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Human Biotin Deficiency

A Case History of Biotin Deficiency Induced by Raw Egg Consumption in a Cirrhotic Patient[1, 2, 3]

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BIOTIN IS SO WIDELY distributed in foods and so abundantly produced by intestinal bacteria that doubt has been expressed concerning the occurrence of a deficiency state in man on the basis of an inadequate dietary intake (1). Interest in biotin has been based on its ability to protect against the egg-white injury factor in animals and man. György et al. (2) in 1941 recognized that avidin, a protein component of raw egg white, is capable of binding biotin so that it becomes nutritionally inactive. Virtually all human and animal studies have employed egg white in some form to produce an artificial biotin-deficiency state, and scant attention has been paid to derangements of biotin metabolism, storage, or excretion in human disease. Sydenstricker et al. (3) in 1942 described the clinical manifestations of biotin deficiency induced in four normal volunteers by feeding diets containing 200 g of desiccated egg white daily. A dry scaly dermatitis developed after 3–4 weeks followed by atrophic glossitis, anorexia, nausea, vomiting, mental depression, pallor, muscle pains, paresthesias, and precordial pain. Laboratory studies revealed anemia, abnormal electrocardiograms, hypercholesterolemia, and hyperbilirubinemia. Prompt improvement occurred within 3–5 days following the injection of 150 μg of biotin daily. Most of the comments in textbooks and medical literature concerning human biotin deficiency are based on this article and very little has been added in the 25 years since it appeared. However, the role of biotin in enzymic reactions has been considerably clarified during the last decade by numerous in vitro and in vivo studies involving animals and bacteria. The biochemistry of reactions involving biotin coenzymes has been discussed in several review articles (4–9).

The opportunity to study human biotin deficiency was recently presented to us by a 62-year-old white female who had consumed six raw eggs and 2 quarts of skim milk daily for 18 months. This diet had been recommended as a dietary supplement by a physician in order to provide a high intake of essential amino acids for liver regeneration, following the histological demonstration of Laennec's cirrhosis by biopsy at the time of cholecystectomy. After a few weeks the patient developed anorexia and dysphagia with soreness of tongue and lips. She was unable or unwilling to eat regular meals. However, she persevered in the daily consumption of six raw eggs and 2 quarts of skim milk. During this period she took a high potency multiple vitamin capsule

[1] From the Division of Nutrition, Department of Medicine, Medical College of Alabama, Birmingham, Alabama.

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[3] With the technical assistance of E. Braverman.

(containing no biotin) daily. She also took several brewer's yeast tablets daily, providing an unknown amount of biotin, and she received 100 μg of vitamin B_{12} by injection monthly. Thus the stage was set, unintentionally, for the development of biotin deficiency due to the avidin content of raw egg whites, uncomplicated by deficiencies of other vitamins or common nutrients.

METHODS

Biotin was determined by bacteriological assay using *Lactobacillus plantarum* (ATCC 8014) in the following manner: (+) biotin, USP,[4] was dried over phosphorus pentoxide in vacuo. A stock solution containing 1.0 ng/ml biotin was prepared and stored under toluene at −20 C. Working standards were prepared daily by dilution in water to obtain 1×10^{-10} g/ml biotin. Standard curves were constructed daily, in triplicate. Calibrated 18 x 150 mm test tubes were charged with measured amounts of biotin over a concentration range from 0 to 5×10^{-10} g/tube. Volumes were next adjusted to 5.0 ml with water and each tube made up to a final volume of 10 ml by the addition of 5.0 ml of Bacto-Biotin[5] assay media. The tubes were autoclaved for 10 min at 15 psi, cooled to room temperature, and inoculated aseptically with 1 drop from an 18-hr culture of *L. plantarum* carried on Micro-Inoculum broth,[5] and diluted 1:50 with sterile, isotonic saline. The tubes were incubated at 37 C and read at 650 mμ with a spectrophotometer[6] after 19 hr growth. The standard curve was then constructed from an average on the triplicate readings. Over this concentration range straight lines were consistently obtained from the standard curves.

