

*light of the pulsation theory.* Professor Grabau went to China in 1920 as China Foundation research professor at the National University and chief paleontologist of the Chinese Geological Survey. In recent years opportunities and facilities for scientific research in China have been seriously impeded. The search of the literature is being made in American libraries by his assistant. \$1,000.

B. L. Clark, University of California at Berkeley, will photograph and study Radiolaria from the Tertiary and Cretaceous formations of western North America to determine their value in stratigraphic correlation. Materials for study are being furnished through cooperation with oil companies of the west coast. \$500.

A. Stoyanow, University of Arizona, will extend his studies of the Cretaceous of southeastern Arizona. Professor Stoyanow finds it necessary to compare fossils of his collection with those collected by others and will confer with paleontologists at Texas universities, at the National Museum in Washington, and at Philadelphia and Albany. \$400.

T. S. Lovering, University of Michigan, will carry out mathematical experimental researches in thermal model theory in advancing his investigation of the flow of heat in the earth's crust during geologic time. The larger investigation has been under way for several years, and the grant will furnish research assistance, materials, and apparatus for establishing closer control of the thermal conductivity measurement. \$900.

Leland Horberg, University of Illinois, will lead an expedition of three men into the eastern part of the Gros Ventre Range of northwestern Wyoming to complete a map, on which he has already spent considerable time, to study overthrust and high-angle faulting and the mechanics of the deformation, and to work out the diastrophic history. The area to be studied is claimed to be excellently located for structural studies, for within it are at least three types of structure related to both eastern and western Rocky Mountain trends. \$430.

John Clark, Carnegie Museum, Pittsburgh, Pennsylvania, will spend four months with two assistants in Utah studying the Uinta formation with special reference to paleogeographic interpretations. He has already devoted two seasons to a study of the struc-

tural history of the Uinta Range, and the new work will contribute information on principles of fluvial sedimentation and stream adjustment. \$800.

F. G. Clapp, New York, N. Y., will prepare illustrations and complete a report on the geology of Afghanistan based on private field work during 1934-1938. \$700.

Eugene N. Cameron, Columbia University, was granted assistance to complete the mapping of the Mount Prospect intrusive complex near Litchfield, Connecticut, and to provide chemical analyses of some of the rocks. The area includes about 12 square miles, and the complex series of rocks resembles the Cortlandt series of New York. The field work will require about 10 weeks. \$475.

Robert T. Hill, Dallas, Texas, will continue his studies of the history of geology in the Southwest. Dr. Hill's popular articles on the Southwest are appearing currently in the *Dallas News*, and he is completing two volumes of more technical character. \$1,800.

Bailey Willis, Stanford University, will undertake the revision of the geological map of North America, estimated to cost \$7,500. The American Philosophical Society is cooperating with a grant of \$1,000, and it is expected that other organizations will support the project. Mr. George W. Stose, of the United States Geological Survey, who collaborated with Professor Willis in preparing the existing map (1911 edition), will edit the revision and direct the drafting. Much new information has become available in the past 30 years in the United States, Canada, Mexico, the West Indies and adjacent parts of Central and South America. This will be classified and assembled in preparing an up-to-date map of the continent. \$3,750.

Chester R. Longwell, Yale University, is directing the drafting of the tectonic map of the United States. A great many geologists have been cooperating with the Committee on Tectonics of the National Research Council, Division of Geology and Geography, in plotting the data available in different sections of the country. The American Association of Petroleum Geologists contributed a grant of \$300 for drafting, and the Geological Society cooperates to make possible the final drafting of the first assembly of the sectional maps. \$175.

