FORMATION OF AMYLOMALTASE AFTER GENETIC TRANSFORMATION OF PNEUMOCOCCUS

SANFORD LACKS* AND ROLLIN D. HOTCHKISS
The Rockefeller Institute, New York, N.Y. (U.S.A.)

(Received May 24th, 1960)

SUMMARY

Transformation of mutant strains of pneumococcus which lack the enzyme amylomaltase with DNA from a wild strain which can produce the enzyme, results in the rapid formation of amylomaltase in the recipient cells. Within 10 min, a small fraction of the generation time, the enzyme is being produced at its maximal rate—as a constant proportion of new protein synthesized.

When parallel cultures of different mutant strains are transformed, although the cells are believed to take up the same amounts of DNA, the rate of formation of amylomaltase is proportional to the extent of permanent genetic change, which is different for the different recipient populations. This suggests that DNA must not only be incorporated but must be permanently integrated in the cell in order to elicit enzyme synthesis.

Transformation of a mutant strain with DNA from another strain mutated at a different but linked site, also results in the rapid formation of amylomaltase. In this case enzyme formation requires that donor DNA interact with host DNA, probably by some form of genetic recombination.

Thus, in the process of transformation, it appears that integration, recombination, and probably replication of newly-introduced DNA, as well as its elicitation of specific protein synthesis, occur very soon after entry of the DNA into the cell.

INTRODUCTION

The genetic transformation of pneumococcus by deoxyribonucleate[1] affords an opportunity to study the manner in which the genetic material effects the synthesis of specific proteins. It has been previously reported that mutant strains of pneumococcus lacking an enzyme essential for the utilization of maltose, when transformed with DNA give rise to maltose-utilizing cells[2]. In the present investigation the appearance of the enzyme in transformed cultures has been followed.

DNA from a maltose-utilizing wild strain is able to transform different mutant strains to maltose-utilization, but for each strain this transformation occurs with a characteristic frequency. Furthermore, in certain cases DNA from one mutant strain

Abbreviations: DNA, deoxyribonucleate; Tris, tris(hydroxymethyl)aminomethane.

* Present address: Biological Laboratories, Harvard University, Cambridge, Mass. (U.S.A.).

can transform cells from another to maltose-utilization, and a genetic analysis based on the frequencies of such recombination indicated that the different mutations represent alterations in different, closely-linked regions of the genetic material[2].

The enzyme which is present in the wild strain but absent in the mutant strains is a transglycosidase which cleaves maltose to give glucose and oligosaccharides[2]. Since its action on maltose appears to be similar to that of the enzyme of *E. coli* called amylomaltase[3], it will also henceforth be referred to as amylomaltase.

EXPERIMENTAL TECHNIQUES

The manner in which the amylomaltase-deficient mutant strains of pneumococcus, Mc, Md, Me, Mf, Mg, and Mi, were obtained has been previously described[2]. DNA preparations were made from R6S, a non-encapsulated derivative of a wild strain which contained, also, a marker for streptomycin-resistance, and from MfS, a mutant strain into which the streptomycin-resistance marker had been incorporated by means of transformation. DNA was prepared essentially according to the method of McCARTY AND AVERY[4].

Cultures of the mutant strains were grown for transformation in 400-ml batches of a casein hydrolysate–vitamins–salts medium supplemented with bovine serum albumin, yeast extract, and glucose as previously described[2]. When the turbidity corresponded to about $4 \cdot 10^7$ colony-forming units/ml, 40 ml of glycerol were added, and the culture was frozen. Within several days, the culture was thawed, centrifuged, and the cells were resuspended in 40 ml of medium. Transformation was accomplished according to the method of FOX AND HOTCHKISS[5]. DNA was added to give a concentration of 5 μg/ml. After either 5, 10, or 20 min at 30° deoxyribonuclease (Worthington Biochemical Co.) was added to give a concentration of 1 μg/ml. Aliquots were then withdrawn into tubes containing medium at 37°. The amount of medium into which each aliquot was withdrawn was chosen so as to allow the cells to remain in exponential growth during the incubation period. After various lengths of time these sub-cultures were chilled in an ice-bath.

