trophs are limited in their growth response to all purines. The pyrimidine requirement, on the other hand, is not affected in mutants derived from pyrimidine requiring auxotrophs.

A close interrelationship between adenine and uracil metabolism is indicated by the fact that both compounds are excreted as a result of resistance to DAP. One possible explanation of this phenomenon would be the possibility of a disturbance in a common regulatory control for both purine and uracil biosynthesis. The reactions involved in formation of a common precursor leading to the synthesis of both adenine and uracil could be altered so that feed-back inhibition is prevented. However, the present knowledge concerning purine and pyrimidine biosynthesis suggests that the regulatory control mechanisms for the two are independent of each other(9,10). Studies to resolve this possibility are described in the succeeding paper (11).

Summary. Resistance to 2,6-diaminopurine in *Salmonella typhimurium* resulted in 2 phenotypically distinguishable mutants. The mutant *dap-r-3* differed from the mutant *dap-r-6* in its cross-resistance to other purine analogues and also in its ability to allow the satellite growth of the parent sensitive strain *LT-2* when plated in presence of inhibitory concentrations of adenine or DAP. The satellite phenomenon could be explained by the fact that adenine and uracil, which were excreted by the mutant *dap-r-3,* prevented the inhibition of the wild type by DAP and adenine respectively. Excretions of these compounds were not directly responsible for the resistance to DAP, since the *dap-r-3* type mutation could also be demonstrated in auxotrophic strains incapable of synthesizing adenine or uracil *de novo.* The mutation to a *dap-r-3* type in a purine requiring auxotroph resulted in an additional defect characterized by poor growth response to all the purines.

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Feedback Inhibition of Purine Biosynthesis in Adenine-Excreting Mutant of *Salmonella typhimurium.** (27178)

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In the preceding article(1) we reported a mutant of *Salmonella typhimurium* which was resistant to 2,6-diaminopurine (DAP) and which excreted adenine into the medium during growth. This phenomenon, whereby resistance to an antimetabolite leads to overproduction of its corresponding competitive metabolite, has been reported for other inhibitory analogues(2-6). Evidence has been accumulated to indicate that the excretion of the metabolites, as well as resistance to the antimetabolites, is the result of faulty feedback control of biosynthesis. Moyed(5) found that a bacterial mutant resistant to 5-

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methyl tryptophane excreted tryptophane and contained an enzyme which was resistant to feedback inhibition by tryptophane or the analogues. A similarly altered enzyme involved in histidine biosynthesis was found in histidine-excreting mutants resistant to the histidine analogue, 2-thiazole alanine(6).

Since purine analogues can apparently mimic the natural purines as feedback inhibitors (7), it was thought that a similar type of faulty feedback control might be operating in the adenine-excreting mutant. This could explain both its overproduction of adenine and its resistance to DAP. To examine this possibility, feedback inhibition by purines was studied by methods previously employed (7-10). These involved the effects of purines on the synthesis of a purine precursor, 5-amino-4-imidazole carboxamide (AICA).

Methods. Methods of isolation and characteristics of DAP-resistant mutants have been described(1). Feedback inhibition of purine biosynthesis was studied with the purine requiring auxotroph *Escherichia coli, strain B 96/1* and *B 96/1 dap*, using accumulation of AICA as an index of purine biosynthesis(8). The mutant, *E. coli B 96/1 dap*, resembled its prototype Salmonella(1). Accumulation of AICA excreted as ribonucleoside was studied with non-proliferating cell suspensions. About 0.5 mg dry weight of cells per ml were incubated in medium E(11) containing 0.2% glucose, 0.1% casein hydrolysate, and varying concentrations of purines. After incubation at 37°C for 3 hours, trichloroacetic acid (TCA) was added to precipitate the cells and AICA in the supernatant fluid was measured as a diazotizable amine by the method of Bratton and Marshall(12). The color was determined with a Klett-Summerson photoelectric colorimeter using green filter.

The AICA accumulation was studied in prototrophic strains of *S. typhimurium* by using sulfadiazine as an inhibitor(9,10). Cultures were grown in medium E and the turbidity of the overnight cultures was adjusted to Klett units of 175. The inoculum was then diluted 1:200 into tubes containing 10 μg/ml of sulfadiazine, 5 μg/ml thiamine, 0.1% casein hydrolysate and varying concentrations of purines. Growth tubes were incubated at 37°C for 18-24 hours until maximum turbidity was reached. Final turbidity in all the tubes was 175 Klett units at the time of analyses. Cells were then precipitated with TCA and the supernatant fluids were assayed for AICA by the Bratton and Marshall test(12), after acetylation with acetic anhydride.