## SPECIAL ARTICLES

### THE POSSIBLE IDENTITY OF VITAMIN H WITH BIOTIN AND COENZYME R

DURING work on the chemistry of vitamin H, the curative factor for egg-white injury, it became apparent that the properties of this vitamin were remarkably similar to those given in the literature for

biotin, a yeast-growth factor, and for coenzyme R, a growth and respiration factor for many strains of the legume nodule organism *Rhizobium*.<sup>1</sup> The similarity in properties of biotin and coenzyme R has been pointed out during the past year by two independent

<sup>1</sup> F. E. Allison, S. R. Hoover and D. Burk, *SCIENCE*, 78: 217, 1933.

groups of workers. West and Wilson<sup>2</sup> concluded from a comparison of the growth effects of concentrates of biotin and coenzyme R on *Saccharomyces cerevisiae* and *Rhizobium trifolii* that biotin and coenzyme R are probably identical. A comparison of the effect of purified biotin and of concentrates of coenzyme R on *Rhizobium* also led Nilsson and co-workers<sup>3</sup> in Sweden to the same conclusion. Thus, any evidence which links vitamin H to one of these factors obviously tends to establish the identity of vitamin H with the other factor. The presumptive evidence derivable from the literature for the possible identity of these three factors rests on the general parallelism in their occurrence and distribution, in the similarity of their solubilities, in their behavior with precipitants and adsorbents and in their stability toward various reagents.

From the standpoint of the distribution there appear to be no data seriously conflicting with the possible identity of the three factors under discussion. It should be kept in mind, however, that the yeast- and *Rhizobium*-growth tests used to detect the presence of biotin and coenzyme R respectively are far more sensitive than the rat assay method used for vitamin H. The presence of traces of vitamin H activity might therefore remain undetected in the concentrations existing in various materials, as a result of which vitamin H might incorrectly appear to be less widely distributed. Liver and yeast are recognized as particularly rich sources of vitamin H, biotin, and coenzyme R. Interestingly enough, the reports indicate that both biotin and vitamin H are present in the liver bound to tissue proteins or other colloids in a water insoluble form and are liberated only after special treatment, as autolysis recorded for biotin<sup>4</sup> and vigorous autoclaving for vitamin H.<sup>5</sup> Other qualitative parallelisms of occurrence in natural materials exist but need not be detailed in this note. We would like to report, however, a recent observation we have made on the vitamin H activity of molasses. Since cane molasses has been reported as a particularly good source of both biotin<sup>4</sup> and coenzyme R,<sup>6</sup> it was very desirable to assay the material for vitamin H activity. Our assays indicate that cane molasses is likewise very potent in vitamin H activity.

From the standpoint of chemical and physical behavior the similarity in the nature of the materials responsible for the individual activities is marked.<sup>5,7,8,9</sup> All three substances are dialyzable, heat stable and

resistant to treatment with acid or alkali. They are soluble in water and alcohol but insoluble in chloroform, ether and petroleum ether. All are adsorbed readily on charcoal. None is precipitated by lead acetate. Treatment of vitamin H or biotin with benzoyl chloride in pyridine results in an inactive product. Nitrous acid brings about inactivation of both substances. Acetylation of vitamin H with ketene, or acetylation of biotin with acetic anhydride in the presence of concentrated sulfuric acid causes inactivation. Reports on the electrophoretic behavior of coenzyme R coincide closely with that observed by ourselves with vitamin H.

With our growing realization of the similarity in properties between vitamin H and these two other factors we thought it would be of interest to test our purest vitamin H preparation for biotin and coenzyme R activity. We had succeeded mainly by means of electro dialysis in increasing the potency of vitamin H preparations from liver concentrates to 215 units per mg, a value greater than any reported hitherto. For comparison one of the less active fractions from the same electro dialysis experiment, assaying somewhat less than 8 units per mg, was selected. These two samples were sent for coenzyme R assay<sup>10</sup> to Dr. F. E. Allison and Francis W. Minor, of the Bureau of Agricultural Chemistry and Engineering of the U. S. Department of Agriculture, to whom we are greatly indebted and wish to express our appreciation. Confirmation and extension of these assays at Cornell was made possible by cultures generously furnished us by Dr. Allison. The biotin assays were carried out simultaneously, employing a modification of the method of Snell, Eakin and Williams.<sup>4</sup> The vitamin H assays were performed at Western Reserve University.<sup>11</sup>

