a diet containing 2% succinylsulfathiazole.

EXPERIMENT NUMBER	DAILY SUPPLEMENT	NUMBER OF ANIMALS	GAIN IN WEIGHT			
			1st week	2nd week	3rd week	3-week period
			gm.	gm.	gm.	gm.
1[1]	None	9	+ 2	— 2	— 7	— 7
1	2 μg. biotin[3]	4	+ 2	+ 2	+ 2	+ 6
1	10 mg. "folic acid" concentrate[4]	2	— 3	+ 5	+ 2	+ 4
1	2 μg. biotin[3] and 10 mg. "folic acid" concentrate[4]	5	+ 14	+ 11	+ 11	+ 36
2[2]	None	3	+ 2	— 6	0	— 4
2	5 μg. biotin[5]	2	+ 13	+ 9	+ 13	+ 35
2	20 mg. "folic acid" concentrate[4]	3	+ 4	+ 13	+ 15	+ 32
2	5 μg. biotin[5] and 20 mg. "folic acid" concentrate[4]	2	+ 23	+ 18	+ 18	+ 59
2	133 mg. grass juice powder[6]	4	+ 8	+ 17	+ 11	+ 36

[1] All animals in experiment 1 were depleted on ration S-7 for 7½ weeks before supplements were started.

[2] All animals in experiment 2 were depleted on ration S-7 for 9 weeks before supplements were started.

[3] SMA biotin concentrate no. 200, 20 μg. per milliliter.

[4] "Folic acid" concentrate 200,000 S-P units per gram.

[5] Crystalline biotin (Merck).

[6] 133 mg. grass juice powder equivalent in "folic acid" to 20 mg. "folic acid" concentrate.

biotin or of the "folic acid" concentrate alone were effective in decreasing the prothrombin time. The concentrate of "folic acid" and biotin together appeared to be superior to either factor alone in restoring the prothrombin time to normal.

In addition to growth inhibition, certain changes in the appearance of the rats were described earlier in this communication. A favorable effect on these changes was exerted by a combination of the "folic acid" concentrate and biotin and occasionally by one or the other factor alone.

TABLE 7

The prothrombin time of rats receiving 2% succinylsulfathiazole [1] and various dietary supplements.

GROUP NUMBER	DAILY SUPPLEMENT	NUMBER OF ANIMALS	DURATION OF DEPLETION	DURATION OF SUPPLEMENT FEEDING	PROTHROMBIN TIME [2]
			days	*days*	*seconds*
1	None	3	79	0	> 240, 134, > 180
			Average		*> 185*
2	5 μg. biotin	3	63	16	105, 60, 55
			Average		*73*
3	20 mg. "folic acid" concentrate	3	63	16	46, 70, 46
			Average		*54*
4	5 μg. biotin and 20 mg. "folic acid" concentrate	4	63	16	49, 46, 42, 42
			Average		*45*
5	133 mg. grass juice powder	4	63	16	45, 54, 41, 45
			Average		*46*

[1] Diet no. S-7 was employed.

[2] Citrated plasma diluted 1:8 with saline (0.1 cc.); freshly restored lyophilized rabbit brain thromboplastin (0.1 cc.); calcium chloride, 0.277% solution (0.1 cc.); all tests run at 37°C.

DISCUSSION

The effect of ingested succinylsulfathiazole on the number and type of organisms present in the intestinal tract of the rat has not yet been studied extensively. In the dog succinylsulfathiazole induces a striking reduction in the coliform count of the feces (Poth and Knotts, '41). However, studies in monkeys indicated that, under the experimental conditions which prevailed, where the predominating flora were made up of lactobacilli, streptococci and anaerobic bacteria, succinylsulfathiazole had a less marked effect on the initially low coliform count of the feces (Welch et al., '42). Apparently succinylsulfathiazole affects primarily the coliform organisms (vitamin synthesizers) and leaves more or less uninfluenced the lactobacilli, streptococci, and anaerobic bacteria (vitamin requirers). Points still to be determined are the extent to

which "folic acid" and biotin promote the synthesis, by the residual intestinal bacteria, of additional essential dietary factors, and the extent to which "folic acid" and biotin are required by the tissues of the rat. Supplementing a succinylsulfathiazole ration with either "folic acid" or biotin decreased the prolonged prothrombin time of the rats to a value approaching that of normal animals, an observation which strongly suggests that a stimulation of the synthesis of vitamin K by intestinal bacteria occurred (Greaves, '39). A combination of "folic acid" and biotin, both of which are required as growth factors by many species of lactobacilli, streptococci (Snell and Peterson, '40; Mitchell et al., '41; Hutchings et al., '41) and anaerobic bacteria (Mueller and Miller, '42), restored the prothrombin time to that found in animals which were given a grass juice supplement high in vitamin K. These findings indicate that at least part of the effect produced by these factors is to promote a certain type of bacterial synthetic activity. However, "folic acid" and biotin might be involved in the utilization of vitamin K by the rat. Presumably biotin and "folic acid" are essential components of certain metabolic systems of the rat; however, further investigation will be required to elucidate the mechanism of action of these substances. Concerning biotin, the sum-total of all available evidence is suggestive of a direct role of this factor in animals; in the case of "folic acid" preliminary evidence supports the same conclusion. Data are accumulating which indicate that succinylsulfathiazole may influence the intestinal synthesis of still other factors which are concerned with growth or other physiological processes in the rat.