Samples were assayed for total cells, maltose-utilizing transformants and streptomycin-resistant transformants by means of colony counts in dilution tubes containing selective media and antibody[2,6]. The contents of the tubes were then centrifuged and resuspended in 5 ml Tris buffer, 0.05 *M*, pH 7.3. Then 0.2 ml were removed for protein determination with the Folin-Ciocalteau phenol reagent (Amend Chemical Co.) according to the procedure of LOWRY *et al.*[7] and following the suggestions of OYAMA AND EAGLE[8]. The suspension was then centrifuged and the cells were assayed for amylomaltase content.

Measurement of amylomaltase depended on the specific oxidation of glucose, one of the products of its action on maltose, by glucose oxidase to give H_2O_2 which in the presence of peroxidase reacted with *o*-dianisidine to give a colored product*. Cells to be assayed were taken up in 0.2 ml Tris buffer, 0.05 *M*, pH 7.3, containing 0.02 *M* NaF, 0.003 *M* glutathione, 0.1 *M* NaCl, and 0.07 % sodium deoxycholate. Addition of glutathione was essential for the preservation of enzyme activity; fluoride inhibited further metabolism of glucose; NaCl and deoxycholate were neces-

* This procedure for measuring glucose and the necessary reagents are marketed by the Worthington Biochemical Company, Freehold, New Jersey, under the trade-name "Glucostat".

sary for lysis of the cells. On incubation at 37° for 10 min the cells were lysed. After chilling the lysates, 0.1 ml of 6% maltose (twice recrystallized from alcohol) was added, and the mixtures were incubated for 2 h at 37°. To end the reaction the samples were heated for 5 min at 100°. Glutathione was destroyed by the addition of 0.05 ml 1% H_2O_2 which in turn was destroyed by subsequently treating with 0.05 ml of 0.02% catalase (lyophilized preparation, Nutritional Biochemicals). Heating again for 5 min at 100° destroyed the catalase. To the samples were added 3 ml of a solution containing 3.75 mg glucose oxidase (Worthington), 0.15 mg horseradish peroxidase (Worthington), and 0.3 mg *o*-dianisidine (Eastman Organic Chemicals) in Tris buffer, 0.05 *M*, pH 8.5. At this pH interference by maltase present in the glucose oxidase preparation was eliminated. After 15 min at room temperature 0.05 ml of 4 *N* HCl was added to end the reaction and stabilize the color. Coagulated protein was removed by centrifugation and the O.D. of the clear supernates was read at 420 mμ in a Beckman spectrophotometer. The proportionality of color to the amount of amylomaltase, even in the presence of large amounts of lysed amylomaltase-deficient cells, is shown in Fig. 1. Amylomaltase activity will be expressed in terms of a standard unit defined as that amount which produces 1 mμmole of glucose/min.

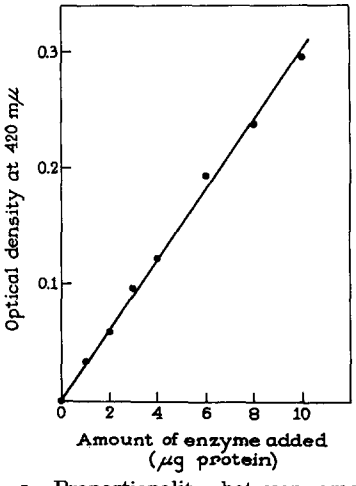

Fig. 1. Proportionality between amount of amylomaltase and color produced in the assay reaction. The indicated amounts of a purified amylomaltase preparation were added to mixtures of NaF, NaCl, glutathione, sodium deoxycholate, and cells of the negative strain, Mc, equivalent to 0.1 mg protein, in 0.2 ml Tris buffer. Lysis of the cells occurred on incubation for 10 min at 37°. Maltose was added and incubation was continued for 1 h. Samples were assayed for glucose as described in the text. The value found for the maltose blank, 0.010, was subtracted from the O.D. readings.