It was found that our highest-purity vitamin H sample was extremely potent in coenzyme R activity. 0.01 gamma produced one billion cells in the assay procedure.<sup>10</sup> The most significant point, however, was the quantitative comparison between the two samples. Within experimental error (*ca.*  $\pm 15\%$ ), the same ratio of coenzyme R potency was obtained as had been found for vitamin H activity. The more highly purified sample was on a basis of dry weight, some thirty times as potent in coenzyme R activity as the less pure sample of vitamin H. The biotin assays were likewise parallel. These results were confirmed and the comparisons extended to other electro dialysis samples, with results of the type obtained as indicated in Table 1. The preparation of the purified vitamin H and the

<sup>2</sup> P. M. West and P. W. Wilson, *SCIENCE*, 89: 607, 1939.

<sup>3</sup> R. Nilsson, G. Bjälfve and D. Burström, *Naturwissenschaften*, 27: 389, 1939.

<sup>4</sup> E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

<sup>5</sup> P. György, *Jour. Biol. Chem.*, 131: 745, 1939.

<sup>6</sup> F. E. Allison and S. R. Hoover, *Jour. Bact.*, 27: 561, 1934.

<sup>7</sup> D. G. Clark, N. Y. (Cornell) Agr. Expt. Sta., *Memoir*, 196: 30 pp., 1936.

<sup>8</sup> S. R. Hoover and F. E. Allison, *Trans. 3rd Intern. Congr. Soil Sci.*, Oxford, 1: 158, 1935.

<sup>9</sup> F. Kögl and B. Tönnis, *Z. Physiol. Chem.*, 242: 43, 1936.

<sup>10</sup> F. E. Allison and F. W. Minor, *Soil Science*, 46: 473, 1938.

<sup>11</sup> Acknowledgment is made to Catharine S. Rose for valuable assistance in the vitamin H bio-assays.

detailed comparative assays will be presented elsewhere.

TABLE 1

CORRELATION OF VITAMIN H, BIOTIN, AND COENZYME R ASSAYS OF VARIOUS LIVER CONCENTRATE\* ELECTRODIALYZATES

CELL	III	IV	V	VI
pH	4.7	3.4	3.1	3.0
SOLIDS mg/cc	4.6	2.7	1.9	1.2
VITAMIN H Units a/mg Units/cc	6-8 30-40	52 140	215 400	160-200 200-250
BIOTIN cc $\beta$	0.00025	0.00005	0.00002	
COENZYME R cc $\gamma$	0.001	0.00025	0.0001	0.00014
RELATIVE CONC. PER MG				
Vitamin H	1	7.4	31	26
Biotin	1	8.5	30	
Coenzyme R	1	6.8	24	26

\* A preparation assaying 20 units of vitamin H per mg was subjected to electrodialysis, in a series of 11 cells separated with Cellophane membranes. The vitamin H preparation was placed in cell III (cathode cell being No. I) and distilled water placed in the other cells. The electrodialysis was allowed to continue until the voltage (initial 4,500) remained constant (1,300) for 24 hours.

a Daily dose, applied for 30 days, required for complete protection against egg white injury.

$\beta$  cc required to produce half maximum growth increase (ca. 500 per cent. increase over inoculum) of baker's yeast in 16 hours at 30° C. in 12 cc yeast growth medium deficient in biotin.

$\gamma$  cc required to produce half maximum growth of *Rhizobium trifolii* strain 209 (ca. 800 million cells/cc) in 4 days at 28° C. in 25 cc synthetic sucrose-mineral-nitrate medium (inoculum negligible).

