TABLE V. Effects of Age of Rat on Excretion and Bone Accumulation of Orally Administered Ca^{45}.

Age, mo	No. of rats	Avg body wt (g)	Fecal excretion of Ca^{45}, % of dose	Femur shaft				Ca45	
				Ca^{45}*	mg Ca/g	Specific† activity	% ash	Fraction	Ca^{45} absorbed
1	9	36	23	.57	90	6.3	25	.74	
3	12	171	33	.45	168	2.7	49	.67	
6½	4	312	56	.30	195	1.5	50	.68	
16	6	310	51	.18	210	.86	56	.37	

* μg of labeled Ca per g fresh tissue based on dosage of 10 μg per 100 g body wt.
† μg of labeled Ca × 10^3 per g of total Ca based on above dosage.

month-old animals; (5) for meaningful studies of this type, interpretations must include consideration of exchange reactions, and care must be given to initial selection of diets and animals, preparation of animals for the experiment, and dietary management during the experimental period.

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Inhibition of Urinary 17-Ketosteroid Excretion Produced by "Benemid".* (19103)

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(Introduced by Walter Fleischmann.)

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Benzoic acid(1,2) and carinamide (4'-carboxyphenylmethanesulfonanilide)(3-6) are known to block the renal tubular secretion of penicillin and thus to enhance plasma penicillin concentrations. A new compound, Benemid,† p-(di-n-propylsulfamyl)-benzoic acid has also been found to increase the plasma concentrations of penicillin and para-aminosalicylic acid (PAS)(7-11). Only 2 g of Benemid per day are necessary to produce consistent elevation of plasma penicillin and PAS levels(7-11), whereas 6 to 24 g per day of carinamide were necessary to produce similar results(3-6).

Bissell and colleagues(12) and Ceresa and

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† The authors are indebted to Sharp and Dohme, Inc., Glenolden, Pa. for the Benemid used in this study.

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Rubino(13) observed that carinamide greatly inhibited the renal excretion of 17-ketosteroids. The latter authors found that the administration of less than 6 g per day to an adult failed to diminish 17-ketosteroid excretion. Because of the similarities in the physiological action of carinamide and Benemid, an investigation has been made of the effect of the latter drug on urinary excretion of 17-ketosteroids, 11-oxycorticosteroids (neutral reducing lipids) and chemically measured estrogens (fluorogenic phenols) in normal man.

Methods. Two healthy, adult males, L.G. (Subject No. 1), age 33 years, and C.M. (Subject No. 2), age 26 years, served as subjects. Diet was unrestricted. Serial 24-hour urine collections were made during control periods and when Benemid was given. Each specimen was preserved with 10 ml of 25% acetic acid. 17-ketosteroids were measured by the meta-dinitrobenzene reaction in ethanolic KOH(14). This was done on urine extracts prepared by acid hydrolysis and subsequently extracted with carbon tetrachloride and washed with 2.5 N NaOH. Spectrophotometric curves to measure dehydroisoandrosterone were made on aliquots of these same extracts by the method of Allen and colleagues(15). A Beckman spectrophotometer, model DU, was used, with cells of 10 mm light path. Neutral reducing lipids were measured by the reaction of phosphomolybdic acid with chloroform extracts which had been washed 3 times with 0.1 N NaOH and partitioned between benzene and water(16). It is believed that the 0.1 N NaOH wash removed from the urine any Benemid which might have interfered with the steroid determinations. Urinary estrogens were measured chemically by a slight modification of Jailer's method(17).

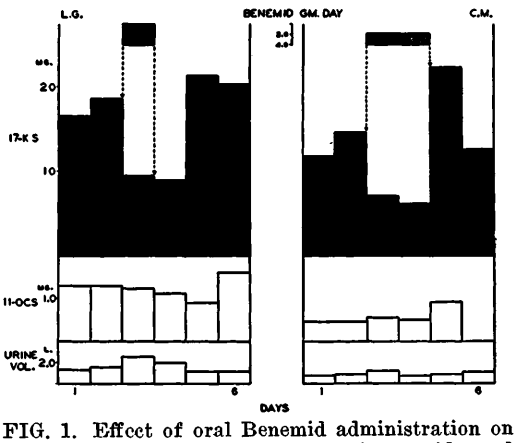

FIG. 1. Effect of oral Benemid administration on urinary 17-ketosteroids, 11-oxycorticosteroids and urine volume in two adults.