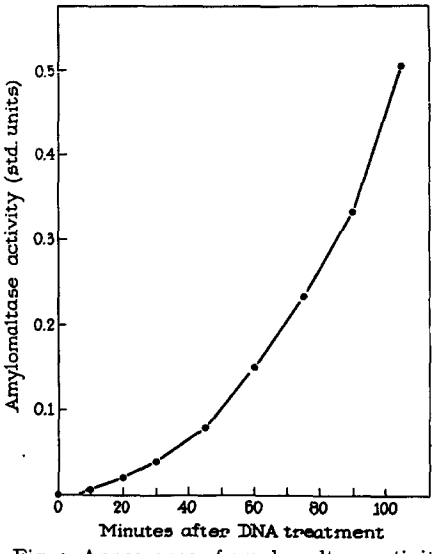

Fig. 2. Appearance of amylomaltase activity in a transformed culture of a negative strain. A suspension of Mg cells was treated with wild-type DNA for 5 min at 30°. After treatment with deoxyribonuclease for 1 min, samples were diluted into fresh medium at 37° and incubated for various lengths of time. Amylomaltase activity in the samples was determined as described in the text.

RESULTS

Soon after a brief exposure to DNA from the wild strain amylomaltase, activity appeared in a transformed culture of a mutant strain. This is shown in Fig. 2 for the

case of strain Mg treated for 5 min with DNA from strain R6S. Although there appears to be a lag of about 6 min, within 10 min enzyme was detectable and the rate of formation augmented with growth of the culture.

When the increase in amylomaltase is examined as a function of the increase in total protein, a linear relationship is observed (Fig. 3). At first, protein increases to the extent of 20 % without the appearance of any enzyme; this increase in protein corresponds to a lag in time of about 6 min. By 10 min, however, and thereafter, amylomaltase is synthesized at its maximal rate, as a constant proportion of new protein.

The increasing rate of enzyme formation, clearly evident by 40 min (Fig. 2) parallels the increasing rate of protein synthesis in the exponentially growing culture (Fig. 3). This correspondence indicates that at least by this time the newly introduced genetic factor is multiplying as rapidly as the genetic factors responsible for protein synthesis in general, that is, the host genome.

It has been reported[2] that cells of different mutant strains are genetically transformed by R6S DNA to maltose utilization with different frequencies even when transformation to streptomycin-resistance occurs with the same frequency in the different strains. It might be expected that although the extent of permanent genetic incorporation varied, the rate of formation of enzyme would be the same if it depended only on the amount of DNA which entered the cell. In order to investigate this possibility parallel cultures of different strains were treated with DNA from the maltose-utilizing strain and the appearance of amylomaltase was followed.

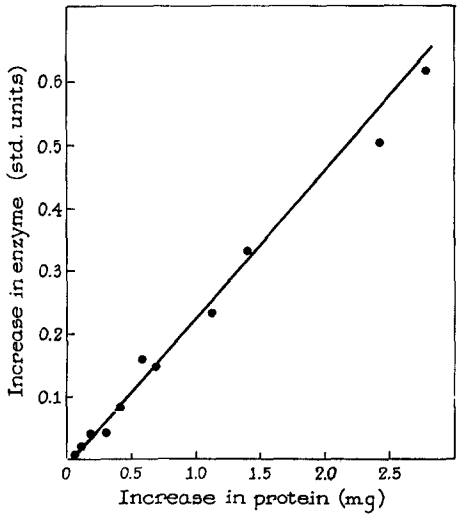

Fig. 3. Formation of amylomaltase with respect to protein synthesis in a transformed culture. Same experiment as in Fig. 2. Amylomaltase and protein were determined as described in the text. Prior to incubation the samples contained 0.27 mg protein and no detectible amylomaltase activity.

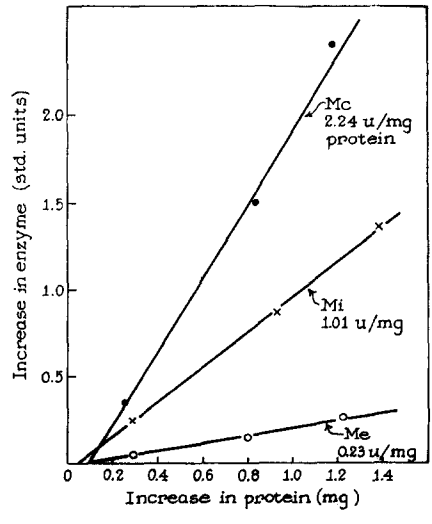

Fig. 4. Rates of formation of amylomaltse in transformed cultures of different mutant strains. Cultures of Mc, Mi, and Me, frozen at the same stage of growth, were thawed and treated with wild-type DNA for 20 min at 30°. After treatment with deoxyribonuclease for 1 min, samples were diluted into fresh medium and incubated 0, 30, 60, and 90 min at 37°. Initially, the protein content of each culture was about 0.3 mg, and no amylomaltase was detectible.

