[6] The Association of Vitamin Chemists, Inc., *Methods of Vitamin Assay*, Interscience Publishers, Inc., 1947, pp. 1–189.

[7] Bonner, D., and Beadle, G. W., "Mutant Strains of *Neurospora* Requiring Nicotinamide or Related Compounds for Growth," *Arch. Biochem.*, **11**, 319–328 (1946).

[8] Tatum, E. L., and Bell, T. T., "*Neurospora*. III. Biosynthesis of Thiamin," *Am. J. Bot.*, **33**, 15–20 (1946).

[9] Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., "Enzymatic Liberation of B Vitamins from Plant and Animal Tissues," *Univ. Texas Pub.* **4237**, 1942, pp. 15–36.

[10] Andrews, J. S., Boyd, H. M., and Gortner, W. A., "Nicotinic Acid Content of Cereals and Cereal Products," *Ind. Eng. Chem., Anal. Ed.*, **14**, 663–666 (1942).

[11] Melnick, D., "Collaborative Study of the Applicability of Microbiological and Chemical Methods to the Determination of Niacin (Nicotinic Acid) in Cereal Products," *Cereal Chem.*, **19**, 553–567 (1942).

[12] Cheldelin, V. H., and Williams, R. J., "The B Vitamin Content of Foods," *Univ. Texas Pub.* **4237**, 1942, pp. 105–124.

[13] Van Lanen, J. M., Tanner, F. W., Jr., and Pfeiffer, S. E., "Composition of Hybrid Corn Tassels," *Cereal Chem.*, **23**, 428–432 (1946).

[14] Hunt, C. H., Ditzler, L., and Bethke, R. M., "Niacin and Pantothenic Acid Content of Corn Hybrids," *Ibid.*, **24**, 355–363 (1947).

[15] Foster, J. W., "Some Introspections on Mold Metabolism," *Bact. Rev.*, **11**, 167–188 (1947).

[16] McIlwain, H., "Interrelations in Microorganisms between Growth and the Metabolism of Vitamin-Like Substances," *Advances in Enzymology*, Vol. 7, Interscience Publishers, Inc., 1947, pp. 409–460.

[17] Meissel, M. N., "Hyper- and Avitaminosis in Microorganisms," *Nature*, **160**, 269–270 (1947).

[18] Mangelsdorf, P. C., and Fraps, G. S., "A Direct Quantitative Relationship between Vitamin A in Corn and the Number of Genes for Yellow Pigmentation," *Science*, **73**, 241–242 (1931).

[19] Iowa Corn Research Institute, Eleventh Annual Report, 1945–1946, pp. 35–36.

A GENE-CONTROLLED REACTION IN NEUROSPORA INVOLVING THE SYNTHESIS OF PANTOTHENIC ACID

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Communicated by J. T. Patterson, June 30, 1948

The postulate that the gene is the ultimate agent controlling biochemical reactions is accepted by many geneticists as a working hypothesis. The evidence is based on the fact that gene changes bring about changes in biochemical reactions, and sometimes create a "genetic block" which makes it impossible for products to be formed. Furthermore, each gene is assumed to be specific for a specific type of biochemical reaction, with the corollary

logically following that there exists a one-to-one relationship between the gene and the enzyme.[1,2] The experimental evidence confirming this relationship is scanty, because its direct investigation is made difficult by inadequate information on known gene-controlled reactions, and the technical difficulties of carrying out many of these reactions *in vitro*.

That the ability to produce a specific enzyme is, in fact, inherited has been demonstrated in at least two cases. The clover, *Trifolium repens* has been shown to possess a genetically determined enzyme, linamerase, which hydrolyzes the glucosides, linamerin and lotaustralin.[3] Some rabbits possess an atropinesterase in their blood which hydrolyzes atropine and monoacetylmorphine, and the ability to produce this enzyme is incompletely dominant to the condition in which the enzyme is absent.[4] In both of these cases the enzyme activity can be demonstrated *in vitro*. The substrate and end-products have been chemically characterized, and the presence or absence of the enzyme shown to be an inherited trait.