Extensive pathological changes in the tissues of rats given either sulfaguanidine or succinylsulfathiazole have been described (Daft, Ashburn and Sebrell, '42; Daft, Ashburn, Spicer and Sebrell, '42). Although growth changes have occupied our particular attention, careful histological study was made of the tissues of two large series of rats given succinylsulfathiazole. In the first series inositol and p-amino-

benzoic acid were not given (S-1); in the second series the basal diet (S-7) contained these compounds. Suffice it to say that in neither case have we been able to find definite changes of an intensity comparable to those described by Daft and Sebrell. No simple explanation of the discrepancy in findings is available at present.

Black et al. ('42) showed that the effects of sulfaguanidine can be markedly antagonized by the administration of PABA, either orally or parenterally. In our experience with succinylsulfathiazole the effect of this sulfonamide-antagonist has not been striking. It will be noted that marked inhibition of growth is produced by succinylsulfathiazole when both PABA and inositol are present in the diet (tables 4 and 5). Extensive study of the effect of the drug in the presence of PABA, but in the absence of inositol, has not been made; however, the effect of 0.2% PABA added to diet S-1 (no inositol) indicates that a slight effect was exerted. Additional attention will be given to this point.

Theories concerning the mechanism of action of succinylsulfathiazole have, for the most part, revolved around the concept of a gradual liberation of sulfathiazole in the intestine, in amounts insufficient to produce a significant concentration of sulfathiazole in the blood and other tissues. Such a splitting might result in a localized bacteriostatic concentration of the sulfonamide (Poth et al., '42; Kirby and Rantz, '42). The possibility should not be overlooked, however, that succinylsulfathiazole per se may have some specific effect on certain enzyme systems.

SUMMARY

The inhibition of growth and increase in prothrombin time brought about by the inclusion of succinylsulfathiazole in highly purified rations adequate in the well-recognized members of the vitamin B complex could be largely overcome by supplements of a "folic acid" concentrate and crystalline biotin.

The relationship of "folic acid" and biotin to bacterial activity in the intestine is discussed.

ACKNOWLEDGMENTS

We wish to express our indebtedness to Dr. Hans Molitor of the Merck Institute for Therapeutic Research, and Dr. W. H. Engels, of the Research Laboratories of Merck and Co., for the gift of crystalline biotin; to Drs. E. L. R. Stokstad and T. H. Jukes of the Lederle Laboratories for a "folic acid" concentrate and to Dr. Richard Graham of the Cerophyl Laboratories, for "Cerophyl" and a powdered grass extract. Dr. David Klein of the Wilson Laboratories kindly supplied the liver extract and the chondroitin sulfuric acid. Dr. Louis Kazal of our laboratories performed the determinations of prothrombin time.

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Wilson-'49
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Growth 13(4): 309-318. 1949.

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THE EFFECT OF BIOTIN ON MITOTIC ACTIVITY IN THE LIVER OF THE MOUSE[1]

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(Received for publication August 20, 1949)

INTRODUCTION

In a previous paper we have reported that mitotic activity may be stimulated in the mouse liver by the intraperitoneal injection of pulped liver, kidney, or egg yolk (Wilson and Leduc, 1947). Boiled or autolysed liver was as effective as fresh liver. Liver, kidney and egg yolk are all high in biotin, and biotin is thermostable. Furthermore, we had used the autolysed liver in the expectation that by liberation of some active agent the effect could be speeded up, but this did not occur; and biotin is not freed from combination in the liver by autolysis (György, *et al.*, 1939). All these facts pointed to the desirability of investigating the effect of biotin on the mitotic activity of the liver.

Our experience in producing biotin deficiency in mice has already been reported (Wilson, Leduc, and Winston, 1949). The present paper is a report of experiments performed to study the mitotic activity in the livers of biotin deficient mice, of mice which are being cured of the deficiency and of non-deficient mice that are being treated with biotin. Our results indicate that biotin is not the factor responsible for the stimulation of mitosis in the liver by the injection of pulped liver, kidney or egg yolk.

MATERIAL AND METHODS

The mice used for these experiments were from our own colony in which several inbred strains are being maintained. They were mostly from new albino strains that are being selected particularly for vigor of growth and reproduction. They are kept in a room with

[1]This investigation was supported by research grants from the National Cancer Institute, U. S. Public Health Service, and the Rhode Island Cancer Society.

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the temperature controlled at 75°-78° F. and fed Purina laboratory chow.

