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.

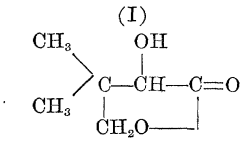
[6] R. J. Williams, SCIENCE, 89: 486, 1939.

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

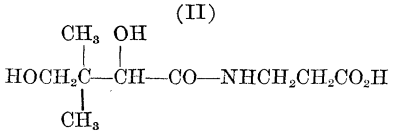
[8] Woolley, Waisman and Elvehjem, *Jour. Am. Chem. Soc.*, 61: 977, 1939.

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.

the present study fragments were used for testing immediately after cutting. After staining, the micronucleus or part of the macronucleus could easily be detected if present in the fragment used, since the micronucleus in this species (especially in the two races used in the present investigation) is large and conspicuous. Apparently each clone of *P. bursaria* retains permanently its characteristic mating type.[2] This permanence of mating type affords uniform and constant material for study. In this species the animals show a tendency to creep slowly over the bottom of the container, a behavior which facilitates cutting with a glass needle to such an extent that large numbers of fragments may be obtained.

Two races of *P. bursaria* (*Gr14* and McD_3) belonging to two different mating types were used in the present investigation. Each of the two races has a single, large, deeply staining micronucleus. After a definite mating reaction had been observed, the fragments were fixed and stained to determine whether the micronucleus or part of the macronucleus was present.

Enucleate fragments of either mating type were found to give the normal mating reaction with whole animals of the other mating type. Mating reaction between enucleate fragments and whole animals seems to be identical with that between whole animals. Control experiments showed that fragments never agglutinate with whole animals of the same mating type.

The mating reaction also occurs between two enucleate fragments belonging to two different mating types. Control experiments showed that the mating reaction never occurs between enucleate fragments of the same mating type. Thus the cytoplasm alone (in the absence of the nuclei) exhibits the reactivity and diversity of mating type. Of course this reactivity may be due to the retention of influence of the nuclei which have just been removed.

Further investigations are projected, and the findings here noted will be presented in detail later.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF SUBSTITUTING PINE SAPWOOD FOR MALT AGAR IN CULTURING TEST FUNGI

THE method described in this article is the result of experiments made in an effort to substitute pine sapwood for malt agar medium in testing the toxicity of preservatives in wood. The results obtained by the use of Ponderosa pine sapwood as a medium for growing the wood-rotting and sap-staining fungi used in the method were so consistently favorable and the reduction in time as well as the low-cost of materials proved so encouraging that it was believed of interest to present the method to other workers for trial and comment.

The containers used are half-gallon, square, wide-mouth Kerr jars, fitted with metal, self-sealing screw caps. Two pieces of Ponderosa pine sapwood, $\frac{1}{4}$ inch thick, $2\frac{3}{4}$ inches wide and $6\frac{7}{8}$ inches long, are placed on a grooved piece of pine sapwood in such a manner as to form a V-shaped trough when the jar is placed on its side. The grooved piece is $\frac{5}{8}$ inch thick, $1\frac{3}{8}$ inches wide and $6\frac{7}{8}$ inches long, with the groove about $\frac{7}{8}$ inch wide and $\frac{1}{4}$ inch deep (see Fig. 1). At the bottom of the V is placed a length of glass tubing $6\frac{3}{4}$ inches long and about $\frac{3}{8}$ inch in outside diameter. The pine test pieces, $\frac{1}{4} \times 1\frac{1}{2} \times 2$ inches in size, rest with the $\frac{1}{4} \times 1\frac{1}{2}$ inch edge on the glass tubing, to prevent contact between the two rows of test pieces.

The wood pieces and the glass tube are placed in position in the jar and sufficient water added (about 100 cc) to equal the weight of the three wood pieces. A pad of cotton is placed in the metal cap, which is loosely screwed to the jar. The jar and contents can then be sterilized. After sterilizing and cooling the jars and contents, the test fungus inoculum can be placed at various points over the surfaces of the two

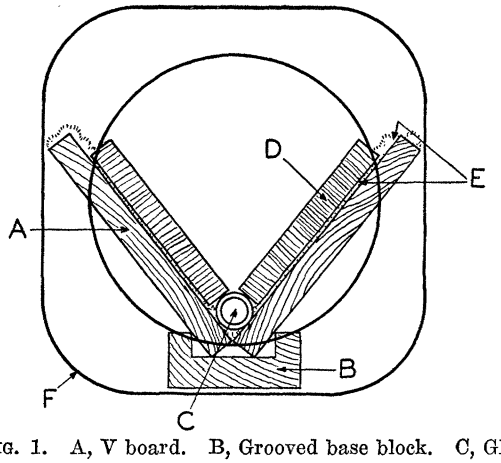

FIG. 1. A, V board. B, Grooved base block. C, Glass tubing. D, Test block. E, Fungus mat. F, Square half-gallon fruit jar.

V boards. Three to six individual inocula to each board serve to cover it with a vigorous growth in from 10 to 15 days. The decay-producing fungus *Lenzites trabea* and the various stain fungi isolated from window sash thrive on this medium. When thicker V boards are used, care must be taken to prevent too high a moisture content in the wood.

[2] See H. S. Jennings, *Genetics*, 24: 202–233, 1939.