More significant, however, was the variation in response exhibited by different strains to altered pH and/or the presence of glucose. On the basis of these responses, the 7 strains studied may be divided into 3 groups. Group 1. composed of *E. coli,* strains Tennessee and Texas, and *P. vulgaris,* exhibited no pH effect, while a definite glucose effect was apparent. Group 2, composed of *Bact. cadaveris* and *A. aerogenes,* was affected by both the increased hydrogen ion concentration and by the presence of glucose, but the carbohydrate effect was far greater than that of the altered pH. Group 3, composed of the Gratia and Crookes strains of *E. coli,* exhibited a reduced aspartic acid deaminase activity when grown at acid pH, but no reduction in enzyme activity when grown in the presence of glucose. Growth in the glucose medium was associated with an increase in hydrogen ion concentration.

Discussion. It is manifest from the results presented that the presence of glucose or other fermentable carbohydrates in the growth medium exerts an adverse effect on the aspartic acid deaminase activity of the harvested cells of many, but not all, bacterial species studied. In those strains which are adversely influenced by the presence of glucose the effect is much greater than can be accounted from the resultant increase in hydrogen ion concentration. As noted earlier, the results with citrate speak strongly for an action not at all related to the concurrent pH change. This is also supported by the observation that the 2 strains not affected by the presence of glucose in the growth medium exhibited a lowered deaminase activity when cultured at a pH close to that which resulted from the utilization of the carbohydrate.

It is pertinent here to comment that in sharp contrast to the carbohydrate effects noted in this study and others where the sugar had been incorporated into the growth medium, the addition of such substances to resting cell suspensions resulted in a pronounced stimulation of the rate of deamination of aspartic acid(4).

Summary. 1. The reduction in aspartic acid deaminase activity in cells harvested from a medium containing glucose has been shown not to be specific to this sugar, but rather is shared by other fermentable carbohydrates. 2. The effect of the carbohydrate varies among the several strains studied, and is not associated with the increase in hydrogen ion concentration due to the production of fermentation acids.

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Uric Acid Production in Normal and Gouty Subjects, Determined by N^{15} Labeled Glycine.* (20019)

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By measuring the isotopic composition of

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urinary uric acid in 2 normal men and one gouty subject after the administration of isotopically labelled (N^{15}) glycine. Stetten and his co-workers(1) were able to demonstrate conclusively that overproduction of uric acid was largely responsible for the hyperuricemia in the gouty subject. They emphasized that because of the expense and labor involved, the study was restricted to a single patient with gout, who was selected on the basis of a large urinary uric acid output and minor tophaceous involvement. Whether the disclosed metabolic defect is seen only in this relatively rare type of case or is manifest in other types and stages of gout could not be answered.

For this reason it was considered desirable to study the rate of uric acid formation in the more commonly encountered type of disease. Therefore, a gouty subject with a normal uric acid excretion was selected for this study along with a normal individual for the sake of comparison. The experimental procedure of Stetten *et al.* was followed. Both subjects were fed N^{15} labeled glycine in a single dose and were maintained on the same constant diet throughout the period of study. Daily urine samples were analyzed for total nitrogen and uric acid and for isotope abundance in both of these fractions.

Procedures. Subjects. H.S., a white male, 44 years of age, weighing 64.1 kg, had experienced recurrent attacks of gouty arthritis for approximately 4 years. He was unaware of a family history of gout. He had had rheumatic fever as a child and had suffered from intermittent claudication for 2 years. He had mitral stenosis and border-line congestive failure at the time this study was made. A small tophus in the right ear showed the typical urate crystals and gave a positive Murexide test. Nonprotein nitrogen and phenolsulfonphthalein excretion tests were both normal. Serum uric acid varied from 9.0 to 9.3 mg %. Daily urinary uric acid excretion was 474 $\pm$ 14 mg. A.F.M., a white male, 30 years of age, weighing 82.0 kg, was apparently free of all disease and gave no family history of gout. His serum uric acid was 3.7 mg %. His daily excretion of uric acid on the same constant diet given patient H.S. was 663 $\pm$ 45 mg.