For the production of the biotin deficiency, glass cages with raised floors of wire mesh were used. The diets have been described and discussed in detail elsewhere (Wilson, Leduc, and Winston, 1949). To avoid any secondary problems that might arise from the use of sulfaguanidine, we have not employed diets containing this drug although we have found them to be the most effective in producing the deficiency. The diet in general use is as follows: G.B.I. vitamin-test casein, 10 per cent; egg white,[3] 20 per cent; Argo corn starch, 29 per cent; Crisco, 20 per cent; Mazola corn oil, 4 per cent; Squibb's cod liver oil, 2 per cent; G.B.I. salt mixture USP XII, #2, 7 per cent; G.B.I. powdered brewer's yeast, 8 per cent. In most experiments the mice are weaned onto this diet when 16 to 19 days of age and the majority of them show the usual signs of deficiency in 4 to 5 weeks. The fact that in some of the animals the deficiency symptoms never develop introduces an element of uncertainty in those experiments where changes in the liver during the development of the deficiency were studied.

The deficiency was cured by the subcutaneous injection of biotin[3] and by the substitution of a basal diet for the diet containing egg white. To determine the changes in mitosis in the livers of the animals so treated, one mouse was killed each day during the course of the experiment and its liver prepared for microscopic examination.

For the determination of the mitotic activity of the liver, the method of Wilson and Leduc (1947) was used. The mice were killed by a blow on the head, decapitated and bled thoroughly. A piece of the large left lateral lobe of the liver was fixed in Bouin's fluid, sectioned in paraffin at 5μ, and stained in Delafield's hematoxylin and eosin. All the mitotic figures in a single section were counted under an oil immersion objective and this number was reduced to a standard area and nuclear density by the following formula:

$$\text{mitotic activity} = \frac{\text{number of mitotic figures}}{\text{area of section} \times \text{nuclear density}}$$

The nuclear density of the section was established by counting all

[3]We are indebted to Henningsen Lamesa, Inc., Lamesa, Texas, for the powdered egg white and to Merck and Company, Inc., Rahway, N. J., for the crystalline biotin.

the nuclei in a standard area, and the area of a standard projection of the section was measured with a planimeter.

Experimental

Two experiments were performed to observe the changes in mitotic activity in the livers of young mice during the development of biotin deficiency. Sixteen-day-old mice were placed on an egg white containing diet and on the corresponding basal diet and they were subsequently killed at intervals through 30 days to determine mitotic activity. No difference between the control and experimental groups was found to which we could assign any significance.

We have treated deficient mice in two ways in order to study the effect of biotin on the mitotic activity of the liver: (1) by substituting basal diet for the egg white containing diet, and (2) by the subcutaneous injection or biotin.

Control diets have been substituted for diets containing egg white in 4 experiments. In experiment 150B (Fig. 1) the mice were placed on an egg white containing diet at 16 days of age and showed mild deficiency symptoms after 20 days. After 29 days on the egg white diet, when deficiency symptoms were definite, they were transferred

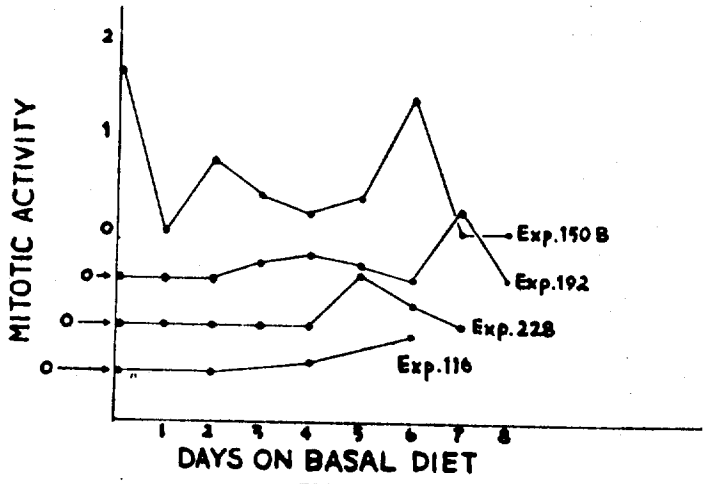

FIGURE 1

Mitotic activity in the livers of biotin deficient mice after a basal diet was substituted for the diet containing egg white. Each point on the curves represents the mitotic activity in the liver of a single animal.

to the basal diet in which the egg white was replaced by casein. The animals were fed *ad libitum*. The average food intake from the twentieth to the twenty-ninth days on the egg white containing diet was 2.5 gm. per day per mouse. The average daily intake when these animals were transferred to the basal diet was 3.7 gm. Mitosis occurred irregularly from the second to the sixth days after the transfer with a peak of activity on the sixth day. In experiments 116 and 192 (Fig. 1) in which the deficient mice were also transferred to the basal diet and fed *ad libitum* no extraordinary mitotic activity occurred in the liver, but mitotic figures were present in greatest numbers on the sixth and seventh days after the transfer. In experiment 228 (Fig. 1) the intake of the basal diet was controlled so that the mice received the same amount of the basal diet (av. 2.8 gm. per day per mouse) as they had previously eaten of the diet containing egg white. The daily ration was fed all at once and it was consumed immediately. It seemed to be insufficient to satisfy their appetites. A small amount of mitotic activity was present in the liver after 12 hours and after 5 and 6 days after transfer to this diet.