Fig. 4 presents the results obtained in comparing strains Mc, Mi, and Me which are permanently transformed to maltose-utilization at frequencies in the ratios of 10:4:1. In all cases the kinetics of enzyme formation was the same: after a brief delay amylomaltase was produced as a constant proportion of total protein. It is clear, however, that the rates were proportional to the extent of permanent transformation of the maltose character. Similar results were obtained in comparing strains Mg and Md (Fig. 5). Table I summarizes these results.

TABLE I

RELATIONSHIP OF THE RATE OF FORMATION OF ENZYME TO THE EXTENT OF PERMANENT TRANSFORMATION

Expt.	*Recipient strain*	*DNA donor**	*Total cells*	*Transformants to:*		*Rate of amylo-maltase formation***	*Relative extent of transformation to mal+*	*Relative rate of enzyme formation*
				Streptomycin resistance	*Maltose utilization*			
1	Mc	++	$1.5 \cdot 10^9$	$3.4 \cdot 10^7$	$5.5 \cdot 10^7$	2.24	1.00	1.00
	Mi	++	$1.7 \cdot 10^9$	$2.7 \cdot 10^7$	$2.1 \cdot 10^7$	1.01	0.38	0.45
	Me	++	$1.5 \cdot 10^9$	$3.3 \cdot 10^7$	$3.3 \cdot 10^6$	0.23	0.06	0.10
2	Mg	++	$3.5 \cdot 10^9$	$2.9 \cdot 10^7$	$4.6 \cdot 10^7$	2.47	1.00	1.00
	Md	++	$3.1 \cdot 10^9$	$2.8 \cdot 10^7$	$1.8 \cdot 10^7$	0.98	0.39	0.40
3	Mc	++	$7.2 \cdot 10^8$	$1.1 \cdot 10^7$	$1.6 \cdot 10^7$	1.29	1.00	1.00
	Mc	MfS	$7.0 \cdot 10^8$	$1.0 \cdot 10^7$	$4.6 \cdot 10^6$	0.29	0.29	0.23

* Wild-type DNA with streptomycin-resistance marker indicated as ++.
** Units of enzyme activity per mg of new protein.

A priori it would be expected that the cells in the different parallel cultures took up the same amount of wild-type DNA. This supposition is confirmed by the observation that the extent of transformation to streptomycin-resistance was the same in the different cases compared, for LERMAN AND TOLMACH[9] and FOX[10] have shown uptake of [^{32}P]DNA to be generally proportional to the number of streptomycin-resistant cells produced. So, although the same amount of DNA containing the wild-type allele of the amylomaltase locus must have been taken up by the cells, the extent of transformation to maltose-utilization depended on the particular recipient and the rate of formation of enzyme from the start was correlated with the extent of this permanent genetic change.

Amylomaltase-deficient strains in certain cases can be crossed to produce maltose-utilizing cells[2]. The appearance of enzyme was followed in one such cross in which Mc was the recipient and MfS the donor of the DNA. Fig. 6 shows that enzyme synthesis was initiated as rapidly with mutant as with wild-type DNA. As in the latter case, the amount of enzyme formed was proportional to total protein synthesized. However, the rate of enzyme formation was reduced, corresponding to the reduced extent of permanent transformation to maltose-utilization, which was in this case occasioned by the necessity for recombination between closely linked sites. Unless this correspondence is fortuitous, it seems probable that the genetic elements of strains Mc and Mf do not complement each other when on separate structures. Observations in which complementation of function occurs between closely-linked sites apparently governing individual enzymes[11,12] have been rare and may not refer

to sites on homologous DNA molecules. Therefore, it is most likely that some form of recombination between different, homologous genetic structures must have occurred very early in order to allow amylomaltase to be synthesized at its maximal rate by 15 min after DNA treatment.

