tions. We forego any discussion of the possible significance of the finding reported herein until further experimental data on these and related aspects are forthcoming.

VINCENT DU VIGNEAUD
JULIET M. SPANGLER
DEAN BURK
DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, N. Y., AND NATIONAL CANCER INSTITUTE, BETHESDA, MD.
C. J. KENSLER
K. SUGIURA
C. P. RHOADS
MEMORIAL HOSPITAL, NEW YORK, N. Y.

PRELIMINARY OBSERVATIONS ON "EGG WHITE INJURY" IN MAN AND ITS CURE WITH A BIOTIN CONCENTRATE[1,2]

IT seemed important to determine whether any of the manifestations of spontaneous avitaminosis in human beings might be related to biotin deficiency. To this end a small group of volunteers ate a diet planned to contain a minimal amount of biotin; to this was added desiccated egg white in amounts sufficient to furnish approximately 30 per cent. of the total caloric intake. The diet was composed of 125 gm of polished rice, 80 gm of patent white flour, 75 gm of farina, 205 gm of cane sugar, 32 gm of lard, 10 gm of butter washed five times in hot water and 25 gm of lean beef. To this was added 200 gm of dehydrated but otherwise unmodified egg white. The basal components contained approximately 387 gm of carbohydrate, 31 gm of protein and 32 gm of fat with a caloric value of 1,960; the egg white contained 160 gm of protein and 32 gm of fat with a value of 928 calories. Such a diet is extremely poor in vitamins except riboflavin which is present in egg white in amounts approximating 10 mg per 100 gm. The daily vitamin supplement was:

Thiamin chloride	6 mg
Riboflavin	9 mg
Nicotinic acid	75 mg
Pyridoxine	5 mg
Ca. pantothenate	5 mg
Ascorbic acid	75 mg
Vitamin A	5,000 units

In addition, 1 gm of ferrous sulfate and 1 gm of calcium lactate were given daily.

It was possible to continue four subjects under observation to a satisfactory conclusion; three were white men, one a Negro woman. During the third and fourth weeks all four developed a fine scaly desquamation without pruritus. This disappeared spontaneously in seven to ten days. Thereafter nothing of significance was noted until the seventh week when one man developed a maculosquamous dermatitis of the neck, hands, arms and legs. During the seventh and eighth weeks all showed a pronounced grayish pallor of the skin and mucous membranes which was out of all proportion to the blood picture. During this same period the white patients showed atrophy of the lingual papillae, it was notable that the tongues remained pale with none of the capillary engorgement seen in pellagra or ariboflavinosis. During the ninth and tenth weeks all subjects showed increasing dryness of the skin with marked reticulation and a return of fine branny desquamation. No ocular or genital lesions were observed.

After the fifth week symptoms were prominent; these were strikingly like those noted in experimental thiamin deficiency. Mild depression progressed to extreme lassitude, somnolence and in two instances a mild panic state. Muscle pains, hyperesthesia without demonstrable neurologic changes and localized paresthesias were complained of by all. After the tenth week anorexia occurred with occasional nausea. Two patients complained of precordial distress and showed definite electrocardiographic changes.

Examinations of the blood at frequent intervals showed a definite diminution in hemoglobin content, the number of erythrocytes and the volume of packed red cells, there was slight increase in bile pigments and striking rise in the serum cholesterol. Determinations of biotin excretion in the urine showed that after seven to eight weeks of the experimental regimen the subjects excreted from 3.5 to 7.3 micrograms of biotin in 24 hours as compared with 29 to 52 micrograms excreted by individuals taking a normal diet.[3]

Treatment with an injectable biotin concentrate[4] has been completed in three of the four volunteers. The daily dose, administered in three aliquots, has varied from 75 to 300 micrograms; 150 micrograms seemed the minimal amount required for prompt relief. Depression, muscle pains, precordial distress and anorexia were abolished on the third to fifth days of treatment. Active distaste for the diet was replaced by willingness, even eagerness to eat it, although there was no significant increase in the amount consumed. The striking ashy pallor of skin and mucous membranes disappeared in four days. The elevated level of serum cholesterol was significantly reduced after

[1] From the University of Georgia School of Medicine and the University Hospital, Augusta, Ga., and the Division of Chemotherapy, National Institute of Health, Bethesda, Md.

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[3] Biotin determinations made by Dr. H. Isbell, of the National Institute of Health.