It is the purpose of the present investigation to make a direct study of the gene-enzyme relationship in the fungus, Neurospora. Two strains of Neurospora obtained from Dr. G. W. Beadle at the California Institute of Technology were used, the Emerson Wild Type 5265A and Pantothenicless 5531A. The pantothenicless mutant is unable to grow unless supplied with pantothenic acid, a vitamin not required by the wild type in its medium. It has been reported that 5531 has the ability to synthesize both β-alamine and pantoyl lactone (*DL*-α-hydroxy-β,β-dimethyl-γ-butyrolactone), and therefore presumably has a genetic block for the reaction coupling these fragments to produce pantothenic acid.[5] This mutant differs from the wild type by a single gene, and the gene locus has been approximately located on one of the seven chromosomes of Neurospora.[6]

Experimental.—Both wild type and pantothenicless Neurospora were grown in basal medium[7] with 1 γ per ml. of calcium pantothenate in the pantothenicless cultures. Mycelial pads were harvested at the end of 72 hours, washed thoroughly with sterile, distilled water and transferred aseptically to sterile flasks containing 25 ml. of distilled water. To one set of flasks 5 ml. each of 0.1 *M* solutions of β-alanine and pantoyl lactone was added. The cultures were incubated at 28°C. with constant shaking. Flasks removed after different intervals of time were steamed for about ten minutes; the mycelium was harvested and dried at 90°C., and then weighed. The fluid contents of the flasks were assayed for pantothenic acid with *Lactobacillus arabinosus* according to the method of Skeggs and Wright.[8] One per cent glucose (instead of two per cent) was used in the assay medium and growth response was measured turbidimetrically after an incubation period of 17 to 18 hours instead of acidimetrically after 72 hours' incubation. The amount of pantothenic acid produced in the medium per mg. dry weight of mycelium was calculated. The data from a 41-

hour run are presented in table 1. They show that the wild type produces considerable amounts of pantothenate in the presence of β-alanine and pantoyl lactone, and very little in their absence, whereas the pantothenicless mutant produces none in either case. There can be little doubt that wild type Neurospora is capable of coupling the pantoyl lactone and β-alanine to form pantothenic acid, and that the pantothenicless mutant has a genetic block at this coupling reaction.

It should be noted, in connection with the experiment reported above, that no attempt was made to control the pH, but that the pH remained at about 6.0 during the course of the incubation. It was also found that toluene, which was used as a preservative in one experiment, completely inhibited the production of pantothenate by wild type.

In vitro experiments were carried out with a preparation made by growing large amounts of mycelium in minimal medium. Growth was permitted to proceed for 48 hours with aeration, and the mycelium then washed with distilled water and four successive portions of dry acetone. After being dried thoroughly the preparation was ground to a fine powder and stored in the refrigerator. The yield is about 20 to 23 g. of acetone-dried mycelium per 15 liters of culture fluid.

TABLE 1

PRODUCTION OF PANTOTHENATE BY INTACT, RESTING MYCELIUM OF WILD TYPE AND PANTOTHENICLESS *Neurospora* ("PRECURSORS" REFERS TO β-ALANINE AND PANTOYL LACTONE)

PERIOD OF INCUBATION (HOURS)	MICROGRAMS PANTOTHENATE PRODUCED PER MG. DRY WEIGHT MYCELIUM			
	WILD TYPE		PANTOTHENICLESS	
	PERCURSORS	NO PERCURSORS	PERCURSORS	NO PERCURSORS
0	0.01	0.01	0.01	0.00
5.5	0.09	0.01	0.01	0.01
16.5	0.35	0.04	0.01	0.03
22.5	1.23	0.11	0.03	0.03
28.5	2.50	0.11	0.02	0.03
41.0	5.00	0.06	0.02	0.02

The effect of hydrogen-ion concentration on the production of pantothenate was tested by using 50 mg. of the dried mycelial preparation per flask suspended in 25 ml. of 0.04 *M* potassium phosphate buffer solution containing 1 ml. each of 0.1 *M* solutions of pantoyl lactone and β-alanine. The solutions were sterilized and the mycelial preparation added aseptically. The reaction mixtures were incubated at 25°C. and shaken constantly for 24 hours. They were then steamed, the solids separated by centrifugation and the centrifugates assayed for pantothenate. Since in alkaline solution there is a certain amount of synthesis of pantothenic acid from β-alanine and pantoyl lactone when these compounds are heated together in solution, a control series of flasks was set up which contained no mycelium. The contents of these flasks were assayed and the values obtained subtracted

from the pantothenate values found in the flasks with mycelium. The results expressed in micrograms pantothenate per mg. acetone-dried preparation are given in table 2. They show that the optimal pH range under the conditions of this experiment lies above pH 6.0.