Determination of Total Whole Blood Biotin Levels

Bound biotin was liberated from whole blood by prolonged digestion with papain, using a modification of the procedure of Baker et al. (10). The papain-buffer solution was made by dissolving 10 g of crude papain[7] in approximately 300 ml distilled water containing 56 ml of 0.2 M Na_2HPO_4 and 44 ml 0.1 M citric acid. The volume was adjusted to 1 liter with water and the solution was blended for 2 min in a Waring Blendor, then filtered. Whole heparinized blood was added to the papain-buffer solution to yield a dilution of 1:50 (1 ml of blood, 49 ml of papain-buffer solution). This was layered with a preservative (1.0 ml of a mixture of chlorobenzene, 1,2-dichloroethane, and *N*-butylchloride (1:1:2) by volume), to prevent bacterial contamination. The flask was then closed and placed in the 37 C incubator for 18 hr.

At the end of this period of incubation the flask was uncovered and autoclaved 30 min at 15 psi to drive off the preservative and coagulate residual protein. After cooling to room temperature the hydrolyzed solution was filtered and assayed for biotin by additions of 5, 2, and 1 ml of the papain hydrolysate to the assay tubes. All assays were set up in triplicate, along with a papain blank. The papain blank was free of biotin. The three tubes were set up as described for the standard curve. Two of the three tubes were inoculated with *L. plantarum* as before, the third tube serving as a blank to zero the spectrophotometer. Concentrations of biotin were read directly from a simultaneously run standard curve.

Determination of Free Whole Blood Biotin Levels

To determine the unbound blood biotin whole blood was diluted 1:50 with the papain buffer without papain. The solution was autoclaved 15 min at 15 psi, coagulated protein was removed by filtration and the filtrate assayed as described for total whole blood biotin.

Determination of Urinary Biotin Levels

Well mixed urine samples (stored under toluene) were diluted 1:500 with distilled water. This diluted solution was then assayed in triplicate at the following levels: 0.5, 1.0, 2.5, 4.0, and 5.0 ml. Since the biotin in urine is unbound, no prior treatment or digestion is required prior to bioassay.

Biotin Clearance Studies

In the course of the studies on the replacement of biotin in a deficient individual, it be-

[4] Biotin, USP, was obtained from the California Corporation for Biochemical Research, Los Angeles, Calif.

[5] Bacteriological assay medium and maintenance broth were supplied by Difco Laboratories, Detroit, Mich.

[6] Spectronic-20, Bausch & Lomb, Rochester, N. Y.

[7] Crude papain was obtained from Sigma Chemical Co., St. Louis, Mo.

came of interest to follow biotin levels in blood and urine following intravenous injection of a test load of biotin.

Biotin was prepared for injection by dilution of the stock solution used for the standard curve preparation in isotonic saline to a level of 50 μg/ml. This solution was autoclaved 15 min at 15 psi. Four milliliters (200 μg) were injected intravenously for the test load.

The patient under study was hospitalized on the night prior to the study and received no breakfast. At 7:45 AM a sample of blood was drawn to serve as a zero-time sample. The 200 μg of biotin were injected at 8:00 AM. Blood samples were drawn at 15 min, 30 min, 1 hr, 2 hr, 5 hr, 8 hr, 12 hr, and 24 hr into heparinized tubes. These blood samples were assayed for free and total whole blood biotin levels as described above.

Upon arising on the morning of the clearance study urine was voided and discarded. Following the biotin injection, urine was collected for 24 hr in two separate containers: one container for the first 5 hr, the second for the remaining 19 hr. Urine so collected was assayed for biotin as described above.

An arbitrary standard of reproducibility was imposed on all microbiological assays by not accepting a reading unless duplicate tubes gave readings within 0.05 OD units of each other. A further safeguard against inhibitors or nonbiotin growth factors was the requirement that virtually identical results should be calculable from two or more dilutions of unknown material.

CASE HISTORY

Patient TB (U.H. E14-53-69), a 62-year-old female was admitted to University Hospital on June 16, 1966, for evaluation of swelling of her abdomen and ankles. The diagnosis of cirrhosis had been made by biopsy of the liver at the time of a cholecystectomy performed at another hospital in June 1964. The patient had been a heavy drinker earlier in life but had drunk little if any alcohol in recent years. There was no history of hepatitis.

