THE METABOLISM OF PURINES IN AEROBACTER AEROGENES: A STUDY OF PURINELESS MUTANTS[1]

MARCUS S. BROOKE AND BORIS MAGASANIK

Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts

Received for publication June 4, 1954

Amino acid deficient bacterial mutants have been of great value in the elucidation of the biosynthesis and the metabolic relationships of amino acids (Davis, 1952). Similarly, purineless and pyrimidineless mutants should be of value for the study of the metabolism of the components of nucleic acids. However, most purineless mutants isolated have been found to be nonexacting in their requirements and consequently their study could throw little light on the metabolism of purines.

The isolation of a nonexacting purineless mutant and of a guanineless mutant from the same parent strain of *Aerobacter aerogenes* has been reported from this laboratory (Ushiba and Magasanik, 1952; Magasanik and Brooke, 1954). The guanineless mutant was capable of synthesizing the purine ring as shown by the accumulation of xanthosine in its culture medium (Magasanik and Brooke, 1954).

This paper presents a comparison of the growth requirements of these two mutants and of their probable genetic blocks and discusses the implications of these observations on the biosynthesis of nucleic acid adenine and guanine in *A. aerogenes*.

MATERIALS AND METHODS

The isolation of the auxotrophic guanineless mutant, strain P-14, of *A. aerogenes* by ultraviolet irradiation of strain 1033 has been described previously (Ushiba and Magasanik, 1952). The purineless mutant strain PD-1 was obtained from the same parent strain by analogous procedures (Magasanik and Brooke, 1954). To obtain back mutants of strain PD-1 the organism was grown for 30 hours in minimal medium containing 6 μg adenine and 25 mμg thiamin per ml, and an aliquot of this culture was then streaked out on minimal agar containing thiamin but no adenine. Back mutants of strain P-14 were obtained by subculturing 3 ml of a 14 hour culture grown in minimal medium containing 6 μg guanine per ml into 50 ml of minimal medium containing 6 μg guanine per ml. This was allowed to grow for 24 hours, and an aliquot then streaked out on minimal medium. Back mutants of strain PD-1 were obtained frequently whereas the occurrence of back mutants of strain P-14 was rare.

The purines, ribosides, and ribotides used were products of the Nutritional Biochemicals Corporation, Cleveland, Ohio, with the exception of 2,6-diaminopurine which was a product of the Krishell Laboratories, Portland, Oregon; the *a* and *b* isomers of adenylic acid which were products of Schwarz Laboratories, Inc., New York, New York; the isoguanine which was kindly given to us by Dr. Aaron Bendich of the Sloan-Kettering Institute for Cancer Research, New York, New York; and the 4-amino-5-imidazole carboxamide[2] which was kindly given to us by Dr. Zelma Miller of the Children's Hospital, Boston, Mass. Inosine-3′-phosphate, inosine-5′-phosphate, and a mixture of xanthosine-2′ and 3′-phosphate were prepared from adenosine-3′-phosphate, adenosine-5′-phosphate, and yeast guanylic acid, respectively, according to the method of Shuster and Kaplan (1953). All of these compounds were tested for purity by spectrophotometric and chromatographic methods.

The medium used has been described (Ushiba and Magasanik, 1952). In experiments with strain PD-1 the medium was fortified with 25 μg vitamin B_1 (Nutritional Biochemicals Corpora-

[1] This work has been supported in part by the William F. Milton Fund of Harvard University, by a grant from the United States Public Health Service, and by the Eugene Higgins Trust. A preliminary report of part of this work was presented at the 53rd annual meeting of the Society of American Bacteriologists, San Francisco, August, 1953.

[2] For simplicity the term carboxamide will be used frequently in referring to the 4-amino-5-imidazole carboxamide.