Injections of biotin were made subcutaneously into biotin deficient mice in 6 experiments. In experiment 139 a group of mice was used that had been placed on an egg white containing diet at 30 days of age and that had developed mild deficiency symptoms in 32 days. After 48 days on the diet when deficiency symptoms were marked, daily injections of 1.5γ of biotin were begun, while the mice continued to receive the egg white diet. Animals were then sacrificed at daily intervals. A small amount of mitotic activity was found on the second day only (Fig. 2). In experiments 143 and 261 (Fig. 2) the biotin deficient mice were given 2γ of biotin daily and in experiment 227 (Fig. 2) a single injection of 10γ of biotin was given. In all of these experiments the effect, though positive, was small, early, and of short duration.

The effect of biotin injections on liver mitosis was also studied in normal animals. Six experiments were conducted using in each 7 to 10 young adult mice, 75 to 111 days of age. In three the animals received 2γ of biotin daily and in three they received a single injection of 10γ of biotin. In experiment 172 mice which were 104 and 106 days of age and which were maintained on laboratory chow were given daily subcutaneous injections of 2γ of biotin. Mitotic activity appeared in the liver of the mouse killed on the third day

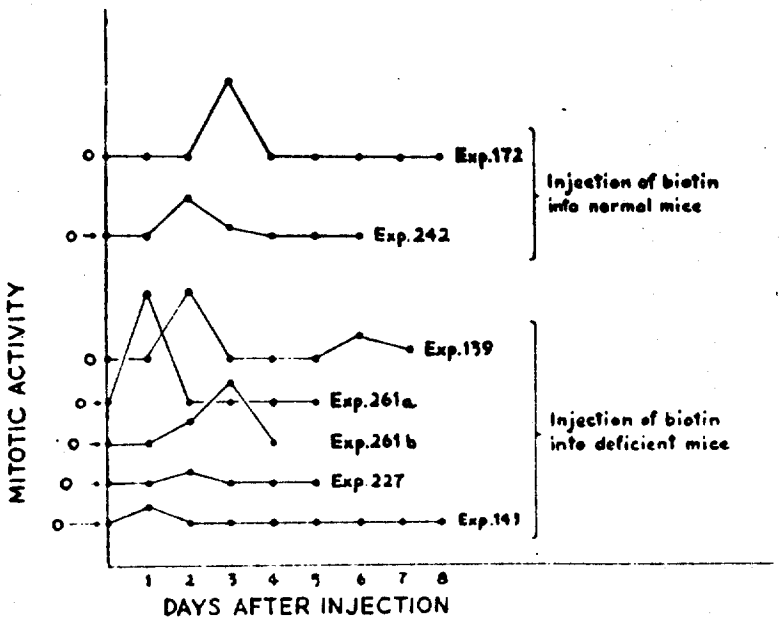

FIGURE 2

Mitotic activity in the livers of normal mice and of biotin deficient mice after the injection of biotin.

of the injection period (Fig. 2). In experiment 242, 70- to 74-day-old mice, maintained on a basal semisynthetic diet containing 10 per cent dried liver which must contain a large amount of biotin, received daily injections of 2γ of biotin. A small amount of mitotic activity was present on the second day (Fig. 2). In the 4 remaining experiments a few mitotic figures appeared in the liver but activity was at most very slight. As in the deficient mice the effect was early, and of brief duration.

Discussion

Biotin is one of the most potent of the growth factors. Extremely small amounts are effective in the growth of the microorganisms that require it and the normal daily requirement for the rat and mouse is of the order of only 2 to 5γ. What part it plays in the multiplication of the animal cell is not known. In our experiments we have found some stimulation when deficient animals are treated with biotin, and some stimulation when biotin is injected into normal, non-

deficient, animals. There have been no spectacular effects, however, and the appraisal of the results requires some caution.

When a group of young mice is placed on a deficiency producing diet, the mitotic activity of the liver persists for some time and then falls off irregularly so that by the time the symptoms appear (after 4 to 5 weeks) no mitosis is found. This is not different, however, from the events in normally growing mice, for mitosis declines in the liver after the fourth week and occurs only sporadically in older mice. Furthermore, as the deficiency symptoms appear the food intake decreases which results in a partial inanition which also brings about a cessation of mitosis, as reported by Leduc (1949). We cannot therefore conclude that the decreased mitotic activity is due to lack of biotin.