Results. In the initial experiments with sulfadiazine bacteriostasis, it was observed that in the presence of casein hydrolysate and thiamine, AICA accumulation was proportional to the concentration of sulfadiazine present in the medium within the range of 0-20 μg/ml. Growth was similar in all the tubes. Since maximum accumulation was obtained with 10 μg/ml of sulfadiazine, this concentration was used for subsequent studies. Fig. 1 shows the effects of purines on accumulation of AICA in the Salmonella mutant *dap-r-3* and the wild type parent *LT-2*. Whereas adenine was less efficient in inhibiting AICA accumulation in the mutant as compared to the parent strain *LT-2*, the feedback control mechanism *per se* was not affected in the adenine-excreting mutant *dap-r-3*. This was particularly evident when hypoxanthine, another purine known to inhibit AICA accumulation in purine requiring auxotrophs(8), was used as a feedback agent. With hypoxanthine as inhibitor, no significant difference was observed between *LT-2* and *dap-r-3*. The major difference between the 2 strains, seen when adenine was used as a feedback agent, is apparently due to some other mechanism rather than a defective feedback control.

The effects of purines on AICA accumulation were also studied in *E. coli B 96/1/dap*, a DAP-resistant mutant resembling *dap-r-3* in all its properties except its ability to synthesize adenine *de novo*. It grew poorly on all purines. The advantage of using this mutant for study of feedback control is that one can study accumulation of AICA independent of growth, since it has an additional growth requirement for tryptophane(8). Fig. 2 presents data obtained from these studies. Adenine inhibited AICA accumulation both in *B 96/1* and *B 96/1/dap*. However, twice as

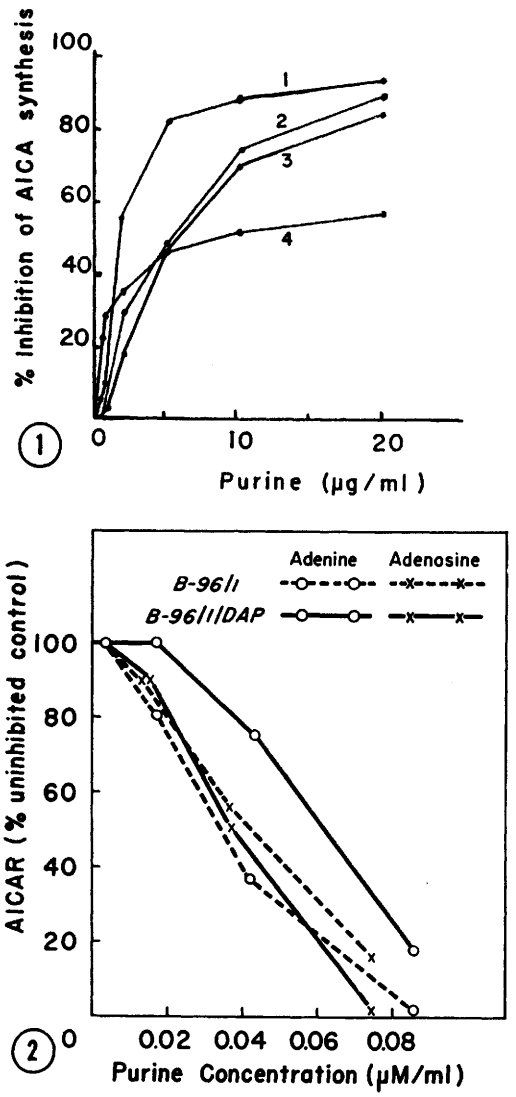

FIG. 1. Effect of purines on synthesis of AICA in sulfadiazine-inhibited cultures of *LT-2* and *dap-r-3* of *S. typhimurium*. (1) *LT-2* with adenine, (2) *dap-r-3* with hypoxanthine, (3) *LT-2* with hypoxanthine, and (4) *dap-r-3* with adenine.