TABLE 1

Response of mutants PD-1 and P-14 to purines and 4-amino-5-imidazole carboxamide

The results are expressed in millimicromoles of nutrilite needed for the production of one unit of cells.*

Nutrilite	Mutant PD-1	Mutant P-14
Isoguanine	55.4 ± 6.6†	No growth
2,6-Diaminopurine	No growth	37.2 ± 7.4
Xanthine	54.1 ± 2.1	No growth
Hypoxanthine	49.3 ± 1.6	No growth
Adenine	52.4 ± 0.2	No growth
Guanine	47.5 ± 0.5	38.8 ± 8.5
Carboxamide	10,300	No growth

* A unit is defined as the number of cells contained in 1.0 ml of a suspension of an optical density of 1.0 at 590 mμ determined in a square cuvette (light path 13 mm) in a Coleman Universal spectrophotometer, model 14. A unit contained about 9×10^8 viable cells and 50 μg of bacterial nitrogen.

† Standard deviation.

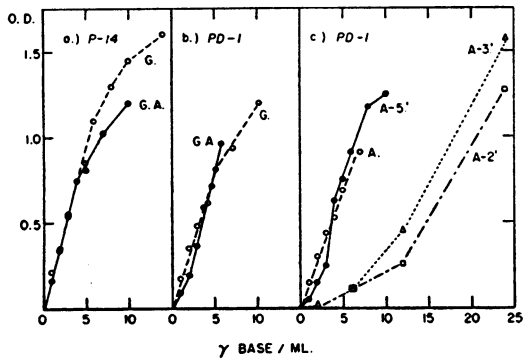

Figure 1. Growth of mutants P-14 and PD-1 on guanine (G.) and guanylic acid (G.A.) and of mutant PD-1 on adenine (A.), adenosine-5′-phosphate (A-5′), adenosine-3′-phosphate (A-3′), and adenosine-2′-phosphate (A-2′).

tion) per liter, or maximum growth was not obtained.

To determine the bacterial growth about 10^7 cells were taken from the growth on a tryptic-digest agar slant, suspended in distilled water, and inoculated into a 125 ml Erlenmeyer flask containing 20 ml of basal medium plus varying amounts of the growth factors. All compounds were tested in concentrations as high as 100 μg per ml with the exception of the carboxamide which was tested at levels up to 600 μg per ml. The solutions of the bases were sterilized by autoclaving, and those of the ribosides, ribotides, and carboxamide were sterilized by filtration or prepared aseptically and added to the medium subsequent to its being autoclaved. The inoculated flasks were shaken on a Brunswick rotary action flask shaker, model V, at 37 C for 18 hours. The turbidity of the cultures was then measured at 590 mμ in a Coleman spectrophotometer, model 14.

RESULTS

Purines. Strain P-14 requires guanine, guanosine, guanylic acid, or 2,6-diaminopurine for growth. Adenine, hypoxanthine, xanthine, the corresponding ribosides and ribotides, isoguanine, or 4-amino-5-imidazole carboxamide all fail to support the growth of this mutant. Strain PD-1 is a nonexacting purineless mutant which grows on adenine, hypoxanthine, guanine, the corresponding ribosides or ribotides, isoguanine, xanthine, or 4-amino-5-imidazole carboxamide. It cannot grow on xanthosine, xanthosine-2′ and 3′-phosphate, or 2,6-diaminopurine. The quantitative response of mutants P-14 and PD-1 to the purines and their derivatives is shown in table 1 and figure 1. It will be noted that in experiments with strain PD-1 vitamin B_1 had to be added to the medium before maximum growth was obtained. In the absence of this vitamin no matter how much purine or purine derivative was added to the medium, poor growth (optical density *circa* 0.1 as opposed to an optical density of 1.8 which represents full growth) resulted. Growth of mutant P-14 on 2,6-diaminopurine was clumpy, and the results variable. These equivocal results might be due to a disturbance of the guanine to adenine ratio in the cell, it being assumed (*vide infra*) that 2,6-diaminopurine is used solely for the synthesis of nucleic acid guanine and not of nucleic acid adenine. Such an effect has been suggested by Bendich *et al.* (1950). It was usually observed that for a given growth increment strain PD-1 required more guanine than did strain P-14 (table 1 and figure 1). The amount of carboxamide required by strain PD-1 for a standard increase of growth was several hundred times greater than the amount of other nutrilites required for the same growth increment. This is in agreement with the findings of other workers (Bergmann *et al.*, 1952; Gots, 1950).