When biotin is injected into animals that are biotin deficient mitotic activity in the liver always occurs but it is always small in amount, apears early and is of short duration (Fig. 2). In 6 groups of non-deficient, normal animals similarly injected with biotin a like amount of mitotic activity occurred in two groups and almost none was found in the other 4. The failure to obtain a spectacular increase in mitotic activity in the liver when biotin deficient animals are treated with biotin suggests that under the conditions of dietary deficiency biotin is not a limiting factor in mitosis. There are a number of observations in the literature that corroborate this. West and Woglom (1942) found that tumors produced large healthy growths when transplanted into mice in an extreme stage of biotin depletion. Lazere, Thomson and Hines (1943) found that in rats maintained on an egg white containing diet and showing the characteristic symptoms of biotin deficiency, nerve regeneration and muscle recovery occur at rates comparable to those of control animals. They conclude that if biotin is essential for neuromuscular regeneration, the requirements must be below the amount still available in a deficient animal. Kornberg, Tabor, and Sebrell (1945) found that in biotin deficient rats suffering a progressive decline in weight terminating in death restoration of red blood cells and hemoglobin after repeated hemorrhages compares favorably with that in animals on an adequate diet.

Biotin seems to have little or no effect on wound healing. Bosse and Axelrod (1948) found that the healing of skin ulcers occurred equally rapidly in rats exhibiting severe symptoms of biotin deficiency and in control rats. We have made similar wounds in the skin of

biotin deficient mice and can corroborate the statement that they heal readily.[4] The result is particularly surprising because the skin changes are among the most striking symptoms of this deficiency. From all of these reports it seems clear that in animals that are biotin deficient the capacity of the tissue cells for mitosis is not impaired. It seems obvious, then, that if biotin is essential for mitosis the symptoms of the disease appear before the level of the cells themselves is reduced to the point where mitosis is impossible.

When biotin is injected into normal young adults on the stock diet, which is abundantly adequate in biotin, mitotic activity in the liver is initiated. Although the effect is slight it is positive and it seems significant for no mitosis would ordinarily be found at this age. The effect is, moreover, a fleeting one and cannot be repeated, for with subsequent daily injections it does not recur. Injection of a corresponding amount of distilled water or of Ringer's fluid was found to have no effect. We have considered the possibility that we were dealing with a condition of near deficiency such as, apparently, was present in the experiments of Nielsen and Black (1944) who obtained improved growth in mice on a diet with very low biotin content. In our experiments, the animals were on a stock diet containing an adequate supply of biotin. It is possible, however, that the biotin of the diet is not all available to the animal. It is conceivable that the intestinal bacteria may compete with the animal for the biotin of the diet and render it unavailable so that even in the presence of an apparent abundance, the animal is on the margin of deficiency. We doubt, however, that the increased mitosis we have observed can be explained in this way for we have been unable to obtain any observable increase in weight or growth rate by the injection of biotin. Furthermore, the increased mitotic activity produced in the liver by the injection of biotin is slight and of brief duration. The effect of the injected biotin on the liver cannot be indirect, through changes in the circulation since it is reported to have no pharmacodynamic effect (Schmidt and Landy, 1942). It would seem then to be a direct effect on the liver cells themselves.

The effect of biotin on isolated cells has been studied in cultures of yeast and bacteria, but its effect on tissue cells of higher animals has been little studied. Hamilton and Plotz (1942) have reported

[4]These observations were made in connection with a study of the skin of biotin deficient mice being conducted by Mr. Harold Rauch of this department.

that it has a stimulating effect on the growth of nerve tissue *in vitro*. They comment on the fact that the medium must have contained considerable biotin to begin with since it contained chick embryo extract. It is therefore surprising that such a slight addition should have an effect. Furthermore active proliferation of cell bodies was observed only in the original cultures and not in subsequent subcultures. Since no evidence of mitotic activity was found, this proliferation is attributed to amitosis. Burt (1943) failed to corroborate these observations and questioned the evidence for cell proliferation. However in two respects the observations of Hamilton and Plotz are like our own on the liver cells of the intact organisms. In the presence of an apparently adequate supply of biotin the addition of a small amount of pure biotin produces a brief increase in cell proliferation which subsides and does not recur. The effect may be due to the immediate availability of the pure biotin. Much of the biotin in tissues and elsewhere is in a combined form from which it is liberated only with difficulty. It is suggestive that the liver cells of animals with extreme symptoms of deficiency still contain relatively large amounts of biotin. West and Woglom (1942) report levels as high as 0.58γ per gram of fresh liver in deficient mice as compared with 3.12γ for normal livers and Wright and Welch (1944), 0.35γ per gram in livers of deficient rats as compared with 1.5γ per gram for normal ones. West and Woglom further report that mice are capable of tolerating enormous doses of biotin either fed or injected without any change in the free or total biotin content of the liver. From these observations we would tentatively conclude that biotin is necessary for mitotic division of the liver cells, that the biotin already present in the tissues and tissue fluids is not as readily available as pure biotin, and that the cell can respond to only a limited amount of additional pure biotin.