TABLE 2

THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE SYNTHESIS OF PANTOTHENATE BY THE ACETONE-DRIED MYCELIUM OF WILD TYPE *Neurospora*

pH	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
5.0	0.01
5.5	0.29
6.0	0.70
6.5	0.79
7.0	0.59
7.5	0.62
8.0	0.59
9.0	0.54

The effect of omitting the precursors, β-alanine and pantoyl lactone, is shown in table 3. In this experiment incubation time was 24 hours at 25°C. The blank contained only phosphate buffer at pH 6.0 and the mycelium. The data demonstrate the dependence of the reaction on the presence of both of the precursors.

TABLE 3

THE EFFECT OF THE PRESENCE OF β-ALANINE AND PANTOYL LACTONE ON THE PRODUCTION OF PANTOTHENATE BY WILD TYPE *Neurospora*

	MICROGRAMS PANTOTHENATE PRODUCED PER MG. MYCELIUM
Pantoyl lactone + β-alanine	0.58
Pantoyl lactone	0.13
β-alanine	0.11
Blank	0.10

Table 4 shows the effect of increasing the concentration of the mycelial preparation on the amount of pantothenate synthesized during an incubation period of 24 hours at 25°C. The total amount of pantothenate produced per flask is given in the table.

The release of pantothenic acid preformed in the wild type mycelium used in the preceding experiments might be expected to have some effect on the results. For this reason intact and acetone-treated mycelia were assayed for their content of the vitamin. Preparations from fresh cultures were homogenized in acetate buffer at pH 4.5 and treated with Clarase and Caroid enzyme mixtures for 24 hours at 37°C.[9] No pantothenate was detected by assay in the case of the intact mycelium, probably because of incomplete digestion. However, the acetone-treated mycelium contained 0.014 γ pantothenate per mg., a concentration very close to the pantothen-

ate content reported for *Oidium lactis*.[10] If we accept this value as a reasonably accurate one for wild type Neurospora then it becomes quite evident that pantothenate must actually have been synthesized, and not merely released after having been formed during the actively growing phase of the mycelium.

Discussion.—The data presented demonstrate that there is an enzyme system present in wild type Neurospora which catalyzes the synthesis of pantothenic acid from β-alanine and pantoyl lactone. This reaction can be demonstrated both *in vivo* and *in vitro*, although the rate is not as great in the latter case. Efforts made to increase the activity of the acetone-dried preparation to at least that of the intact mycelium have not met with consistent results. At present it appears that glucose and coenzymes such as adenosine triphosphate, pyridoxal phosphate and Coenzyme I may stimulate the synthesis of pantothenate *in vitro*, but the proper conditions have not yet been worked out.

TABLE 4

THE EFFECT OF CONCENTRATION OF THE MYCELIAL PREPARATION ON THE SYNTHESIS OF PANTOTHENATE

MG. MYCELIUM PER FLASK	MICROGRAMS PANTOTHENATE PRODUCED PER FLASK
25	37
50	55
75	93
100	175

The present investigation indicates that the reaction leading to the production of pantothenate from β-alanine and pantoyl lactone in Neurospora should be useful in studying directly the gene-enzyme relationship in this organism.

The authors gratefully acknowledge the interest shown in their work by Dr. H. K. Mitchell of the California Institute of Technology whose suggestion concerning the possible usefulness of the pantothenicless mutant of Neurospora provided the initial stimulus for this investigation.

[1] Wright, S., *Physiol. Rev.*, **21**, 487–527 (1941).
[2] Beadle, G. W., *Chemical Rev.*, **37**, 15–96 (1945).
[3] Atwood, S. S., and Sullivan, J. T., *J. Hered.*, **34**, 311–320 (1943).
[4] Sawin, P. B., and Glick, D., these PROCEEDINGS, **29**, 55–59 (1943).
[5] Tatum, E. L., and Beadle, G. W., *Ann. Missouri Bot. Gard.*, **32**, 125–129 (1945).
[6] Beadle, G. W., *Am. Scientist*, **34**, 31–54 (1946).
[7] Horowitz, N. H., and Beadle, G. W., *J. Biol. Chem.*, **150**, 325–333 (1943).
[8] Skeggs, H. R., and Wright, L. D., *Ibid.*, **156**, 21 (1944).
[9] Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *Univ. of Texas Pub. No.* **4237**, pp. 15–36 (1942).
[10] Woods, A. M., Taylor, J., Hofer, M. J., Johnson, G. A., Lane R., and McMahan, J. R., *Ibid.*, pp. 84–86 (1942).