Purine ribosides. The results with the ribosides

of adenine, hypoxanthine, guanine, and xanthine were, both qualitatively and quantitatively, essentially the same as those with the free bases except that strain PD-1 did not grow on xanthosine.

Purine ribotides. The response of both strains to guanylic acid was similar to that to guanine (figure 1). Strain PD-1 grew poorly on low levels (up to 3 μg per ml) of adenosine-5′-phosphate, but subsequently the response to increasing amounts of the ribotide was similar to that obtained with the free base. Analogous but accentuated results were obtained with adenosine-2′-phosphate and adenosine-3′-phosphate, the response to these compounds being poor until more than 12 μg per ml of nutrilite was added to the medium. Similar results were obtained with inosine-3′-phosphate and inosine-5′-phosphate. The poor growth response at low levels of these compounds remains unexplained. It is possible that under these conditions the growth rate is slower, either because the nutrilite cannot easily penetrate the cell or because it has to be dephosphorylated before it can be used. It was not possible to extend the period of growth to see whether higher levels of growth could be reached with smaller amounts of nutrilite because the emergence of back mutants obscured the results.

Sparing effect of 2,6-diaminopurine. Strain PD-1 could not grow in a minimal medium supplemented only with 2,6-diaminopurine. However, 2,6-diaminopurine spared adenine when the latter compound was used to support the growth of the organism as illustrated in figure 2. It can be seen that 2,6-diaminopurine had quantitatively the same growth promoting activity as adenine, but full growth could not be obtained with less than about 5 μg per ml of adenine, irrespective of the amount of 2,6-diaminopurine used. Guanine could be substituted for adenine with the same results. 2,6-Diaminopurine did not inhibit the growth of strain PD-1 or of the parent strain 1033 when added to the medium in a concentration of 25 μg per ml.

Inhibitory effects. Strain P-14 will grow on guanine but not on adenine; however, when both guanine and adenine are supplied in the medium, growth is inhibited, a mixture of 6 μg of guanine and 1 μg of adenine per ml of culture fluid resulting in approximately 80 per cent inhibition. Similar results were obtained when hypoxanthine was used instead of adenine. When adenine was supplied as adenosine rather than as the free base, 3 μg per ml of adenine were needed to obtain 80 per cent inhibition. Adenosine-3′-phosphate or adenosine-5′-phosphate in concentrations equivalent to 12 μg per ml of adenine failed to cause any inhibition. The same results were obtained when guanosine, guanylic acid, or 2,6-diaminopurine was substituted in these experiments for guanine. In no case was the inhibition relieved by increasing the concentration of guanine, guanosine, guanylic acid, or 2,6-diaminopurine. The results of these experiments were reproducible only if an inoculum of standard size was used (10^7 cells per flask). Large inocula (i.e., 10^9 cells of an 18 hour culture) obscured the inhibition. Fries (1949) using a mutant of *Ophiostoma multiannulatum* obtained similar results with guanine and guanosine, on the one hand, and adenine or hypoxanthine, on the other hand, although the inhibitions he ob-

