(2) The Chattons[2] and Zweibaum[3] have maintained that conjugation is entirely determined by environmental conditions. Others (Sonneborn and Cohen[4] and Sonneborn and Lynch[5]) have shown that genetic factors are also involved. The present results show the overwhelming importance of genetic factors in some stocks.

(3) Calkins and Gregory[6] long ago attempted to show that the descendants of the four products of the first two fissions after conjugation in P. caudatum (where four instead of two macronuclei are formed in each exconjugant) were genetically diverse. The present work confirms this principle in a striking way.

It may perhaps be said that with the present work the genetics of Paramecium enters the quantitative and predictable stage, with tools and methods of analysis which should lead rapidly into a systematic, coherent body of knowledge in close touch with the rest of genetic science.

[1] Sonneborn, T. M., *Genetics*, **21**, 503–514 (1936).

[2] Chatton, E., and M. Chatton, *C. R. Acad. Sci.* (Paris), **193**, 206-209 (1931).

[3] Zweibaum, J., *Arch. Protistenk.*, **26**, 275–393 (1912).

[4] Sonneborn, T. M., and B. M. Cohen, *Genetics*, **21**, 515–518 (1936).

[5] Sonneborn, T. M., and R. S. Lynch, *Genetics*, **22**, 284–296 (1937).

[6] Calkins, G. N., and L. H. Gregory, *Jour. Exptl. Zoöl.*, **15**, 467–525 (1913).

[7] Further work has shown that in a small proportion of the exconjugants sex may segregate at the second fission after conjugation; and this has been correlated with the fact that in a similar proportion of the exconjugants three or four instead of two macronuclei are formed.

THIAZOLE AND THE GROWTH OF EXCISED TOMATO ROOTS

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We have reported earlier[1] that excised tomato roots do not grow in a nutrient solution of mineral salts and pure cane sugar. They grow satisfactorily, however, in the same solution to which dried brewer's yeast is added. We have found that the substitution of crystalline vitamin B_1 for the yeast permits growth to occur and we have grown excised tomato roots in a nutrient solution consisting of mineral salts, cane sugar and crystalline vitamin B_1 for over seven months through seven subcultures. It appears that unlimited growth of excised tomato roots is possible in this medium. We have, therefore, been able to define more exactly the nutrient requirements of tomato roots by substituting for the numerous and varied constituents of brewer's yeast a single definite chemical compound, namely vitamin B_1, and thus to prepare a synthetic solution of known constituents which is adequate for their growth. Bonner[2] has reported that a solution of mineral salts, cane sugar and vitamin B_1 is adequate for the growth of excised roots of pea. Previously Schopfer[3] had found vitamin B_1 essential for the growth of *Phycomyces Blakesleeanus.* Our results demonstrate that a medium containing mineral salts and pure cane sugar is inadequate for the growth of excised tomato roots, while one of mineral salts, cane sugar and crystalline vitamin B_1 is adequate.

We have been interested in determining whether vitamin B_1, as such, is essential for the growth of excised tomato roots or whether other chemical compounds might replace it. There is some indirect evidence from our experiments that this is a possibility. For example, we have found that dried brewer's yeast which has been heated in solution for 12 hours at 120°C. at pH 9.0 does not lose its potency as a constituent of the nutrient medium for excised tomato roots. Since vitamin B_1 is destroyed by high temperatures in an alkaline medium, the above result is taken to indicate, either that the vitamin was not entirely destroyed by the treatment and the small amount which remained after the heating was adequate for the growth of the roots, or that some constituent of yeast which is heat stable in alkaline solution is as effective or nearly as effective as vitamin B_1 in permitting growth. Furthermore, we have found the effective material in brewer's yeast to be soluble in water and in 80% alcohol, but extracts of yeast made with absolute alcohol have not proved, in our experiments, as beneficial as the 80% alcoholic extracts. Vitamin B_1 is soluble in absolute alcohol and it was our expectation that if this vitamin were the effective material in brewer's yeast it would be extracted with absolute alcohol. It is possible that vitamin B_1 is the effective material in yeast and that a satisfactory explanation may eventually be found for the observations given above which are not in accord with that assumption.[4] However, because of these observations and the hope that substitutes for B_1 might elucidate its function, we have searched for a compound which would replace the vitamin in a synthetic medium adequate for the growth of excised tomato roots.

The following substances or combination of substances were not found in our experiments to replace vitamin B_1 in a solution adequate for the growth of excised tomato roots: vitamin G or B_2 (lactoflavine), cysteine hydrochloride, pantothenic acid, indole-3-acetic acid, inositol, urea, asparagin, yeast ash, zinc and boron salts, Hoagland's A to Z mixture containing salts of lithium, copper, zinc, boron, aluminum, tin, manganese, nickel, cobalt, titanium, iodine and bromine. It is possible that one or more of these substances might be effective at other concentrations and under other conditions than those we have used. Gautheret[5] found cysteine hydrochloride beneficial to the growth of excised corn roots. However, the results of our experiments did not encourage us to believe that any of the above would take the place of vitamin B_1.