[4] Biotin concentrate supplied by the S.M.A. Corporation, Chagrin Falls, Ohio.

one week. Insufficient time has elapsed for evaluation of other evidences of correction of physiologic disturbances.

Immediately after administration of 150 micrograms of biotin concentrate the urinary excretion rose from a deficiency level of 3 to 5 micrograms of biotin a day to a level approximating 55 micrograms. This excretion was maintained until the dose was increased to 300 micrograms when there was a further step-like increase in biotin excreted to 140–150 micrograms. When medication was discontinued there was an immediate fall in excretion to a level slightly lower than that found in persons eating a normal diet.

SUMMARY AND CONCLUSIONS

It has been possible to maintain human volunteers on a diet extremely poor in vitamins and in which approximately 30 per cent. of total calories was supplied by desiccated egg white. This was supplemented by seemingly adequate amounts of vitamins, iron and calcium. These individuals developed definite symptoms and signs, some of which were strikingly similar to those of spontaneous avitaminosis.

Symptoms and signs were rapidly cured by the parenteral administration of a biotin concentrate in doses representing 150 to 300 micrograms of biotin per diem.

V. P. SYDENSTRICKER
S. A. SINGAL
A. P. BRIGGS
N. M. DEVAUGHN

AUGUSTA, GA.

H. ISBELL

BETHESDA, MD.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

MICROBURETTE

THE principle of operation of the burette is that mercury is displaced by the spindle of a micrometer or by smaller spindles replacing the original. Moving an ordinary micrometer spindle through 25 mm displaces around ¾ cc, which is precisely divided into 2,500 parts. Since the measurement is based on the displacement of dry mercury, it is not affected by the amount of solution which adheres to the walls of the burette.

The anvil of the micrometer is replaced with a set screw (1). The lower part of the burette is a cylindrical reservoir with a plane ground, thick bottom and plane ground opening and an internal diameter just enough to clear the spindle. On the closed end of the reservoir is stuck a fiber ring (2) and next that a steel disk (3) with a center punch to receive the set screw. To the open end is stuck a fiber washer (4) greased with a heavy stopcock grease. The reservoir is fastened in the micrometer frame. Mercury will not leak through the spindle bearing when it is properly greased. If a small spindle is used, the fiber washer (4) must be reamed to a perfectly tight fit around the spindle which will be proof against leakage of mercury.

The micrometer frame is clamped to a convenient stand and mercury is sucked in to fill the reservoir. It is essential for calibration that no air bubbles remain in the reservoir. The last traces can be removed by applying vacuum suction to the burette tip. When the spindle is screwed out flush with the fiber washer, the enlarged bubbles can then readily be brought up into the funnel-shaped opening to the capillary. The reservoir is air free when there is no visible bouncing of the capillary column of mercury when the micrometer spindle is quickly moved and when no deviation from a mark occurs if the burette is tilted from vertical to horizontal.

For relative calibration it is convenient to have two ringmarks (5, 6) on the burette capillary at the same level. The volume between the marks is read on the micrometer by moving the mercury meniscus

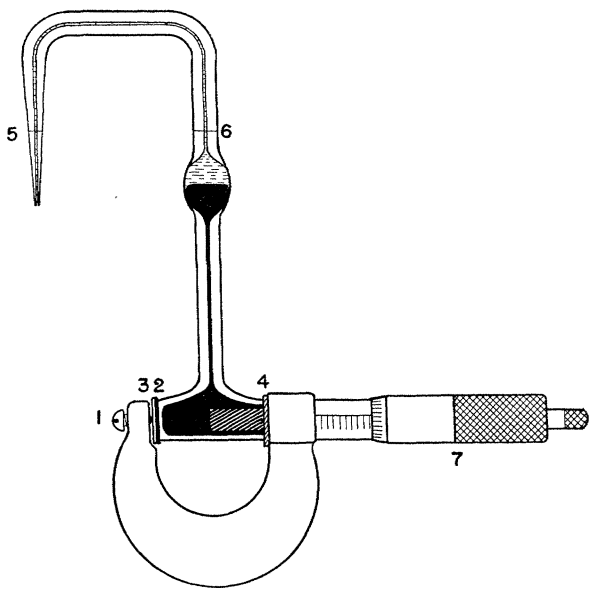

FIG. 1

from one mark to the other in the dry burette. Some mercury is discharged and a new measurement taken, until the whole range has been tested.

The micrometers tested at our laboratory always came out with an accuracy corresponding to one fifth of the smallest divisions of the micrometer, *i.e.*, as