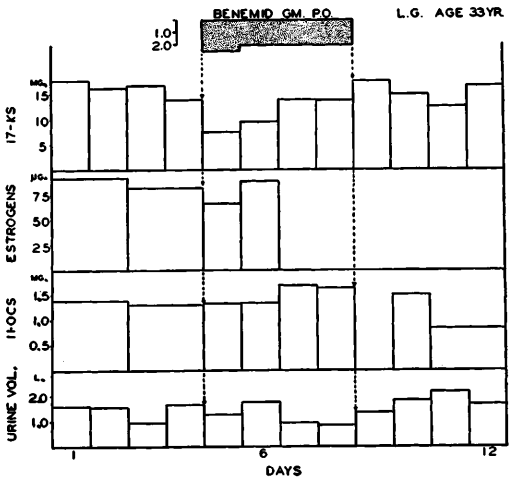

FIG. 2. Effect of oral Benemid administration on urinary 17-ketosteroids, estrogens, 11-oxycorticosteroids and urine volume in an adult.

Results. Fig. 1 shows the data obtained from a 6-day experiment on each of the 2 subjects. Administration of 4 g Benemid to subject No. 1 over a single 24-hour period (1 g each 6 hr) produced approximately 50% reduction in urinary 17-ketosteroid excretion. This reduction persisted for 48 hours. Subject No. 2 was given 2 g Benemid per day for 2 days (0.5 g each 6 hr). The relative diminution of urinary 17-ketosteroids was about as great as in Subject No. 1, but immediately upon withdrawal of the drug there was a return to normal. Benemid had no observable effect on the excretion of neutral reducing lipids in either subject.

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TABLE I. Effect of Oral Benemid on Urinary Excretion of Estrogens in Subject C. M.

Day of exp.	Benemid, g/day	Urine vol, ml/day	Urinary estrogens, μ/day
1	0	740	20.7
2	0	820	17.4
3	2	1050	15
4	2	750	12
5	0	810	27.2
6	0	1050	24

Fig. 2 presents information concerning a 12-day experiment on Subject No. 1. On the first day 2.5 g Benemid were given, followed by 2.0 g per day for 3 more days. There was an appreciable drop in urinary 17-ketosteroids on the first day of treatment. These values gradually rose during the remaining 4 days of treatment. Neutral reducing lipids showed no change on this regimen.

Urinary estrogen excretion has been adequately studied in only one subject receiving Benemid. Table I shows the diminution in urinary estrogen when Subject No. 2 was given 2 g of Benemid per day. Fig. 2 shows a few urinary estrogen values on Subject No. 1, but the data are insufficient for any conclusion.

Because of the possibility that the total 17-ketosteroid inhibition observed might involve retention of certain 17-ketosteroids and not others, a qualitative study by the method of Allen and colleagues was carried out on the neutral 17-ketosteroid urine residue(15). This spectrophotometric technique sharply distinguishes between androsterone and dehydroisoandrosterone.‡ Fig. 3 shows the spectrophotometric absorption curves on urine extracts of Subject No. 1 studied with this reaction. The peak at 390 mμ may be produced by androsterone or related 3-alpha-hydroxyketones, whereas the peak at 600 mμ may be produced by dehydroisoandrosterone, one of the 3-beta-hydroxyketones. It is seen that there is no significant difference in configuration between the control curve and the Benemid curve.

‡ The authors are indebted to the Schering Corp. for crystalline androsterone and dehydroisoandrosterone acetate used as standards in this determination.

Comment. It is of considerable interest to observe the very selective manner in which certain systems are inhibited by the renal blocking agents carinamide and Benemid. In previous studies it has been observed that Benemid increased plasma concentrations of para-aminosalicylic acid but had no effect on plasma concentration of the structurally similar compound, acetylsalicylic acid(8). The current observations concerning inhibition of urinary 17-ketosteroid excretion as contrasted with lack of effect on urinary neutral reducing lipids would support this principle.