For the first 6 months postoperatively, she had been placed on a low-fat, bland diet. Then in February 1965 a diet consisting of six raw eggs and 2 quarts of skim milk daily had been prescribed in order to increase the intake of high-quality protein. She was placed on daily multivitamin (Theragran) and yeast tablets and was given 100 μg of a vitamin B_{12} injection monthly.

Although the patient had begun to notice mild dryness of the skin and occasional mild soreness of her mouth and lips in the fall of 1965, it was not until several months prior to admission that these symptoms increased in severity. Her tongue became sore and reddened. Her lips became fissured and encrusted and occasionally bled. She also noted an increased tendency to bruise easily. Approximately 6 months prior to admission she noted the onset of mild dysphagia which was occasionally associated with nausea and vomiting. Associated with these symptoms were increasing lassitude, malaise, exertional dyspnea, and a tendency to tire easily. She also noted a recurrent sensation of substernal pressure which was not related to eating but which was partially relieved by belching. Mild abdominal swelling had been present during the past 2 years, and became more severe during the 2 months prior to admission. Swelling of the ankles had developed 2 weeks prior to admission.

The patient's past history included scarlet fever and acute glomerulonephritis in childhood, a hysterectomy, and bilateral salpingo-oophorectomy for "chocolate cysts of the ovaries" in 1942, recurrent urinary tract infections, and mild diabetes mellitus discovered 2 years prior to admission. The family history was remarkable in that her father and a sister had diabetes mellitus and a brother died of "nephritis."

On physical examination the patient was found to be an elderly, moderately obese, white female who appeared chronically ill. Vital signs on admission were blood pressure of 150/84, a pulse of 88/min, a respiratory rate of 16/min, and a temperature of 98.8 F. The skin was dry and covered with fine scales. This was particularly prominent over the dorsum of the hands. Spider angiomata were present over the neck and thorax. Palmar and plantar erythema were present. Several small ecchymoses were scattered over the extremities. The lips were dry, fissured, and encrusted. The oral mucosa was reddened and the tongue magenta in color. There was no papillary atrophy but the fungiform papillae were hyperemic. The liver was firm and smooth and descended four fingerbreadths below the right costal margin on deep inspiration, while the upper border was normally placed. The

