ing practically all of the material must pass first through the liver before it is put into the general circulation; hence the oxidation values of 48-63% are obtained. It is of interest to note that Dominguez, Corcoran and Page(4) in evaluating the use of mannitol in renal function concluded from their studies that the utilization of mannitol in humans, although small after IV injection, is presumably metabolic.

Although metabolites are routinely given parenterally in many experimental studies in order to by-pass the intestine, it is obvious from the results reported here that data obtained from parenteral injection should be examined with comparable data obtained after oral administration for a true evaluation of the metabolic pathway.

Summary. Radioactive mannitol has been administered to rats with the following results: 1. When given orally, about 50% of the mannitol carbon is recovered in the expired CO_2 in 12 hours. 2. With intraperitoneal injection, the mannitol is largely recovered in urine with only 2-3% of the mannitol carbon recovered in the expired CO_2. 3. By injection directly into the portal system by way of the spleen, oxidation comparable to or greater than that obtained by oral administration is obtained. It is concluded from these results that mannitol can be oxidized by the body if it is administered by a route that makes it first available to the liver, as in oral feedings.

1. Olmsted, William H., *Diabetes,* 1953, v2, 132.
2. Todd, W. R., Meyers, Jane, West, E. S., *J. Biol. Chem.,* 1939, v127, 275.
3. Smith, W. W., Finkelstein, Norma, Smith, H. W., *ibid.,* 1940, v135, 231.
4. Dominguez, R., Corcoran, A. C., and Page, I. H., *J. Lab. and Clin. Med.,* 1947, v32, 1192.
5. Elkinton, J. R., *J. Clin. Invest.,* 1947, v26, 1088.
6. Clark, J. K., and Barker, H. C., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 152.
7. Carr, C. J., and Krantz, J. C., *J. Biol. Chem.,* 1938, v124, 221.
8. Wick, Arne N., Almen, Mary C., and Joseph, Lionel, *J. Am. Pharm. Assn.,* 1951, v40, 542.

Received January 4, 1954. P.S.E.B.M., 1954, v85.

Urinary Oxalate Excretion by Man Following Ascorbic Acid Ingestion.* (20827)

MERTON P. LAMDEN AND GLORIA A. CHRYSTOWSKI. (Introduced by H. B. Pierce.)
From the Department of Biochemistry, College of Medicine, University of Vermont, Burlington.

Oxalic acid has long been known to be a product of the *in vitro* oxidation of ascorbic acid(1-5). *In vivo,* Scheinkman(6) showed that ascorbic acid ingestion by guinea pigs resulted in increased urinary oxalate. Burns *et al.*(7) found that one of the end products of the metabolism of radioactive 1-C^{14}-L-ascorbic acid administered to guinea pigs was urinary oxalate. Since large doses of ascorbic acid are being prescribed by physicians(8,9), an investigation of the effect of ascorbic acid on the urinary oxalate excretion of man is warranted. This study is concerned with that investigation.

Procedure. In the first experiment, 11 healthy adult males on their regular diet collected 24 hour control urine samples. Each subject then ingested, in addition to his regular diet, 3 g of pure ascorbic acid at each mealtime (9 g/day) for 2 days, collected a 24 hour urine sample on the second day and another 24 hour control urine sample one week later. The results indicated that there was increased oxalate excretion with the daily ingestion of 9 g of extra-dietary ascorbic acid. A second experiment was conducted to determine at what minimum dosage of supplementary ingested ascorbic acid an increase in urinary oxalate occurred. Forty adult males were placed into groups ingesting different amounts of ascorbic acid ranging from 0.25 g to 8 g (divided into 3 doses as in the first experiment) daily for one week at the end of

* Supported in part by a grant from Hoffmann-LaRoche Inc., Nutley, N. J.