FIG. 2. Effect of purines on synthesis of AICA in nonproliferating cells of *E. coli B 96/1* and *B 96/1/dap*.

much adenine was required for 50% inhibition of AICA synthesis in the mutant *B 96/1/dap*. When adenosine was used as a feedback agent, no significant difference was obtained between the mutant and its parent strain. The results, therefore, strongly suggest that differences between *B 96/1* and *B 96/1/dap* with respect to adenine could be due to different rates of utilization of adenine rather than any defect in feedback control of its biosynthesis.

Discussion. Recent studies on mutants which excrete metabolites have led to the general belief that in all these instances the overproduction is due to some defect in mechanisms involved in control of biosynthesis of these metabolites(3-6). The results presented here suggest that in explaining the excretion of adenine in the mutant *dap-r-3*, one must consider mechanisms other than a mere defect in the control of purine biosynthesis. The much lower inhibition of AICA synthesis by adenine in the mutant *dap-r-3*, compared with the wild type, could be due to either incorporation or conversion of adenine to some active feedback agent as a limiting factor in the mutant. This is substantiated by our recent observations that the mutant *dap-r-3* incorporates adenine at a much lower rate than *LT-2* and that it is also deficient in adenylic pyrophosphorylase activity(13). On the other hand incorporation of hypoxanthine and other purines is practically the same in both the mutant and wild type strains. Thus, inability of the resistant mutant to form adenylic acid as efficiently as the wild type could account for the poor inhibition of AICA when adenine is used as a feedback agent. This defect was not observed when hypoxanthine was used as a feedback agent, since the mutant *dap-r-3* easily synthesized inosinic acid from hypoxanthine(13). In the mutant *B 96/1/dap*, adenylic acid is apparently synthesized from adenosine *via* a reaction other than adenylic pyrophosphorylase.

Alternative explanations must be sought to account for the excretion of adenine in the *dap-r-3* mutant. It is interesting to note that free adenine rather than its ribosylated derivative is excreted by the mutant. If the mutant enzymes can degrade adenylic acid to adenine as rapidly as it is made *de novo*, this could result in interference with the feedback control of purine biosynthesis provided adenylic acid and not free adenine is the active form of feedback inhibitor. The salvage mechanism for reutilizing adenine for growth *via* adenylic pyrophosphorylase, which is normally operating in the wild type, is absent in

the mutant strain. Whatever the mechanism is for the excretion of adenine by the mutant strain, it is not *per se* responsible for the resistance to DAP(1).

Summary. The mechanism of excretion of adenine by the mutant *dap-r-3* of *Salmonella typhimurium* selected for resistance to DAP was studied. The mechanism for control of purine biosynthesis by feedback inhibition is unaffected in this mutant as indicated by (1) inhibition of AICA synthesis in sulfadiazine-inhibited prototrophic strains *dap-r-3* and *LT-2* by hypoxanthine and adenine; and (2) inhibition of AICA synthesis by adenine and its nucleoside in nonproliferating suspensions of *Escherichia coli B 96/1/dap,* a DAP-resistant mutant derived from a purine requiring auxotroph *B 96/1* resembling *dap-r-3* mutant in its properties. In general, more adenine was required for feedback inhibition in the resistant mutant than in the parent strain. The differences have been ascribed to poor utilization of adenine by the resistant strain.

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Thyroid Activity and Heart Rate.* (27179)

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The increase in heart rate occurring in artificially induced hypothyroidism apparently results from local changes in the heart in addition to the indirect effects of increased metabolism in other tissues. This is suggested by the increased oxygen consumption found in myocardial tissue(1,2). Furthermore, elevated sinus rate after administration of thyroid or thyrotropic hormone, thyroxine, triidothyronine and some of its derivatives persists when the heart muscle is beating spontaneously in the isolated state(3-8). In the hypothyroid state, with decreased metabolic rate, heart rate may be decreased but this is not necessarily the case(9). The present study was performed to compare heart rates in intact animals and beat rate of isolated heart muscle preparations of rats treated with l-thyroxine or l-triiodothyronine or thyroidectomized with radioactive iodine; a preliminary report was previously published in abstract form(10).

Methods. Wistar strain rats of both sexes weighing 150 to 340 g served as experimental animals. Test rats and corresponding control animals were of the same age and, at the beginning of experiment, of the same size. Heart rate determinations in intact rats were performed electrocardiographically in a wooden box divided by partitions into 10 sections, each for one animal. Each rat stood on 2 metal plates, one under the front legs, the other under the hind legs. The animals were allowed to become quiet for a half hour before any recordings were performed. Re-

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