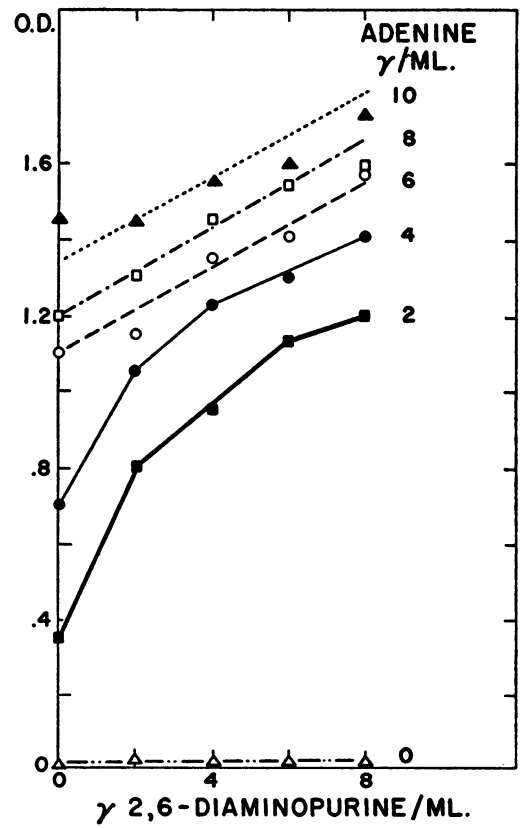

Figure 2. Growth of mutant PD-1 in a medium containing adenine supplemented with 2,6-diaminopurine.

tained were not as marked as those obtained in the present work.

Accumulation of compounds. In culture fluids of strain P-14 a compound strongly absorbing ultraviolet light was observed, isolated, and characterized as xanthosine (Magasanik and Brooke, 1954). Until now, no compound has been found to accumulate in culture fluids of strain PD-1; the Bratton-Marshall test which would detect the presence of 4-amino-5-imidazole carboxamide, its riboside, or ribotide (Gots and Chu, 1952) was negative.

DISCUSSION

The two purineless mutants of *A. aerogenes*, strain 1033, produced by ultraviolet irradiation differ in several respects from the parent strain. Strain PD-1 expresses its changed genotype by being unable to grow in the absence of purines, ribosides, ribotides, or 4-amino-5-imidazole carboxamide, and by being unable to reach full growth in the absence of thiamin. It is nonexacting in its purine requirement; of all the purines and their derivatives used in this work only xanthosine, xanthosine-2′ and 3′-phosphate, and 2,6-diaminopurine failed to support its growth. The latter compound could, however, spare the organism's requirement for guanine or adenine.

Strain P-14 differs from the wild type by being unable to grow unless provided with guanine, guanosine, guanylic acid, or 2,6-diaminopurine; by accumulating xanthosine in its culture medium; and by its sensitivity to adenine, to hypoxanthine and, to a lesser degree, to adenosine.

According to the hypothesis that ultraviolet irradiation causes mutation by the absorption of a single quantum of energy by an affected cell, the metabolic changes observed in these auxotrophic mutants should be the consequences of a single genetic change resulting in the deletion of one discrete enzymatic step. The isolation from both mutant P-14 and mutant PD-1 of back mutants, which phenotypically were identical with the wild strain 1033, supports this view. The back mutant from strain P-14 did not accumulate xanthosine and was not inhibited by adenine. The back mutant from strain PD-1, although selected on a medium containing thiamin, did not require this vitamin for growth. At present, not all the changes observed can be correlated with the loss of a single enzyme. However, the observations made give a reasonably good indication of the position of the metabolic blocks in mutants PD-1 and P-14.