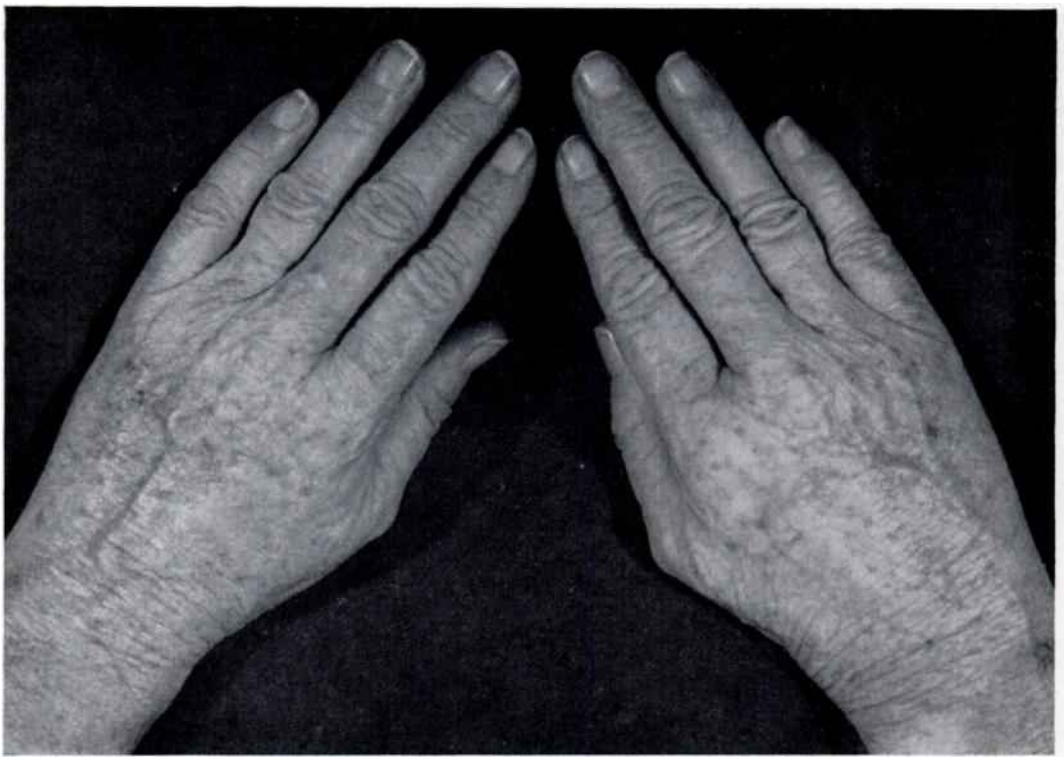

FIG. 1. Appearance of the hands prior to therapy, illustrating the shiny, dry, scaly dermatitis.

spleen was firm and descended two fingerbreadths below the left costal margin. Mild pitting edema of the ankles was noted. No other abnormalities were noted.

The following laboratory studies were within normal limits: hemoglobin, hematocrit, red cell count, red cell morphology, white blood count, differential, urinalysis, electrolytes, blood urea nitrogen, uric acid, bilirubin, alkaline phosphatase, and fasting blood glucose. Whole blood folate was normal by *L. casei* assay. The total cholesterol determinations were also normal, 195 mg/100 ml and 165 mg/100 ml on two different days. The platelet count was 157,000/mm^3. Two-hour postprandial blood glucose determinations were 257 mg/100 ml and 141 mg/100 ml. The thymol turbidity was 5.6 units. Serum glutamic oxaloacetic transaminase determinations were 64 and 58 units. The prothrombin time determination revealed 45% activity. The serum electrophoretic pattern was that of chronic inflammation. The chest X-ray, barium swallow, and electrocardiogram were normal.

In addition to moderately advanced cirrhosis, the patient was suspected of having biotin deficiency. This was borne out by a total whole blood biotin determination of 0.25 ng/ml. She was then given 2 cc of a multivitamin preparation[8] containing 200 μg of biotin/2-cc vial intramuscularly daily while being maintained on a diet of six raw eggs and 2 quarts of skim milk daily. An improvement in the condition of the skin, lips, and tongue was noted within 2 days after biotin therapy was initiated. After 4 days of therapy the tongue appeared normal, the lips were smooth and of normal texture, and the scaliness on the arms and dorsum of the hands had completely cleared (Fig. 1). She was at that time placed on a regular diet. Upon completion of studies she was discharged on a high-protein diet, hydrochlorothiazide, 50 mg three times weekly, and a multivitamin which did not contain biotin. She was instructed not to eat uncooked eggs.

Following discharge from the hospital the patient did well for 3 months, when she again

[8] Berocca-C, Roche Laboratories, Nutley, N. J.

noted the onset of lassitude, soreness of mouth and lips, and dryness of skin. The patient's husband had been found to have incurable carcinoma and had died 2 weeks prior to her second admission in mid-October. The patient had been under considerable emotional stress during this period and received very little rest. She ate poorly and developed a fever and a cough. She consulted her private physician who prescribed capsules of tetracycline and novobiocin for 1 week and also gave her two injections of penicillin. Although the cough and sputum production subsided, she continued to have increasing lassitude, malaise, and soreness of the mouth.

Physical examination again revealed a dry, scaly skin, spider angiomata, palmar and plantar erythema, reddened oral mucosa and tongue, cracked and encrusted lips, mild angular cheilosis, and hepatosplenomegaly. In addition, a few coarse rales were present over the left lung posteriorly. No other significant abnormalities were noted and the chest X-ray was normal.

Pertinent laboratory findings included a normal white blood count, hematocrit, and hemoglobin, a platelet count of 132,500/mm³, total cholesterol of 186 mg/100 ml, serum carotene of 130 mg/100 ml, and liver function studies which were essentially unchanged since her first admission. Marked improvement in clinical manifestations again followed parenteral biotin supplementation.