Our general conclusion is then that although biotin does effect mitotic activity in the liver cell, this effect is slight even in animals that have developed severe symptoms on a biotin deficient diet. This seems to be due to the fact that the animal will die before the biotin supply of the tissues is lowered to a point where normal mitotic activity is impossible and that the cure of the deficiency involves other factors, such as increased food intake and more efficient food utilization, that in their effect tend to obscure the direct effect of biotin

itself. Apparently the effect of the biotin is always slight and fleeting and not commensurate with the size of the dose. A dose as high as 100γ was found to produce no striking effect.

In comparison with injected liver, previously reported, the effect of the biotin is always earlier and much less. The timing might be attributed to the fact that the biotin of the liver is in combination from which it must first be liberated. However, if West and Woglom's (1942) figures are approximately correct, the biotin content of the liver injected in our experiments would be about 2γ per mouse, but the effect of the injected liver exceeds that obtained with any amount of biotin. We conclude, therefore, that the stimulating effect of injected liver on the mitotic activity of the liver is due to something besides its biotin content.

Summary

1. Mitotic activity has been studied in the livers of mice during the development of biotin deficiency and during treatment by injection of biotin or by adjustment of the diet. Mitotic activity declined during the development of the deficiency and was stimulated by treatment, but no effects were found that could not be attributed to other factors than biotin.
2. A slight stimulation of mitotic activity was found in the livers of normal mice after subcutaneous injection of biotin. This is attributed to a direct effect of pure biotin even in the presence of an abundance of biotin in combined form.
3. It is suggested that under the condition of dietary deficiency the biotin content of the tissues does not fall so low that it is insufficient to support cell division.
4. The effect of injected liver in stimulating mitotic activity in the liver, previously reported, must be due to something other than its biotin content.

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Wright-'44
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Proc. Soc. Exptl. Biol. Med. 56:95-98. 1944.

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Determination of Biotin with *Lactobacillus arabinosus.*

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Snell and Wright reported that biotin is an essential growth factor for *Lactobacillus arabinosus.*[1] It was stated that the microbiological determination of biotin may be accomplished through the use of the same basal medium employed in the microbiological determination of nicotinic acid, provided that biotin is excluded and nicotinic acid is added. Since then several procedures have appeared for the microbiological determination of biotin with *Lactobacillus casei.*[2,3,4]

The growth factor requirements of *L. arabinosus* are less complex than those of *L. casei,* since luxuriant and rapid growth of *L. arabinosus* may be obtained in a medium composed only of certain purified amino acids, glucose, purines and pyrimidines, inorganic salts, and several synthetic members of the vitamin B complex. Growth stimulation by an eluate factor from tomato juice of unknown composition[5] occurs only under certain conditions where the basal medium and the medium for inoculation are prepared from very highly purified constituents.[6] On the other hand, *L. casei* is stimulated by a variety of unknown factors and, in addition, requires the presence of "folic acid."[7] Desthiobiotin has an antibiotin activity for *L. casei* but it does not affect the growth of *L. arabinosus.*[8,9] Difficulty has been reported in the preparation of an adequate basal medium for the determination of biotin by one of the methods using *L. casei* but certain improvements in procedure have been described.[4]

Saccharomyces cerevisiæ has been used widely as a test organism in the determination of biotin.[10] It has been shown, however, that yeast responds to peroxide-treated biotin,[11] as well as to certain forms of biotin which do not combine with avidin (vitamers[12]) and while such yeast methods may be useful in studies of biotin metabolism they may be criticised because of lack of specificity.

Clostridium butylicum[13] and *Staphylococcus aureus*[14] have been recommended as test organisms for the determination of biotin. These bacteria have not, however, been widely used.

Since *L. arabinosus* has been used successfully for the determination of biotin in these laboratories for some time, the details of the procedure are presented here.

Procedures for the use of *L. arabinosus* in the microbiological determination of pantothenic acid, *p*-aminobenzoic acid, nicotinic

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acid, and a variety of amino acids have appeared.

Procedure. The details of the procedure resemble those described for other microbiological methods involving lactic acid bacteria.

L. arabinosus 17-5* is carried in 1% glucose, 1% yeast extract, 1.5% agar stabs. Transfers from previous stock cultures are made monthly. The fresh transfers are incubated at 30°C for 3 days and then are held in the refrigerator.

One day prior to the procedures leading to the determination of biotin an inoculum is prepared by transfer of the organism from stock culture to a medium of 1% glucose and 1% yeast extract. The latter is incubated at 30°C for 18-36 hours before use. After growth the cells are centrifuged and washed once with sterile saline and a faintly visible saline suspension prepared for seeding the unknown and the standard tubes.