The study of the biosynthesis of the purine components of nucleic acid in bacteria (Sutton and Werkman, 1953; Koch *et al.*, 1952), yeast (Abrams *et al.*, 1948), and higher organisms (Barnes and Schoenheimer, 1943; Heinrich and Wilson, 1950) has provided convincing evidence that both the guanine and adenine of both types of nucleic acids arise from common precursors of small molecular size, and that a derivative, presumably the riboside-5′-phosphate of 4-amino-5-imidazole carboxamide, is a key intermediate in their synthesis (Greenberg, 1953*a*,*b*; Gots, 1953). The free carboxamide can be used for the synthesis of nucleic acids by some micro- and higher organisms although much less efficiently than the purine bases (Bergmann *et al.*, 1952; Gots, 1950). The finding that strain PD-1 responds to comparatively large quantities of the carboxamide agrees with these results and suggests that the genetic block in this mutant is before the biosynthesis of the carboxamide derivative. This organism thus is not capable of any *de novo* synthesis of nucleic acid purines and must use the preformed purines, supplied in the medium, for the synthesis of both nucleic acid guanine and nucleic acid adenine. Growth of this mutant on adenine, guanine, hypoxanthine, or xanthine is evidence of its ability to convert any one of these compounds to both the purines of nucleic acids (table 1). The interconversion of guanine to nucleic acid adenine and of adenine to nucleic acid guanine varies, both qualitatively and quantitatively, in different species of microorganisms, and a complete spectrum of purine interchange has been observed. This has been reviewed recently by Brown (1953). Thus, some microorganisms (*Tetrahymena geleii* and *Lactobacillus leichmannii*) can grow only on guanine which they convert to nucleic acid adenine whereas others (*Torulopsis utilis*) can grow only on adenine which they convert to nucleic acid guanine. The present results agree with the observation that *A. aerogenes* (Hamilton *et al.*, 1952) and the closely related *Escherichia coli* (Koch *et al.*, 1952; Bolton and Reynard, 1954) interconvert both adenine and guanine although with different degrees of efficiency.

In contrast to the other purines, 2,6-diaminopurine does not support the growth of strain

PD-1 but spares guanine or adenine when either of the latter compounds is supplied in the medium. This observation suggests that 2,6-diaminopurine can be used for the synthesis of either nucleic acid adenine or nucleic acid guanine, but not for both. The present finding that 2,6-diaminopurine alone supports the growth of the guanineless mutant P-14, as well as the results of Bendich and Brown (1948) showing that 2,6-diaminopurine is incorporated into nucleic acid guanine but not into nucleic acid adenine in the rat, makes it highly probable that in the nonexacting mutant PD-1, 2,6-diaminopurine is used only for the synthesis of nucleic acid guanine and not for that of nucleic acid adenine. An alternative theory is that 2,6-diaminopurine is inhibitory and that the inhibition is relieved by the addition of adenine or of guanine. Such inhibitions are known (Elion and Hitchings, 1950; Balis *et al.*, 1952) but are unlikely to be in effect because the wild strain, 1033, is not inhibited by 2,6-diaminopurine and increasing the amount of 2,6-diaminopurine to the relatively high level of 25 μg per ml in the presence of adenine or guanine in cultures of strain PD-1 did not cause inhibition.

The observation that strain P-14 accumulates xanthosine (Magasanik and Brooke, 1954) is proof of its ability to synthesize the purine ring. This, together with the finding that the organism grows only on guanine, guanosine, guanylic acid, or 2,6-diaminopurine, suggests that it cannot make nucleic acid guanine. We postulate, however, that there is a *de novo* synthesis of nucleic acid adenine. The observation that the response of this strain to guanine is more efficient than that of strain PD-1 supports this hypothesis (figures 1 and 2).

The foregoing results are the basis of the tentative scheme for the biosynthesis of purines in *A. aerogenes* in figure 3.

The synthesis of purines is thought to proceed *via* the ribotide of 4-amino-5-imidazole carboxamide (Greenberg, 1953*a*; Buchanan and Schulman, 1953; Williams and Buchanan, 1953). The reasons for assuming the genetic block in mutant PD-1 to be before the carboxamide have already been discussed. It is proposed that the genetic block in mutant P-14 is caused by the lack of an enzyme which converts a derivative of xanthosine to one of guanosine. Xanthosine itself is unlikely to be the true intermediate as strain PD-1 cannot utilize this compound nor xanthosine-2′ and 3′-phosphate. The possibility remains that the active intermediate is in reality xanthosine-5′-phosphate and that the accumulation of xanthosine is the result of phosphatase activity. A similar finding is the accumulation of carboxamide-riboside in strains of *E. coli* inhibited by sulfonamides rather than of the biologically active carboxamide-ribotide (Greenberg, 1953*b*).