RESULTS

The results of biotin assays in normal whole blood and urine are presented in Tables I and II. During the first admission the patient excreted an average of 8.4 μg of biotin in the urine daily covering a 3-day collection period while consuming six raw eggs daily. Her initial blood biotin level was 0.25 ng/ml, and as indicated in Fig. 2, showed a return to normal while receiving parenteral biotin injections and the same diet. Daily urinary biotin excretion values were 247, 215, and 138 μg, respectively, during therapy. Figure 3 presents the clearance curves following administration of 200 μg of pure biotin intravenously to the patient and two normal subjects. Table III presents the urinary biotin results for the 24 hr following biotin

TABLE I

Biotin content, normal whole blood

	Nanograms per milliliter
Range	0.82-2.7
Mean	1.47
SD	±0.63

n = 12.

TABLE II

Biotin content, normal urine

	Micrograms/24 hr
Range	24-81
Mean	42.4
SD	13.6

n = 20.

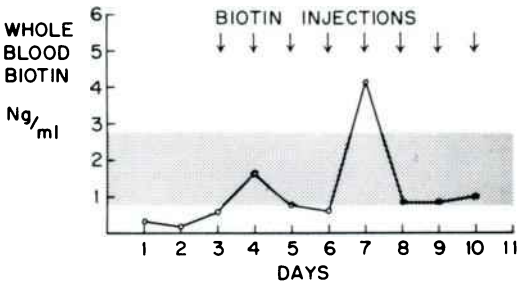

FIG. 2. Sequential changes in the patient's whole blood biotin concentration during 3 days of baseline observations and a week's therapy with daily injections containing biotin. The patient continued to consume six raw eggs daily during this period. The range of variation of normal whole blood biotin is indicated by the stippled area.

administration as well as the day before and the day after the test.

The mean value of 1.47 ng/ml (Table I) compares favorably with the values reported by others (11, 12). However, other workers (10, 13) using the flagellate *Ochromonas danica* report values considerably lower than those for whole blood in the present study. These investigators have reported the normal range of biotin concentration in the urine to be 0.006 to 0.032 μg/ml (10) which is approximately com-

parable to the concentrations reported here. The biotin content of the normal 24-hr urine specimens of the present study is shown in Table II. The mean value of 42.3 μg/day agrees with the results reported by others: 31.7 (14), 49.5 (15), and 39.5 (16) μg/day. The values observed in the present group of normal subjects also conform to the range of 29–62 μg/day observed by Sydenstricker (3); to the range of 21–70 μg/day observed by Rhoads and Abels (17); and to the concentration of 0.032 μg/ml reported by Wright and Skeggs (18). Swendseid and co-workers (19) reported a daily average urinary biotin excretion of 2–6 μg during a prolonged period of starvation but no consistent decrease in relation to the duration of starvation.

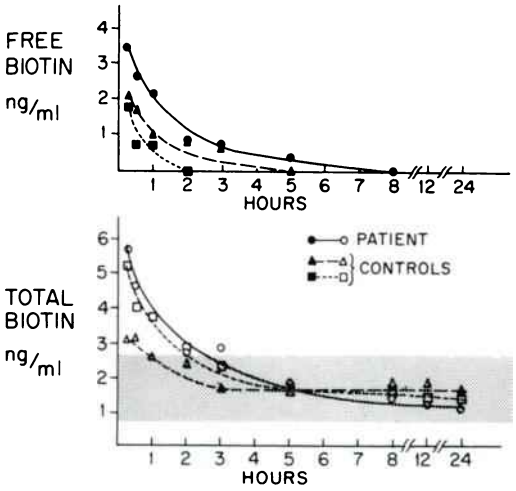

FIG. 3. Variations in the free biotin and total (free plus bound) biotin concentrations of whole blood following intravenous injection of 200 μg of USP biotin in the patient and two normal controls. The curves suggest delayed clearance of free biotin by the patient. Comparison of urinary biotin excretion during this 24-hr period with the day before and the day after indicates that the patient excreted 91% of the dose while normal subjects excreted 27 and 55% of the dose. Presumably normal subjects were better able to divert injected biotin into tissue stores (see text).