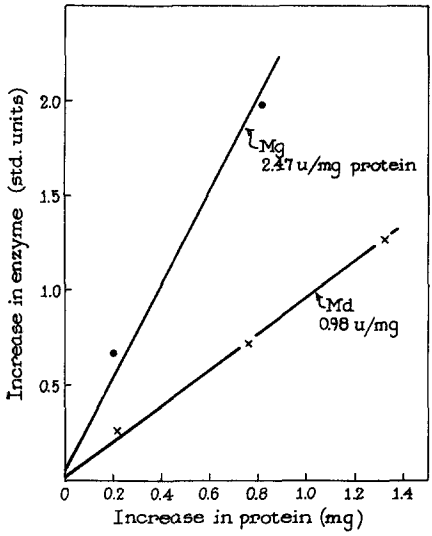

Fig. 5. Rates of formation of amylomaltase in transformed cultures of strains Mg and Md. Procedure was similar to the experiment depicted in Fig. 4. Initially, the cultures contained 0.4 mg of protein.

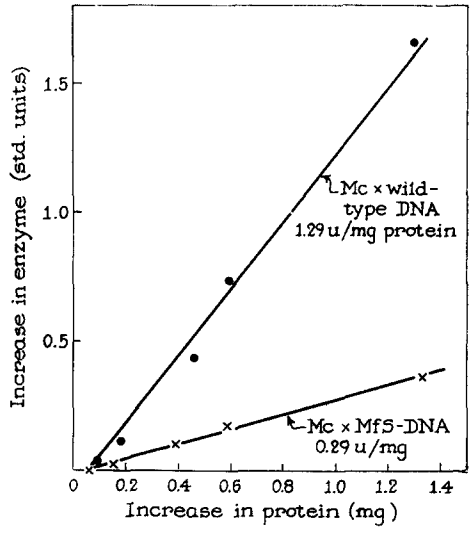

Fig. 6. Formation of amylomaltase after transformation with DNA from wild-type and mutant strains. A suspension of Mc cells was divided into two portions which were respectively treated with equivalent amounts of DNA from strains R6S and MfS for 10 min at 30°. After treatment with deoxyribonuclease for 1 min, samples of each portion were diluted into fresh medium and incubated at 37° for 0, 15, 30, 45, 60, and 90 min. Initially, 0.25 mg protein was present and no amylomaltase was detectible.

DISCUSSION

Amylomaltase is rapidly formed subsequent to the introduction of DNA conveying the genetic capacity to produce the enzyme into cells previously unable to synthesize it. Within 6 min its synthesis has begun and within ten minutes is proceeding at a maximal rate—as a constant proportion of protein synthesis. However, synthesis of the new protein appears to involve the prior integration of the genetic factor carried by the donor DNA. When strains which show different frequencies of permanent transformation of the maltose character are transformed, the rates of enzyme formation are proportional to the extent of this transformation, even though the same amount of donor DNA was taken up by the cells in the different cases. Thus, "free" DNA in the cell does not seem to give rise to enzyme synthesis. It appears that some sort of permanent integration must be necessary for synthesis to occur. Such integration must occur early inasmuch as the early attainment of the constant rate of enzyme formation with protein synthesis precludes the possibility of any long delay in the integration of the genetic factor. Furthermore, the persistence of this rate of enzyme formation indicates that there is no loss of the new factor once it is integrated; that is, no temporary transformation has occurred.

The proportionality between permanent transformation and amylomaltase synthesis holds as well for crosses between two mutant strains. Here enzyme synthesis demands not only integration of the transforming DNA molecule into an organized, functional state, but, presumably before that, recombination (either direct or dependent on replication) of genetic factors in two molecules of DNA. Yet the rapidity of onset and subsequent constancy of enzyme production with respect to protein formation attest that all recombination must have occurred very soon after DNA treatment and that "recombined" DNA is being reproduced not long thereafter.

The various results present a consistent pattern underlying the development of enzyme activity after introduction of DNA into the cell. (a) Following entry into the cell, free DNA must be integrated before it gives rise to enzyme synthesis. (b) Such integration is rapid since enzyme synthesis begins less than 10 min after DNA treatment. (c) Recombination between parts of donor and host DNA molecules to produce a new phenotype also must occur within this early period. (d) Replication of the new genetic factor must occur very soon since the constant rate of enzyme synthesis with respect to protein appears to be reached shortly thereafter; hence, any integration necessary for replication has already occurred. Thus, three different events–integration with respect to phenotypic expression, genetic recombination, and integration prerequisite to replication–all seem to occur within the same brief period. They may well be manifestations of a single process– of incorporation into the genome.

