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	6–8	52	215	160–200
Units/cc	30–40	140	400	200–250
BIOTIN				
cc *β*	0.00025	0.00005	0.00002	
COENZYME R				
cc *γ*	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.

β 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.

γ 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[12] and that it definitely functions in intermediate carbohydrate metabolism, as do various members of the so-called vitamin B complex, 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[1,2] that the chick antidermatitis vitamin or pantothenic acid is composed of a hydroxy acid united in amide linkage with the amino group of β-alanine. While β-alanine has been isolated from concentrates of the vitamin,[1,2] neither the intact pantothenic acid nor the acid fragment has been obtained in pure crystalline condition. The discovery[3] 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.[4,5] The sodium salt was then formed and acetylated, and the acetyl acid was converted to its acid chloride with $SOCl_2$, 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.[3] Parenthetically it should be stated that the active substance of the liver fraction referred to previously[3] 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,[6] 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-

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

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[1] 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.

[2] D. W. Woolley, H. A. Waisman, C. A. Elvehjem, *Jour. Am. Chem. Soc.*, 61: 977, 1939.

[3] D. W. Woolley, *Jour. Biol. Chem.*, 130: 417, 1939.

[4] D. W. Woolley, H. A. Waisman and C. A. Elvehjem, *Jour. Biol. Chem.*, 129: 673, 1939.

[5] J. J. Oleson, D. W. Woolley, C. A. Elvehjem, *Proc. Soc. Exp. Biol. and Med.*, 42: 151, 1939.

[6] D. W. Woolley, B. L. Hutchings, *Jour. Bact.*, 38: 285, 1939.

bered that a portion of the weight of the crystals is made up of the acetyl groups added. Maximum effect was obtained when 1 to 2 micrograms were added per cubic centimeter.

The mother liquor from the crystals (after hydrolysis) was approximately one fifth as active as the crystals. Recrystallization from alcohol-ether or from ethyl acetate-acetone or sublimation in high vacuum did not alter the activity detectably. A few milligrams of the crystals were hydrolyzed and the hydroxy acid was recombined with β-alanine, as previously described.[2] The product was highly active in promoting growth of *Lactobacillus casei*[7] (maximum effect wtih 0.1 micrograms per cc) as well as in causing growth response in rats fed a synthetic ration.[5,8]

While it is not impossible that the crystals are a mixture, the above facts make this possibility seem remote. It thus appears that both fragments of the pantothenic acid molecule have been obtained in a crystalline state.

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THE STRUCTURE OF PANTOTHENIC ACID

STUDIES on the structure of pantothenic acid were originated and carried forward in the laboratories of one of us[1,2,3,4,5] to the point where β-alanine[5] was recognized as one of its cleavage products and considerable information, in addition to that published, was obtained regarding the other portion of the molecule. A partial synthesis, using β-alanine ester, was also accomplished.[6]

Work on the chick anti-dermatitis factor was in progress in the Merck Research Laboratories when the announcement was made by Jukes[7] and Woolley, Waisman and Elvehjem[8] on the probable identity of the chick anti-dermatitis factor with pantothenic acid, and a cooperative arrangement was proposed to one of us (R.J.W.). By this arrangement all the techniques and experiences gained in the pantothenic acid studies were made available to the Merck Research Laboratories, where the crystalline lactone (cleavage product) was isolated and degraded, the exact structure of pantothenic acid determined, and its synthesis accomplished.

[7] Snell, E. E., F. M. Strong, W. H. Peterson, *Jour. Am. Chem. Soc.*, 60: 2825, 1938.

[8] G. H. Hitchings and Y. Subbarow, *Jour. Nutrition*, 18: 268, 1939.

[1] Williams, *et al.*, *Jour. Am. Chem. Soc.*, 55: 2912, 1933.

[2] R. J. Williams and Robin Moser, *Jour. Am. Chem. Soc.*, 56: 169, 1934.

[3] Williams, *et al.*, *Jour. Am. Chem. Soc.*, 60: 2719, 1938.

[4] *Ibid.*, 61: 454, 1939.

[5] Weinstock, *et al.*, *Jour. Am. Chem. Soc.*, 61: 1421, 1939.

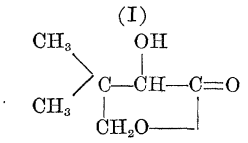
[6] R. J. Williams, SCIENCE, 89: 486, 1939.

[7] T. Jukes, *Jour. Am. Chem. Soc.*, 61: 975, 1939.

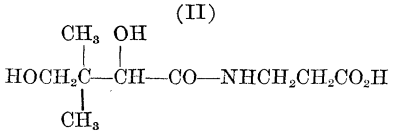
[8] Woolley, Waisman and Elvehjem, *Jour. Am. Chem. Soc.*, 61: 977, 1939.

The study was pursued in the Merck Research Laboratories with large amounts of liver concentrate. Purification methods were devised which gave concentrates containing 3 to 40 per cent. of barium pantothenate, from which the pure crystalline lactone (m.p. 91–92°) was obtained readily. Its composition corresponded to $C_6H_{10}O_3$, and its structure was shown by degradation to be that of α-hydroxy-β,β-dimethyl-γ-butyrolactone (I) which has been synthesized and condensed with β-alanine to produce physiologically active pantothenic acid (II).

(I)



(II)



This work in the Merck Research Laboratories was done by Drs. E. T. Stiller, J. C. Keresztesy and J. Finkelstein, and the results in detail will be published elsewhere under their authorship. An accompanying paper will present the unpublished data up to the point where the cooperation began.

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PRELIMINARY STUDIES ON MATING REACTIONS OF ENUCLEATE FRAGMENTS OF PARAMECIUM BURSARIA

RECENT studies by several investigators[1] have shown that there are distinct mating types in various species of Paramecium. Under appropriate conditions, individuals belonging to different mating types will agglutinate when they are mixed and later form pairs. Such agglutination has been called the "mating reaction." The purpose of the present investigation was to answer the question: Do enucleate fragments of Paramecium exhibit the mating reaction?

Paramecium bursaria—the green Paramecium—is especially favorable for the present study because of (1) the viability of fragments, (2) large size of the micronucleus, (3) permanence of mating type and (4) ease of cutting. Enucleate fragments of this species may remain alive for as long as four days. These fragments show a surprisingly normal behavior. In

[1] T. M. Sonneborn, *Proc. Nat. Acad. Sci.*, 23: 378–385, 1937; R. F. Kimball, *Proc. Nat. Acad. Sci.*, 23: 469–474, 1937; H. S. Jennings, *Proc. Nat. Acad. Sci.*, 24: 112–120, 1938; T. M. Sonneborn, *Proc. Amer. Phil. Soc.*, 79: 411–434, 1938; H. S. Jennings, *Genetics*, 24: 202–233, 1939.