Figure 2 presents data on the changes in blood biotin concentration in the deficient subject on successive days during a course of parenteral biotin administration. The subject was consuming six raw eggs daily during the entire period represented in the figure. Blood samples for biotin assay were scheduled for collection each morning 1 hour prior to administration of biotin for that day. It must be conceded that the high value observed on *day 7* may have been due to collection of the sample after biotin injection. The assays were repeated on three occasions with essentially identical results. The gradual upward slope of most values toward the low normal range is thought to be the significant trend in this figure. It may be noted that the patient experienced an improvement in general sensorium, attitude, and appetite within 48 hr after beginning

TABLE III

Urine biotin values before and after biotin loading

	Prior to Injection	After Injection				Value Corrected for Base-Line Excretion	Percent of Test Load Retained
	1 24 hr	2 5 hr	3 19 hr	4 (column 2 plus column 3) 24 hr	5 24–48 hr	6 (column 4 minus column 1)	7
Patient	29.2	136.8	74.4	211.2	59.5	182	9%
Normal control, no. 1	42.7	55.6	40.7	96.3	38.7	53.6	73.4%
Normal control, no. 2	40.0	106.5	43.4	149.9	40.0	109.9	45%

Values are expressed as micrograms excreted during collection period. See text for details.

therapy. The tongue lost most of its redness and the lips became less scaly. The tongue and lips were virtually normal after the 4th day, and the dry scaly dermatitis was beginning to improve. By this time the patient expressed gratitude for great improvement in her general sensation of well-being after having previously resigned herself to a state of chronic illness. She had not realized the extent of her malaise until improvement took place.

Figure 3 illustrates biotin clearance studies performed on two normal young men and on the patient after 3 months of a regular diet. Table III presents the urinary biotin excretion values of the same subjects for the 24-hr periods immediately prior to and following biotin administration. It may be recalled that for several weeks prior to this study the patient had eaten poorly because of the recent death of her husband and had experienced a mild recurrence of earlier symptoms. These included soreness of the tongue and desquamation of the lips. Nevertheless, she excreted over 90% of the administered biotin and appeared able to retain only 9% for utilization or storage, or both. In contrast the normal subjects retained 73 and 45%, respectively, of the injected vitamin. These excretion rates compare favorably with those observed by Sydenstricker et al. (3). When therapy was instituted at the end of induced deficiency, their subjects retained 63% of a 150-μg dose, and 50% of a 300-μg dose given parenterally.

It may be seen that the free biotin reached higher levels and remained elevated longer in the patient than in either of the two controls. This cannot be attributed to differences in blood volume, since the patient weighed 150 lb. and the normal subjects weighed 155 and 166 lb., respectively. The data indicate that normal subjects removed free biotin from the circulation within 3–5 hr of injection. Approximately one-fourth to one-half of the injected 200-μg load could be accounted for in the normal urine. Presumably the remaining one-half to three-fourths of the load was retained in tissue stores whereas the patient retained less than one-tenth. It is known that the liver is a biotin reservoir (10).

DISCUSSION

The manifestations of avidin-induced biotin deficiency in man as described by Sydenstricker and co-workers in 1942 (3) included: nonpruritic, scaly dermatitis; atrophic glossitis; anorexia and nausea; pallor; muscle pains and localized paresthesia; lassitude, somnolence, and depression; anemia; hypercholesterolemia; precordial pain; and electrocardiographic abnormalities. Biotin deficiency was induced in the four subjects of that study by the administration of biotin-binding protein, avidin, in the form of 200 g of desiccated egg white daily. Since biotin is found abundantly in many foods, it is doubtful that a true dietary deficiency has ever occurred in human adults capable of utilizing it. It is not particularly surprising that the patient described in the present report developed many of the features of induced biotin deficiency after consuming six raw eggs daily for 18 months. However, there are several points which deserve further comment. First, the illness was unwittingly precipitated by the well-intended dietary instructions of a physician who was not aware of the deleterious effect of prolonged ingestion of raw egg white. There seems to be a popular misconception that raw eggs are nutritionally superior to cooked eggs. Secondly, the patient differed in some respects from earlier case descriptions, perhaps on the basis of more massive vitamin supplementation, preexisting liver disease or a relatively low intake of dietary fat (estimated 40 g daily). She had normal hemoglobin, serum cholesterol values, and electrocardiograms in contrast to the subjects observed by Sydenstricker et al. (3).