On the assumption now discussed that a derivative of xanthosine is intermediate between inosinic acid and nucleic acid guanine, it appears that stemming from inosinic acid there is a dichotomy, one branch leading to nucleic acid adenine and the other to nucleic acid guanine. On chemical grounds the conversion of inosinic acid to a derivative of adenine by amination and of inosinic acid to a derivative of xanthine by oxidation is most plausible. The biological activity of the carboxamide-ribotide and the accumulation of xanthosine support the assumption that the free purine bases are not normal intermediates of the biosynthesis of nucleic acids. The bases may be expected to exert their growth promoting activities by being converted to one of the active intermediates shown in the scheme (figure 3). The existence of an enzyme which will form riboside-5′-phosphate from the free base and ribose-1,5-diphosphate has been described by Saffran and Scarano (1953). Bacterial enzymes are known to convert adenine to hypoxanthine (Friedman and Gots, 1953), and isoguanine to xanthine (Friedman and Gots, 1951). 2,6-Diaminopurine, on the other hand, would seem to enter the scheme very close to nucleic acid

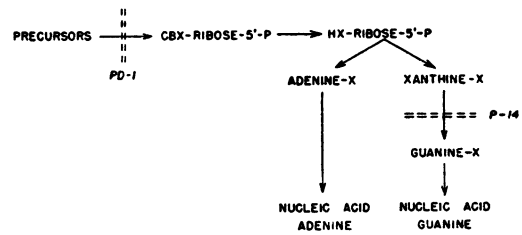

Figure 3. The pathway of nucleic acid synthesis in *Aerobacter aerogenes*. CBX = 4-amino-5-imidazole carboxamide; HX = hypoxanthine; −X = an active intermediate. The purines of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are presumably formed by the same pathway (Balis and Lark, 1954). The dotted double lines represent the genetic blocks.

guanine and not to be converted to nucleic acid adenine or one of the earlier derivatives.

The suggestion that the active intermediates are identical with the riboside-5′-phosphates finds some support in the observation that growth of strain PD-1 on adenosine-5′-phosphate is better than growth on the 2′- or 3′-phosphate. However, this may be a question of permeability, particularly as yeast guanylic acid (a mixture of the 2′- and 3′-phosphates) was utilized as efficiently as guanine or adenosine-5′-phosphate. Further, the finding that the riboside-5′-phosphate supports growth better than the riboside-2′- or 3′-phosphate is by no means a general observation. With mutants of *Bacillus subtilis* Guthrie and Lu (1953) obtained as good growth on adenosine-3′-phosphate as on adenosine-5′-phosphate; growth on adenosine-2′-phosphate was poor. Balis *et al.* (1953) and Elion *et al.* (1953) working with the wild strain of *Lactobacillus casei* and a 2,6-diaminopurine resistant mutant of *L. casei*, respectively, obtained poor growth with adenosine-2′-phosphate and poorer growth with adenosine-5′-phosphate. The best results were obtained with the 3′-isomer.

The inhibition of strain P-14 by adenine and hypoxanthine and the requirement of strain PD-1 for thiamin remain unexplained. Neither of these phenomena is unique. Fries (1949) found that guanineless mutants of *Ophiostoma multiannulatum* were inhibited by adenine or hypoxanthine. Pomper (1952) reported that guanine inhibited the growth of *Saccharomyces cerevisiae* mutants requiring adenine or hypoxanthine. Mutants of *B. subtilis* which grew on 2,6-diaminopurine were inhibited by adenine (Guthrie and Lu, 1953).

The requirement by strain PD-1 of thiamin in addition to purines is analogous to the observations of Lederberg (1952) and Davis (1954, *personal communication*) with purineless mutants of *E. coli* and of Cheng and Pratt (1954, *personal communication*) with a purineless mutant of *Mycobacterium phlei*. These problems will be the subjects of further investigations.