The constituents of the basal medium are shown in Table I. It is convenient to prepare, in one solution of double strength, all the ingredients of the medium with the exception of glucose and the synthetic vitamins. This solution may be preserved under benzene without previous heat sterilization. A stock solution is prepared containing in each 100 ml: thiamin chloride, riboflavin, pantothenic acid, nicotinic acid, 4 mg of each; pyridoxine hydrochloride, 8 mg; and para-aminobenzoic acid, 0.4 mg.

TABLE I.
Basal Medium.

Hydrochloric acid hydrolyzed, norite-treated, vitamin free casein (16)	0.5	%
Tryptophane	0.01	"
Cystine*	0.01	"
Glucose	2.0	"
Sodium acetate (anhydrous)	0.6	"
K_2HPO_4	0.05	"
KH_2PO_4	0.05	"
NaCl	0.001	"
$MgSO_4 \cdot 7H_2O$	0.02	"
$FeSO_4 \cdot 7H_2O$	0.001	"
$MnSO_4 \cdot 4H_2O$	0.001	"
Adenine sulfate†	5	ppm
Guanine hydrochloride†	5	"
Xanthine†	5	"
Uracil†	5	"
Thiamine	1.0	"
Calcium pantothenate	1.0	"
Pyridoxin hydrochloride	2.0	"
Riboflavin	1.0	"
Nicotinic acid	1.0	"
Para aminobenzoic acid	0.1	"

* Dissolved separately with the aid of a small amount of concentrated hydrochloric acid.

† Dissolved separately by heating with hydrochloric acid.

Scrupulously clean pyrex bacteriological test tubes are used and are supported in a wire rack. Appropriate amounts of a biotin solution are pipetted into a series of tubes to establish a standard curve representing the response of *L. arabinosus* to varying amounts of biotin. Various amounts of the materials to be assayed are pipetted into additional tubes. All tubes are then diluted to 5 ml with distilled water. An amount of medium (double strength) sufficient to supply 5 ml for each assay tube is supplemented with 4 g of glucose and 5 ml of vitamin solution per 100 ml. Five ml of the complete medium is then added to each assay tube. The tubes are plugged with cotton and the test autoclaved at 15 lb pressure for 15 minutes. After cooling to room temperature each tube is seeded aseptically with one drop of the bacterial suspension for inoculation. The test is incubated at 30-33°C for approximately 72 hours. Titration of the lactic acid produced, using bromthymol blue as the indicator, is used as a measure of the response of the organism to biotin. From the response of *L. arabinosus* to pure biotin (Fig. 1), which must be determined with every test, the biotin content of the unknown samples is calculated. We have found it convenient to assay unknown materials at 4 different levels. In a successful assay there should be good agreement between the four levels of unknown material assayed.

Results. Comparable results obtained when a variety of natural materials were microbiologically assayed for biotin by the proposed procedure employing *Lactobacillus arabinosus*, and by the biotin procedure of Landy and Dicken[2] with *Lactobacillus casei* are given in Table II. Both procedures gave similar results when applied to materials autoclaved with acid and to some untreated materials. With certain other water-soluble materials, particu-

* Cultures of this organism may be secured from the American Type Culture Collection, Georgetown University Medical School, Washington, D.C., where it is listed as No. 8014.