In view of the above it would not be surprising to discover that the numerous 17-ketosteroid compounds measured by the Zimmerman reaction might be differentially influenced by Benemid administration. The spectrophotometric evidence here presented serves only to compare the excretion of androsterone and/or related steroids with the excretion of dehydroisoandrosterone and/or related steroids. There is no indication that this ratio has been altered by Benemid administration.

Summary and conclusions. (1) The data presented demonstrate that Benemid in doses of 2 g per day per adult produced approxi-

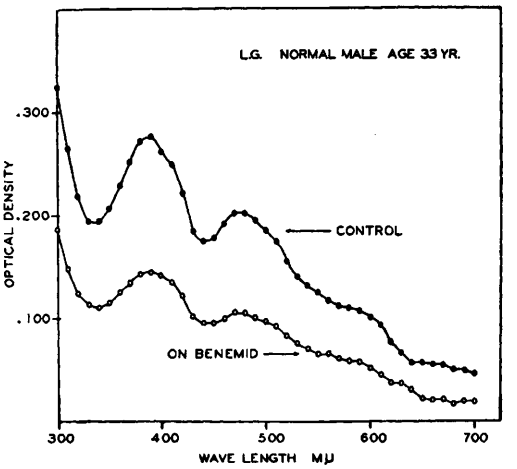

FIG. 3. Spectrophotometric absorption curves of color reaction for dehydroisoandrosterone done on urine extracts of subject L. G. Aliquots of .098 mg and .059 mg 17-ketosteroid were used for the upper and lower curves respectively in a final volume of 7 ml.

mately 50% diminution in urinary 17-ketosteroids. This diminution was not exaggerated by increasing the dose of Benemid to 4 g per day. (2) Spectrophotometric studies showed that Benemid did not change the androsterone-dehydroisoandrosterone ratio of urinary 17-ketosteroids. (3) Urinary estrogens were diminished in one subject. In another subject this diminution was questionable. (4) Urinary 11-oxycorticosteroids (neutral reducing lipids) did not appear to be affected by Benemid.

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Microbiological Assay Method for Thiamine Using *Lactobacillus fermenti* 36 as Test Organism.* (19104)

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Lactobacillus fermenti 36 has been proposed by Sarett and Cheldelin, and Cheldelin *et al.* for thiamine assay of foodstuffs(1,2). In studies with this method, it was observed that a striking stimulation in growth resulted when filbert nut meal extract was added to the assay medium containing either a suboptimum or an optimum amount of thiamine. Growth stimulants have been shown to exist in certain foodstuffs for the growth of *L. casei* in the microbiological assays for riboflavin and pantothenic acid when a suboptimum amount of vitamin is present in the assay medium by Bauernfeind *et al.*(3). Chu and Williams(4) and Pollack and Lindner(5) also reported the existence of stimulatory factors for *L. casei*. Wright and Skeggs(6) and Colio and Babb(7) have demonstrated the existence of a stimulatory growth factor in trypsinized vitamin free casein and malt sprouts extract, respectively, for *Streptococcus lactis R.* In this laboratory a series of experiments showed that there are a number of substances which will stimulate the growth of *L. fermenti* 36 in the presence of any level of thiamine.

The experiments reported here demonstrate the stimulatory effects of maltose and taka-diastase digested starch on the growth of *L. fermenti* 36 in thiamine assay medium. It also demonstrates that one may obtain an erroneous thiamine value by this method if the substances used are high in starch.

Experimental. In the microbiological assay of thiamine reported herein the procedure of Sarett and Cheldelin(1,2) was employed. The test organism used in this assay was *L. fermenti* 36. The extent of growth was determined quantitatively by measuring the turbidity after 16 hours of incubation at 37°C with a Klett-Summerson photo colorimeter, using a 54 filter. The readings are expressed in Klett units. In order to get a low blank value in this assay, a boiled alkali treated peptone was used in the medium. The

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