Materials and methods. N^{15} labeled glycine was synthesized from potassium phthalimide which contained approximately 55 atom % N^{15}. The Schoenheimer and Ratner(2) method was used with the following modification: The ethyl phthalimido-acetate was split as indicated, the phthalic acid filtered off, and the filtrate brought to dryness *in vacuo*. The residue was extracted with a small amount of cold water. This solution, which contained the glycine-hydrochloride, was passed over a Duolite A 4 Anion exchanger column (50 to 60 g) instead of treating it with silver carbonate and then with hydrogen sulfide. Before the exchanger column was used it was treated with approximately 2-3 N NaOH and then washed until a neutral pH was obtained. The glycine which was not adsorbed by the column was washed out with small amounts of distilled water (total volume 600 to 800 ml). The water-clear eluate was brought to a very small volume *in vacuo*, and the glycine precipitated with alcohol. The yield was 97%. The elementary analysis for nitrogen checked within the experimental error. The isotope abundance was 55.15 atom % excess. Both subjects were maintained on the same constant, 2000-calorie diet, poor in purine (140 mg) and relatively low in protein (60 g). After 4 days on this diet both subjects were given tagged glycine well mixed with breakfast (100 mg per kg of body weight). The 24-hour urine samples were collected over a few milliliters of concentrated acetic acid and kept at 4° C. All urine specimens were worked up immediately. The total nitrogen was determined on each 24-hour sample by the micro-Kjeldahl method. The uric acid was determined by differential enzymatic spectrophotometry, as outlined by Kalckar(3) and adapted for clinical use by Praetorius(4). The highly purified uricase was prepared by a relatively simple method(5) which followed the general principles outlined by Holmberg (6). Uric acid was isolated from fresh urine according to Talbott *et al.*(7) with minor modifications. The nitrogen content and the ultraviolet analysis for uric acid checked within the experimental error. Isotope abundance of total nitrogen and uric acid was determined according to Sprinson and Ritten-

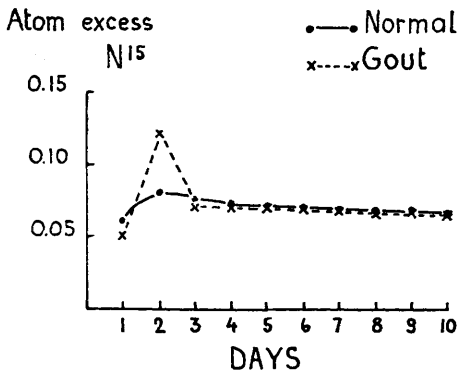

FIG. 1. Abundance of N^{15} in excreted uric acid after ingestion of tagged glycine.

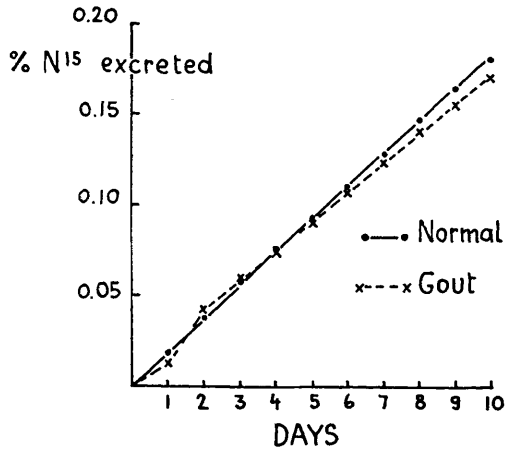

FIG. 2. Cumulative excretion of N^{15} as uric acid expressed as percentage of administered dose.

berg(8).

Results. Total urinary excretion of N^{15} of both subjects was similar to that found by Stetten *et al.* By the 10th day 46.4% of the total dose administered had been excreted by the normal subject and 55.3% by the gouty man.