Thirdly, her illness brings up for consideration possible interrelationships between Laennec's cirrhosis and biotin metabolism. A fundamental property of biotin appears to be its ability to "fix" carbon dioxide in an activated form and donate the molecule to a variety of acceptors. It is clear that biotin is the functional group of a number of enzymic processes in which the transfer of carbon dioxide occurs (20–22). It is of interest that methyl malonyl-CoA may be formed either by the biotin-mediated carboxylation of propionyl-CoA, or by the isomerization of succinyl-CoA which involves vitamin B_{12}. Such reactions may be significant in view of the report by Marchetti and Testoni (23) that supplementary vitamin B_{12} ameliorates the manifestations of biotin deficiency in rats. Evidence of involvement of biotin in animal carbohydrate metabolism comes from a variety of sources (4, 5). Mistry et al. (24) observed impaired glucose utilization in biotin-deficient rats. Pilgrim, Axelrod and Elvehjem (25) reported as early as 1942 that pyruvate oxidation is diminished in livers from biotin-deficient rats. More recently, Wagle (26) has shown that increasing degrees of biotin deficiency in rats are associated with a progressive decline in the incorporation of pyruvate into glucose.

The relationship of biotin deficiency to the postprandial hyperglycemia observed in our patient is obscured by the fact that there was a definite family history of diabetes. Although our patient had normal electrocardiographic tracings she did complain of substernal discomfort not related to meals and she did have exertional dyspnea, ascites, and ankle edema. In view of the role of pyruvate and fatty acid as energy sources in myocardial metabolism (27), it is not unreasonable to suspect impaired cardiac function in biotin deficiency. These symptoms disappeared after biotin administration in our patient as in those reported elsewhere (3).

Sydenstricker and his colleagues (3) observed hypercholesterolemia in each of their four subjects after induction of biotin deficiency. The persistence of normal serum cholesterol values in our patient may have been due to the coexistence of impaired hepatic function and a limited intake of total dietary lipid. Because of the fact that biotin is actively involved in the synthesis of fatty acid and seems to be a factor in the production of nutritional fatty liver, Marchetti and Puddu (28) examined the role of biotin in the induction of fatty livers in rats by the administration of orotic acid. It is of considerable interest that the feeding of orotic acid led to a significant reduction in the liver content of biotin. Biotin deficiency, induced by egg-white feeding, led to a subnormal content of total liver lipid after orotic acid feeding. Conversely, the administration of biotin enhanced the formation of liver fat to values higher than those observed with orotic acid alone. Okey (29) also observed a diminution of total lipid and cholesterol in livers of rats after induction of biotin deficiency by feeding avidin. She suggested that avidin feeding may even help to remove cholesterol already deposited. These observations suggest that biotin deficiency in humans might be expected to lower cholesterol values rather than to raise them. However, there are conflicting views and contradictory results which are discussed in the review by Terroine (4).

The reason for the absence of anemia in our patient is not clear. Landi et al. (30) have presented evidence that both biotin and folate may be involved in the utilization of the β-carbon of serine. Liver homogenates prepared from rats deficient in both biotin and folate showed less methionine synthesis from homocysteine and serine than in extracts prepared from animals deficient in only one or the other. However, it may be noted that our patient received vitamin B_{12} supplementation by parenteral injections and folic acid in the form of yeast tablets. These factors were not included in the vitamin supplement

employed in the other study (3) and may have prevented the occurrence of a nutritional macrocytic anemia.

SUMMARY

1) A 62-year-old white female developed clinical manifestations of biotin deficiency after consuming a diet containing six raw eggs daily for 18 months. The diet was advised by a well-meaning physician to aid liver regeneration following a diagnosis of Laennec's cirrhosis.

2) Clinical manifestations included anorexia, nausea, vomiting, glossitis, pallor, depression, lassitude, substernal pain, scaly dermatitis, and desquamation of the lips. All symptoms cleared or improved markedly after 2–5 days of parenteral vitamin therapy providing 200 μg of biotin daily, while she continued her pretreatment diet. In contrast to other case reports, she did not exhibit anemia, muscle pains, hypercholesterolemia, or electrocardiographic abnormality.

3) Normal subjects were observed to remove free, injected biotin from the blood within 3–5 hr, excreting 55 and 27% in the urine in 24 hr after injection. In contrast, free biotin did not disappear from the patient's blood for approximately 8 hr, and 91% of the dose appeared in the urine. These results suggest that hepatic cirrhosis prevented the proper storage and utilization of biotin in this patient.

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