SUMMARY

Two purineless mutants of *Aerobacter aerogenes*, strain 1033, were isolated. Strain P-14 is a guanineless mutant which will grow only on guanine, guanosine, guanylic acid, or 2,6-diaminopurine. Strain PD-1 is nonexacting in its purine requirement and grew on all the purines, ribosides, and ribotides used in this work with the exception of 2,6-diaminopurine, xanthosine, and xanthylic acid. It also grew on 4-amino-5-imidazole carboxamide. Although it will not grow on 2,6-diaminopurine alone, this compound spares guanine or adenine when either of the latter compounds is present in the culture medium. In order to obtain maximum growth of strain PD-1 thiamin was required. Strain P-14 accumulates xanthosine in culture fluids, and its growth on guanine, guanosine, guanylic acid, or 2,6-diaminopurine is inhibited by adenine or hypoxanthine and to a lesser extent by adenosine. It is not inhibited, at the levels tested, by either adenosine-3′-phosphate or adenosine-5′-phosphate. On the basis of these results the genetic blocks in both these mutants are postulated, and the pathway of purine synthesis in *A. aerogenes* is tentatively suggested.

REFERENCES

ABRAMS, R., HAMMERSTEN, E., AND SHEMIN, D. 1948 Glycine as a precursor of purines in yeast. J. Biol. Chem., **173**, 429–430.

BALIS, E. M., AND LARK, C. T. 1954 Synthesis of PNA and DNA in *E. coli*. Federation Proc., **13**, 178–179.

BALIS, E. M., LEVIN, D. H., BROWN, G. B., ELION, G. B., VANDERWERFF, H., AND HITCHINGS, G. H. 1952 The incorporation of exogenous purines into pentose nucleic acid by *Lactobacillus casei*. J. Biol. Chem., **196**, 729–747.

BALIS, E. M., LEVIN, D. H., BROWN, G. B., ELION, G. B., VANDERWERFF, H., AND HITCHINGS, G. H. 1953 The utilization of purine ribotides by *Lactobacillus casei*. J. Biol. Chem., **200**, 1–6.

BARNES, F. W., JR., AND SCHOENHEIMER, R. 1943 On the biological synthesis of purines and pyrimidines. J. Biol. Chem., **151**, 123–139.

BENDICH, A., AND BROWN, G. B. 1948 2,6-Diaminopurine, a precursor of nucleic acid guanine. J. Biol. Chem., **176**, 1471–1472.

BENDICH, A., FURST, S. S., AND BROWN, G. B. 1950 On the rôle of 2,6-diaminopurine in the biosynthesis of nucleic acid guanine. J. Biol. Chem., **185**, 423–433.

BERGMANN, E. D., BEN-ISHAI, R., AND VOLCANI, B. E. 1952 Role of 4-aminoimidazole-5-carboxamide in purine synthesis by *Escherichia coli*. J. Biol. Chem., **194**, 531–537.

BOLTON, E. T., AND REYNARD, A. M. 1954 Utilization of purine and pyrimidine com-

pounds in nucleic acid synthesis by *Escherichia coli*. Biochim. et Biophys. Acta, **13**, 381–385.

BROWN, G. B. 1953 Nucleic acids, purines and pyrimidines. Ann. Rev. Biochem., **22**, 141–178.

BUCHANAN, J. M., AND SCHULMAN, M. P. 1953 Biosynthesis of the purines. III. Reactions of formate and inosinic acid and an effect of the citrovorum factor. J. Biol. Chem., **202**, 241–252.

DAVIS, B. D. 1952 Intermediates in the biosynthesis of proline, ornithine, histidine, lysine, and the aromatic amino acids. Second International Congress of Biochemistry, Paris. Symposium on the metabolism of micro-organisms, Sedes, Paris, 32–40.

ELION, G. B., AND HITCHINGS, G. H. 1950 Antagonists of nucleic acid derivatives. IV. Reversal studies with 2-aminopurine and 2,6-diaminopurine. J. Biol. Chem., **187**, 511–522.