There was no significant difference in the *urinary uric acid isotope concentrations* of the gouty and the normal subject (Fig. 1). There was, however, a sharp rise in the isotope concentration of the gouty subject between the first and second days and an equally rapid fall on the second and third days. The cumulative excretion of N^{15} in uric acid, expressed as percentage of the total dose administered, was the same in the normal and in the gouty subject (Fig. 2). A comparison of the actual figures revealed excellent agreement between the values on our 2 subjects, gouty and normal, and the 2 normal males reported by Stetten *et al.*

Discussion. This study was designed specifically to determine the production of uric acid from a known purine-precursor, glycine, in a gouty subject with a normal uric acid excretion. For this reason the subject selected was an individual with proven gout characterized by recurrent attacks of gouty arthritis, a hyperuricemia, and minor tophaceous involvement. The control subject was a normal man. The results are quite in contrast to those reported by Stetten *et al.*, in that they show that the actual amount of the precursor incorporated in uric acid is no different in the gouty subject and the normal individual. These experiments, however, do not tell us whether in spite of the same over-all production, the 2 individuals handle glycine the same way qualitatively, *i.e.*, whether they use the same intermediary pathways in purine synthesis. The exact significance of the sharp rise and a subsequent rapid fall in the uric acid isotope concentration in the gouty patient remains unexplained (Fig. 1). The results, however, do show that an increased production of uric acid, at least as far as the incorporation of the nitrogen of dietary glycine in the purine ring is concerned, is not always an essential finding in gout.¶ From these data it cannot be decided whether this implies that patient H.S., in comparison with the case reported by Stetten *et al.*, is simply in a different stage of the disease or displays a different disease mechanism. Additional studies will be required in order to establish whether overproduction of uric acid is an inconstant and nonessential mechanism for the hyperuricemia of gout.

Summary. 1. Glycine was synthesized by the Schoenheimer and Ratner method with a modification (ion exchange resins) which in our hands gave better yields. 2. N^{15} tagged glycine was fed to a normal and to a gouty subject while they were on the same constant, low protein, low purine, 2000-calorie diet. Daily urine samples were analyzed for total

¶ Similar observations have also been made by Stetten *et al.*

nitrogen and uric acid, as well as for isotope concentration in these 2 fractions. 3. No significant difference could be found between the 2 subjects in the isotope distribution of the total nitrogen or uric acid. The percentage of N^{15} excreted as uric acid was no greater in the gouty than in the normal subject. The significance of this finding in relation to the pathogenesis of gout is discussed.

We wish to thank Dr. Gordon L. Brownell in whose laboratory the isotope analyses were carried out.

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Inhibition of Thyroid Function by Cortisone and ACTH in Hypophysectomized Rats.* (20020)

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There is experimental and clinical evidence that administration of cortisone or ACTH suppresses thyroid function(1-5). Similar findings have been obtained under experimental conditions which are known to be accompanied by increased secretion of cortical hormone, including injection of epinephrine(6) or formalin(7) and exposure to abnormal temperature(8). Some investigators believe that this inhibition of thyroid function is caused by suppression of secretion of TSH(1,2); however, differences between the inhibition produced by adrenal hormone and by lack of TSH have been pointed out(9,10).

The experiments reported in this paper were undertaken in order to investigate the possibility that the inhibiting action of cortical hormone is exerted at the thyroid rather than at the pituitary level. A similar investigation was reported by Woodbury *et al.*(11).

Material and methods. Hypophysectomized male rats of the Sprague-Dawley strain were divided into 4 groups. Group one served as control. Group 2 was injected for 5 days with either ACTH¶ or cortisone.¶ Group 3 received daily injections of a TSH preparation¶ for 5 days. Group 4 was treated with both TSH and either ACTH or cortisone. The hormone dosage is indicated in the tables. After 4 days of treatment all animals received 4 μc of I^{131} (carrier free)¶ by intraperitoneal injection. Twenty-four hours later the rats were killed; the thyroid glands were dissected free of connective tissue and weighed on a Roller Smith torsion balance. The glands were then macerated in 5% NaOH; aliquots were plancheted and the radioactivity determined by means of a Geiger Counter.

Results. Results of experiments with ACTH are presented in Table I, those with cortisone in Table II. It is evident that the amount

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¶ The TSH preparations were generously supplied by Dr. Daniel McGinty of the Parke Davis Co., ACTH was obtained from Armour and Co. through the courtesy of Dr. E. E. Hays, and Cortisone from Merck and Co. through the courtesy of Dr. E. Alpert. The I^{131} was obtained from the Atomic Energy Commission, Oak Ridge Laboratories.