The above conclusions depend on the promptness of phenotypic expression found with the amylomaltase system. Other characters such as pyrimidine-independence, sucrose utilization, and sulfonamide-resistance may also be expressed promptly, since transformants to these traits will survive early selection. However, studies of the expression of streptomycin-resistance in pneumococcus have shown that this character does not manifest itself until, on the average, sixty minutes after incorporation of the DNA[13,14]. Perhaps streptomycin-resistance does not involve simply the presence of several molecules of enzyme, but rather more substantial changes, such as in the composition of the cell wall which would require several generations in order to have altered its dominant motif. Or looked at in another way, streptomycin-sensitivity may result from the presence of certain substances which would have to be diluted out of the cells by division before any cells could become resistant. These possibilities would be consistent with the finding that in diploid cells of *E. coli* streptomycin-resistance behaves as a recessive character[15]. (However, evidence has been presented that the contrary is true for pneumococcus[16].)

That DNA must be integrated in permanent fashion before it can exercise its function was an unexpected finding. Precisely what the nature of this integration is remains undetermined. It may be that DNA can act only when incorporated into a nuclear organization, or it may simply have to be integrated to protect it from degradation. The inability of DNA incorporated but not integrated in the cell to produce phenotypic expression, differentiates transformation from transduction in *Salmonella* where the occurrence of "abortive transduction" indicates an activity of genetic material not permanently incorporated into the host genome[17,18]. It may be that the transduced genes are in an adequate state of organization to function, that is, more highly organized than free DNA. This is suggested by the fact that a transducing particle usually contains a number of linked genes and may thus constitute an appreciable segment of a bacterial chromosome. Such an organized segment may be

able to persist indefinitely as a genetic structure even though not replicated.

One of the conclusions stemming from the present work is that the genetic factor in the transforming DNA as soon as it is integrated begins itself to be reproduced by the recipient cell. Replication of the new factor as manifested by increasing enzyme formation occurs within 40 min after incorporation, A prior estimate of the time of earliest replication as 55 min[19] is based on the multiplication of streptomycin-resistant colony-forming units which is observed within the second hour after introduction of DNA[19,20].

It is of interest to compare the manner in which amylomaltase is synthesized subsequent to transformation with specific protein synthesis elicited in other systems, for there seems to be a general pattern governing synthesis of new protein. One of the first such systems studied was induction of β-galactosidase in *E. coli* upon addition of an inducer substance[21]. Synthesis of the β-galactosidase begins at once and continues as a constant proportion of total protein synthesis. Again in *E. coli*, the release of a constitutive enzyme, ornithine carbamylase, from repression by lowering the arginine concentration also results in prompt appearance of the enzyme, its production being proportional to total synthesis[22].

Most similar to the present one is the system studied by PARDEE, JACOB AND MONOD[23] in which β-galactosidase is produced in *E. coli* cells which have undergone conjugation, for here also the eliciting mechanism is the introduction of genetic material, albeit presumably in a more organized, chromosomal, form. In this case the enzyme is produced very quickly after a brief period of conjugation and at a linear rate with time. In another case in which genetic material is introduced into a bacterial cell, when T-even bacteriophages infect cells of *E. coli*, an enzyme essential for the growth of the virus, deoxycytidylate hydroxymethylase, is produced a few minutes after infection[24].

Observations from these quite different systems are in agreement that (a) protein synthesis is a very rapid process, even when it must of necessity be *de novo* due to prior absence of a genetic factor, and (b) increase in a particular protein is proportional to increase in total protein. The level of an enzyme appears in general to play no direct role in governing its synthesis. Rather, if it is being formed, its rate of formation seems to be part of a fixed order in the cell.