ELION, G. B., VANDERWERFF, H., HITCHINGS, G. H., BALIS, E. M., LEVIN, D. H., AND BROWN, G. B. 1953 Purine metabolism of a diaminopurine-resistant strain of *Lactobacillus casei*. J. Biol. Chem., **200**, 7–16.

FRIEDMAN, S., AND GOTS, J. S. 1951 Deamination of isoguanine by *Escherichia coli*. Arch. Biochem. and Biophys., **32**, 227–228.

FRIEDMAN, S., AND GOTS, J. S. 1953 The purine and pyrimidine metabolism of normal and phage-infected *Escherichia coli*. J. Biol. Chem., **201**, 125–135.

FRIES, N. 1949 Effects of different purine compounds on the growth of guanine-deficient *Ophiostoma*. Physiol. Plantarum, **2**, 78–102.

GOTS, J. S. 1950 The accumulation of 4-amino-5-imidazolecarboxamide by a purine-requiring mutant of *Escherichia coli*. Arch. Biochem. and Biophys., **29**, 222–224.

GOTS, J. S. 1953 Occurrence of 4-amino-5-imidazolecarboxamide as a pentose derivative. Nature, **172**, 256–257.

GOTS, J. S., AND CHU, E. C. 1952 Studies on purine metabolism in bacteria. I. The role of *p*-aminobenzoic acid. J. Bacteriol., **64**, 537–546.

GREENBERG, G. R. 1953*a* Mechanisms involved in the biosynthesis of purines. Federation Proc., **12**, 651–659.

GREENBERG, G. R. 1953*b* Isolation of 4-amino-5-imidazolecarboxamide riboside from the culture medium of sulfonamide-inhibited *Escherichia coli*. J. Am. Chem. Soc., **74**, 6307–6308.

GUTHRIE, R., AND LU, W. C. 1953 Biochemical antagonism in purineless mutants of *Bacillus subtilis*. Bacteriol. Proc., **1953**, 90.

HAMILTON, L., BROWN, G. B., AND STOCK, C. C. 1952 Biosynthesis of nucleic acids studied in unicellular systems. J. Clin. Invest., **31**, 636.

HEINRICH, M. R., AND WILSON, D. W. 1950 The biosynthesis of nucleic acid components studied with C^{14}. I. Purines and pyrimidines in the rat. J. Biol. Chem., **186**, 447–460.

KOCH, A. L., PUTNAM, F. W., AND EVANS, E. A., JR. 1952 The purine metabolism of *Escherichia coli*. J. Biol. Chem., **197**, 105–112.

LEDERBERG, E. M. 1952 Allelic relationships and reverse mutation in *Escherichia coli*. Genetics, **37**, 469–483.

MAGASANIK, B., AND BROOKE, M. S. 1954 The accumulation of xanthosine by a guanineless mutant of *Aerobacter aerogenes*. J. Biol. Chem., **206**, 83–87.

POMPER, S. 1952 Purine-requiring and pyrimidine-requiring mutants of *Saccharomyces cerevisiae*. J. Bacteriol., **63**, 707–713.

SAFFRAN, M., AND SCARANO, E. 1953 Mechanism of the incorporation of adenine into adenosine monophosphate. Nature, **172**, 949–951.

SHUSTER, L., AND KAPLAN, N. O. 1953 A specific *b* nucleotidase. J. Biol. Chem., **201**, 535–546.

SUTTON, W. B., AND WERKMAN, C. H. 1953 The carbon and nitrogen precursors of bacterial purines. Arch. Biochem. and Biophys., **47**, 1–7.

USHIBA, D., AND MAGASANIK, B. 1952 Effects of auxotrophic mutations on the adaptation to inositol degradation in *Aerobacter aerogenes*. Proc. Soc. Exptl. Biol. Med., **80**, 626–632.

WILLIAMS, W. J., AND BUCHANAN, J. M. 1953 Biosynthesis of the purines. IV. The metabolism of 4-amino-5-imidazolecarboxamide in yeast. J. Biol. Chem., **202**, 253–262.