These experimental results add much strength to all the evidence that we have already collated indicating that these activities are various biological manifestations of the same substance. One must conclude from these data that they are either identical or indeed closely related compounds. We should like to emphasize that we do not regard the evidence adduced here as proving the identity of vitamin H with biotin and coenzyme R, but that it makes it indeed highly probable, and since it may be some time before final proof can be brought to bear on the question we feel that it is worth while to call attention to the probable identity involved. Obviously, final proof must await the testing of the crystalline compounds for these biological activities. We are attempting to obtain sufficient crystalline biotin for vitamin H assays. These assays of course require comparatively large amounts in terms of biotin activity.

It may be added that if the suggestion be substantiated that vitamin H is identical with biotin and coenzyme R, it will show that the two latter substances are significant in the vital economy of the mammal. Likewise, in view of the demonstrated role of coenzyme R in the respiration of *Rhizobium*, it is likely that vitamin H acts in ways other than simply to protect against egg white injury<sup>12</sup> and that it definitely functions in intermediate carbohydrate metabolism, as do various members of the so-called vitamin B complex,

<sup>12</sup> The possibility that vitamin H might be identical with an anti-dermatitis factor for chick has been suggested by D. M. Hegsted, J. J. Oleson, C. A. Elvehjem and E. B.

to which vitamin H, biotin and coenzyme R may well belong. The possible relationship or even identity of the latter to the gray-hair factor or other unisolated factors in the B complex remains to be determined.

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## ISOLATION OF A CRYSTALLINE DERIVATIVE OF PANTOTHENIC ACID

It has been shown<sup>1,2</sup> that the chick antidermatitis vitamin or pantothenic acid is composed of a hydroxy acid united in amide linkage with the amino group of  $\beta$ -alanine. While  $\beta$ -alanine has been isolated from concentrates of the vitamin,<sup>1,2</sup> neither the intact pantothenic acid nor the acid fragment has been obtained in pure crystalline condition. The discovery<sup>3</sup> of an organism which responds to the acid fragment alone has materially aided final solution of this substance.

Concentrates of the lactone of the hydroxy acid fragment were made essentially as previously described.<sup>4,5</sup> The sodium salt was then formed and acetylated, and the acetyl acid was converted to its acid chloride with  $\text{SOCl}_2$ , and this was poured into concentrated ammonia. The alcohol-soluble fraction of the reaction product was slowly crystallized from a small volume of acetone and alcohol as long needles.

These crystals were hydrolyzed with NaOH and tested by the use of hemolytic streptococcus, strain H69D.<sup>3</sup> Parenthetically it should be stated that the active substance of the liver fraction referred to previously<sup>3</sup> has been identified as nicotinic acid; it is thus possible to use a purely synthetic basal assay medium. Potency of added materials was judged both by quantitative estimation of turbidity,<sup>6</sup> as well as by titration of the acid produced during growth. The crystals were approximately half as active as 1.2 times an equal weight of alkali-treated "80 per cent. pantothenic acid" of Williams. This is not surprising when it is remem-

Hart in an abstract just come to hand (*Proc. Amer. Soc. Biol. Chem.*, New Orleans meeting, 1940).

<sup>13</sup> General Biochemicals Fellow.

<sup>1</sup> R. J. Williams, J. H. Truesdail, H. H. Weinstock, E. Rohrmann, C. M. Lyman and C. M. McBurney, *Jour. Am. Chem. Soc.*, 60: 2719, 1938.

<sup>2</sup> D. W. Woolley, H. A. Waisman, C. A. Elvehjem, *Jour. Am. Chem. Soc.*, 61: 977, 1939.

<sup>3</sup> D. W. Woolley, *Jour. Biol. Chem.*, 130: 417, 1939.

<sup>4</sup> D. W. Woolley, H. A. Waisman and C. A. Elvehjem, *Jour. Biol. Chem.*, 129: 673, 1939.

<sup>5</sup> J. J. Oleson, D. W. Woolley, C. A. Elvehjem, *Proc. Soc. Exp. Biol. and Med.*, 42: 151, 1939.

<sup>6</sup> D. W. Woolley, B. L. Hutchings, *Jour. Bact.*, 38: 285, 1939.