REFERENCES

1 O. T. AVERY, C. M. MACLEOD AND M. MCCARTY, *J. Exptl. Med.*, 79 (1944) 137.
2 S. LACKS AND R. D. HOTCHKISS, *Biochim. Biophys. Acta*, 39 (1960) 508.
3 J. MONOD AND A. M. TORRIANI, *Compt. rend.*, 227 (1948) 240.
4 M. MCCARTY AND O. T. AVERY, *J. Exptl. Med.*, 83 (1946) 97.
5 M. S. FOX AND R. D. HOTCHKISS, *Nature*, 179 (1957) 1322.
6 R. D. HOTCHKISS, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 49.
7 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FAIR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
8 V. I. OYAMA AND H. EAGLE, *Proc. Soc. Exptl. Biol. Med.*, 91 (1956) 305.
9 L. S. LERMAN AND L. J. TOLMACH, *Biochim. Biophys. Acta*, 26 (1957) 68.
10 M. S. FOX, *Biochim. Biophys. Acta*, 26 (1957) 83.
11 N. H. GILES, C. W. H. PARTRIDGE AND N. J. NELSON, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 305.
12 J. A. PATEMAN AND J. R. S. FINCHAM, *Heredity*, 12 (1958) 317.
13 M. S. FOX, *J. Gen. Physiol.*, 42 (1959) 737.
14 M. ABE AND D. MIZUNO, *Biochim. Biophys. Acta*, 32 (1959) 464.
15 J. LEDERBERG, *J. Bacteriol.*, 61 (1951) 549.
16 R. D. HOTCHKISS, *Enzymes: Units of Biological Structure and Function*, Henry Ford Hospital International Symposium, Academic Press, New York, 1956, p. 119.

[17] H. OZEKI, *Genetic Studies with Bacteria*, Carnegie Inst. Wash. Publ., 612 (1956) 97.
[18] B. O. D. STOCKER, *J. Gen. Microbiol.*, 15 (1956) 575.
[19] H. EPHRUSSI-TAYLOR, *Recent Progress in Microbiology, Symposia held at VII Intern. Congr. for Microbiology, 1958*, p. 51.
[20] R. D. HOTCHKISS, in W. D. MCELROY AND B. GLASS, *The Chemical Basis of Heredity*, The Johns Hopkins Press, Baltimore, 1957, p. 321.
[21] J. MONOD, A. M. PAPPENHEIMER AND G. COHEN-BAZIRE, *Biochim. Biophys. Acta*, 9 (1952) 648.
[22] L. GORINI AND W. K. MAAS, *Biochim. Biophys. Acta*, 25 (1957) 208.
[23] A. B. PARDEE, F. JACOB AND J. MONOD, *J. Mol. Biol.*, 1 (1959) 165.
[24] J. G. FLAKS, J. LICHTENSTEIN AND S. S. COHEN, *J. Biol. Chem.*, 234 (1959) 1507.

Biochim. Biophys. Acta, 45 (1960) 155–163

STUDIES ON THE BIOSYNTHESIS OF 5-RIBOSYLURACIL PHOSPHATE IN *NEUROSPORA CRASSA* 36601

JOHN B. HALL AND FRANK WORTHINGTON ALLEN
Department of Biochemistry, University of California, School of Medicine, San Francisco, Calif. (U.S.A.)

(Received June 27th, 1960)

SUMMARY

(1) While the pyrimidine portion of 5-ribosyluracil phosphate has been found to be derived from orotic acid in yeast, it has been found to be derived largely from cytidine of the medium in the pyrimidine requiring mutant Neurospora, *Neurospora crassa* 36601.

(2) Experiments with [2-^{14}C]uracil indicate that uracil is not an intermediate in the conversion of cytidine into 5-ribosyluracil phosphate.

(3) Reasons are presented for the belief that a uridine phosphate, such as uridine 5′-phosphate, is an intermediate in the biosynthesis of 5-ribosyluracil phosphate.

(4) The possibility is discussed that 5-ribosyluracil phosphate is normally derived from a pathway in which orotic acid is first incorporated into uridine 5′-phosphate, which then forms an unknown intermediate and is finally converted to 5-ribosyluracil phosphate.

INTRODUCTION

Studies by HALL AND ALLEN[1] have shown that orotic acid is an effective precursor of 5-ribosyluracil phosphate in yeast. When yeast was grown in the presence of [6-^{14}C]orotic acid it was found that, within the limits of experimental error, 5-ribosyluracil phosphate and uridylic acid were equally labeled by ^{14}C and that cytidylic acid was less highly labeled than the two uracil-containing nucleotides. There seem to be two pathways that could explain these results (Fig. 1). Possibly there is yet another orotic acid pathway, similar to these, in which orotic acid reacts with 5-phosphoribose 1-pyrophosphate, or with some other activated ribose, to give 5-ribosylorotidyl