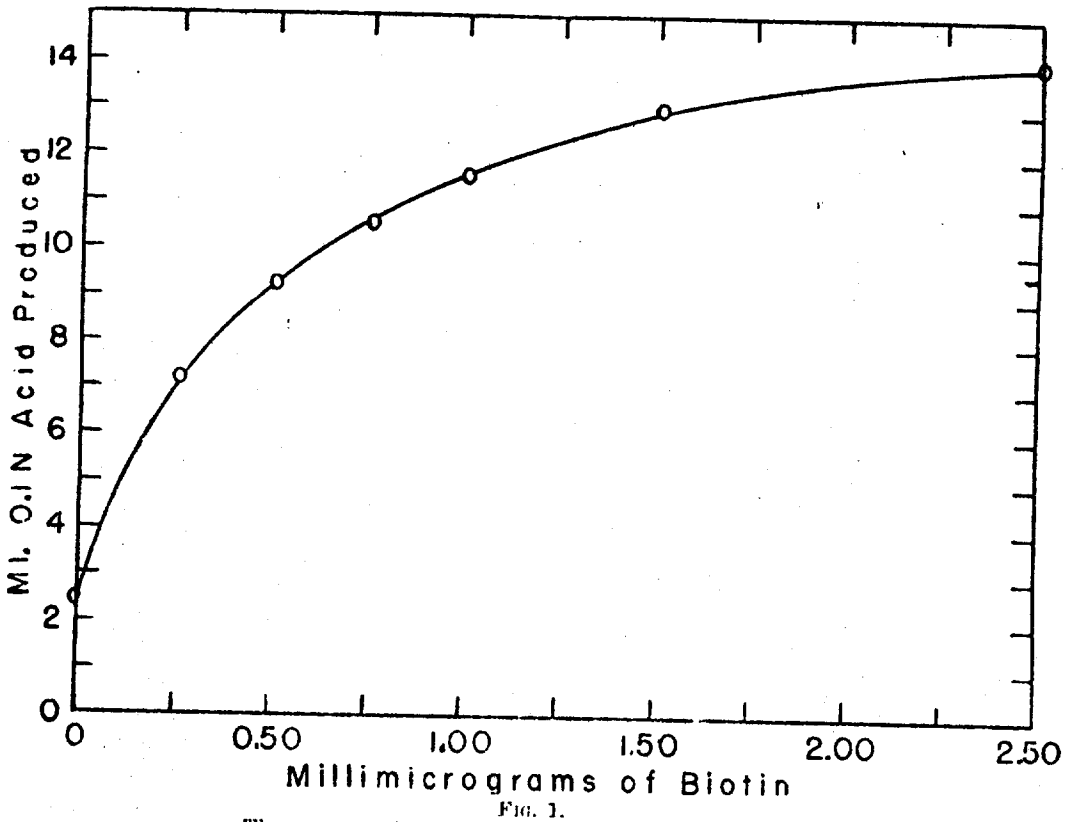

FIG. 1.
The response of *Lactobacillus arabinosus* to added biotin.

TABLE II.
Biotin Content of Various Materials as Determined by *L. arabinosus* and by *L. casei*.

Material	Biotin content	
	L. arabinosus μg/g or ml	*L. casei* μg/g or ml
Curbay powder, untreated	3.7	3.9
Valentine's meat extract, untreated	.038	.038
Bacto peptone, untreated	.15	.16
" " acid autoclaved	.17	.17
Proteose peptone, untreated	.11	.44
" " acid autoclaved	.37	.40
Urine, untreated	.032	.032
Purina chow, acid autoclaved	.19	.24
Yeast extract, untreated	.090	1.6
" " acid autoclaved	1.5	1.8
Malt extract, untreated	.045	.065
Trypsinized vitamin free casein	.00081	.010
Grass juice powder, untreated	.34	.53
Tryptone, untreated	.16	.22
Klim, untreated	.16	.20
Liver powder 1:20, untreated	.22	.27
" " 1:20, acid autoclaved	.29	.21

larly autolyzed or enzyme-treated substances, higher values were obtained with *L. casei*. Acid hydrolysis (6 N H_2SO_4 at 15 lb pressure for 1 hour) did not affect the amount of biotin found with *L. casei* but increased the observed biotin content when determined with

L. arabinosus to a value equal to that found with *L. casei*. It would appear that there exists in certain enzymatically prepared materials a form of biotin which may be utilized for growth and acid production by *L. casei* but not by *L. arabinosus*. Using yeast extract as an example, it was possible to show that this form of biotin, available to *L. casei* but not to *L. arabinosus*, was avidin-combinable. Acid autoclaving converts this form of biotin to one used equally well by the two organisms. This need not seriously interfere with the use of *L. arabinosus* as an assay organism for biotin since it is well known that there exist in many natural materials combined forms of biotin which are not available to bacteria. Preliminary acid hydrolysis has become an accepted procedure before biotin determination by any method.

The recoveries obtained when biotin was added to several naturally occurring materials are given in Table III. Quantitative recovery of biotin was readily obtained. It is believed that better agreement at varying assay levels for the biotin content of various materials is obtained with the *L. arabinosus* method than is possible with the *L. casei* method of Landy and Dicken.[2]

Since pimelic acid is capable of being utilized in lieu of biotin by the diphtheria bacillus,[15] the ability of *L. arabinosus* to utilize similar dicarboxylic acids was studied. Adipic, pimelic, suberic, azelaic, and sebacic acids were found to possess no biotin-like activity for *L. arabinosus*.

TABLE III.
Recovery of Biotin* Added to Various Materials.

Sample	Biotin content μg/g or ml	Recovery %
Proteose peptone†	0.11	100
Bacto peptone†	0.15	100
Malt extract†	0.050	100
Rat liver‡	0.48	101

* Biotin added to the final solution for assay in an amount which approximately doubled that originally present.

† A solution of these materials assayed without previous treatment.

‡ Autoclaved with 6 N H_2SO_4 at 120°C for 1 hour before determination.

Summary. The microbiological determination of biotin, using *Lactobacillus arabinosus* as the test organism, was studied. The existence of a water-soluble, avidin-combinable form of biotin in certain enzymatically prepared materials which is available to *L. casei*, but not to *L. arabinosus*, was demonstrated. When natural materials are autoclaved with 6 N H_2SO_4, prior to determination of their biotin content, comparable results are obtained with the *L. arabinosus* method and with a method using *L. casei*.

[15] du Vigneaud, V., Dittmer, K., Hague, E., and Long, B., *Science*, 1942, **96**, 186.

[16] *The University of Texas Publication*, 1941, **4187**, 82.