Williams and Cline[6] synthesized vitamin B_1 by combining 2-methyl-5-bromo-methyl-6-aminopyrimidine with 4-methyl-5-β-hydroxyethylthiazole. The question as to whether either of these intermediates could replace the vitamin for the growth of tomato roots is a natural one. Through the courtesy of Dr. Williams and of Merck and Company, samples of 2-methyl-5-bromomethyl-6-aminopyrimidine, 2-methyl-5-ethyoxymethyl-6-amino-pyrimidine and 4-methyl-5-β-hydroxyethylthiazole were secured. These compounds were added at a concentration of 0.1 γ per cc. to a nutrient solution containing mineral salts and pure cane sugar with the following results:

The excised tomato roots did not grow in the solutions containing the pyrimidines; growth occurred in the presence of the thiazole and in solutions containing a mixture of the thiazole and either of the pyrimidines. Subcultures of the roots which had grown in the solution containing mineral salts, cane sugar and thiazole grew in a solution of the same composition. These results indicate that 4-methyl-5-β-hydroxyethylthiazole will replace vitamin B_1 in the growth of excised tomato roots. Since the roots grew in the solutions containing a mixture of the pyrimidine and the thiazole it is evident that failure of growth in the solutions containing pyrimidine but no thiazole is not the result of the toxicity of the pyrimidine. We have not yet determined whether the thiazole is as effective as the vitamin nor have we carried tomato roots through a series of subcultures in a medium consisting of mineral salts, cane sugar and thiazole as we have in the same medium containing crystalline vitamin B_1 instead of thiazole. It appears, however, that 4-methyl-5-β-hydroxyethylthiazole will replace vitamin B_1 in a synthetic liquid medium for the growth of excised tomato roots; and that the thiazole but not the pyrimidine radical of the vitamin is effective in permitting growth of these roots in a synthetic medium. These findings do not explain the original observation on the apparent relative insolubility in absolute alcohol of the growth-promoting factor or factors in yeast.[4] The thiazole is soluble in absolute alcohol. They would lead us to modify our original statement[1] that the parasitic relationship of the tomato root to the top involves both carbohydrate and vitamin B_1. It appears now that this relationship involves carbohydrate and the thiazole radical of vitamin B_1.

Does the thiazole actually replace vitamin B_1 in the metabolism of the tomato root or does the root produce sufficient of the necessary pyrimidine to permit synthesis of the vitamin when the thiazole is supplied? How does the thiazole function and will other compounds be found which will replace the thiazole or vitamin B_1? These questions we are not prepared to answer at the present time.

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937).

[2] James Bonner, *Sci.*, **85**, 183–184 (1937).

[3] W. H. Schopfer, *Ber. Deutsch. Bot. Gesell.*, **52**, 308–313 (1934).

[4] Since the preparation of this manuscript Williams has called our attention to the fact that vitamin B_1 is sparingly soluble in absolute alcohol and is not extracted from yeast and foodstuffs by this solvent unless an excess of mineral acid is present. This explains our failure to secure a beneficial effect with extracts of yeast made with absolute alcohol.

[5] R. Gautheret, *Compt. Rend. Acad. Sci. Paris*, **197**, 85–87 (1934).

[6] R. R. Williams and J. K. Cline, *Jour. Am. Chem. Soc.*, **58**, 1504–1505 (1936).

PYRIMIDINE AND THIAZOLE INTERMEDIATES AS SUBSTITUTES FOR VITAMIN B_1

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In studying the nutrient requirements of excised tomato roots two of the authors (Robbins and Bartley) have found that the roots do not grow in a liquid medium containing mineral salts and pure cane sugar but grow in the same medium to which a small amount of crystalline vitamin B_1 has been added.[1] They have found, further, that 4-methyl-5-β-hydroxyethylthiazole will replace vitamin B_1 in a synthetic liquid medium for the growth of excised tomato roots but that 2-methyl-5-bromomethyl-6-aminopyrimidine is not effective.[2] Williams and Cline[3] synthesized vitamin B_1 by combining these two compounds. It appears from these results that the thiazole radical of the vitamin is the effective agent so far as the growth of tomato roots is concerned.

If thiazole can be substituted for vitamin B_1 in a nutrient medium suitable for the growth of tomato roots, can it be used to replace vitamin B_1 for other organisms?

Our results indicate that neither the thiazole nor the brompyrimidine relieve vitamin B_1 deficiency in pigeons, but that sufficient quantities of the pyrimidine and the thiazole given together by mouth are effective. The pyrimidine and the thiazole used in these experiments were secured through the courtesy of R. R. Williams and of Merck and Company. The pigeons used were maintained on ration No. 1669 containing sucrose, casein (treated with alcohol), the Osborne and Mendel salt mixture, celluflour and cod liver oil until they showed symptoms of polyneuritis, at which time they were given the thiazole or pyrimidine in capsules by mouth. Our results follow.

Pigeon No. 1602. Showed definite signs of polyneuritis. Given 100 gamma of thiazole. Little or no improvement noted the next day. Given