which each member collected a 24 hour urine sample. All subjects collected 24 hour control urine samples prior to receiving extra-dietary ascorbic acid and at the end of the first and second weeks following cessation of the ingestion of extra-dietary ascorbic acid. The groups taking 0.25, 0.50, and 2 g of ascorbic acid daily each consisted of 7 subjects. The groups ingesting 4 and 8 g of ascorbic acid each consisted of 6 subjects. The method for determining urinary oxalate was essentially that of Powers and Levatin(10). Care was taken to prevent the *in vitro* conversion of urinary ascorbic acid to oxalic acid. Urine was voided into a large bottle containing 225 ml of stabilizing solution (20% metaphosphoric acid and 3.3% thiourea). In order to test the procedure, 9 g of ascorbic acid were added to a composite urine sample containing the stabilizing solution. This preparation was then allowed to stand 24 hours at room temperature during which time the oxalate content increased only 1.2 mg (as oxalic acid) which amount was within the experimental error of the method. All urine samples were analyzed individually. The values for the oxalate content of the control urines for each subject were averaged. This average was subtracted from the value for the urinary oxalate excretion of the subject following ascorbic acid ingestion to determine the change, whether positive, negative, or zero.

Results. The mean oxalic acid excretion of 51 males without extra-dietary ascorbic acid was 38.3 $\pm$ 1.7 mg (standard error). This value was based on 142 determinations. The range of urinary oxalate output among the controls was 16 to 64 mg oxalic acid. Values reported in the literature for the normal 24 hour urinary oxalate excretion of man range from 14.0 to 56.0 mg with averages of 30 to 35 mg oxalic acid(2,11).

The remarkable constancy of the oxalic acid excretion for an individual on his regular diet without supplementary ascorbic acid is indicated by the average of the maximum variation among controls for each individual which was only 5.2 mg oxalic acid. The greatest variation among controls for an individual was 19 mg.

The effect of ingested ascorbic acid on urinary oxalate excretion is shown in Fig. 1

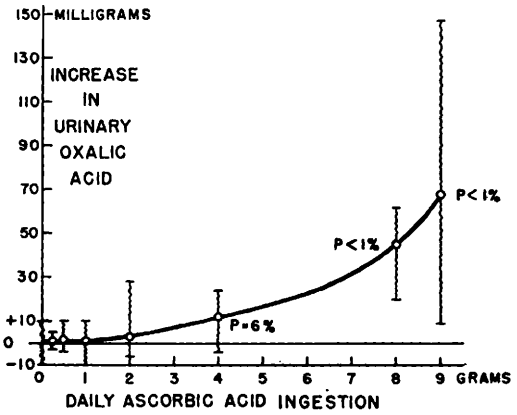

FIG. 1. Effect of amount of ascorbic acid ingested daily on increase in urinary oxalate (as oxalic acid). Circles on curve represent avg increase in excretion of urinary oxalic acid for each group. "Increase" in urinary oxalic acid is the difference between 24 hr urinary oxalic acid excretion of an individual following ingestion of ascorbic acid and avg of the values of his control urines collected when he received no extra-dietary ascorbic acid. The 0.25, 0.50, 1 and 2 g groups consisted of 7 subjects; 4 and 8 g groups, 6 subjects, and 9 g group, 11 subjects. Vertical wavy lines indicate maximum and minimum individual increases (sometimes negative) found within each group except in the case of controls (0 g extra-dietary ascorbic acid ingested) where they indicate the greatest variation between controls of a single individual in the whole study. P = probability level.

in which the increase in 24 hour urinary oxalic acid in mg is plotted against ascorbic acid ingested daily in grams. The ingestion of 4 g of ascorbic acid results in an average increase of 12 mg of excreted oxalic acid significant at the 6% level of probability; 8 g, an average increase of 45 mg of oxalic acid, and 9 g, an average increase of 68 mg of oxalic acid, these last two increases being statistically highly significant. The ingestion of less than 4 g of ascorbic acid daily resulted in a negligible increase in urinary oxalate.

Further investigation is being conducted on laboratory animals to determine whether urinary oxalate calculi and kidney changes may be a consequence of massive ascorbic acid ingestion over a long period of time.

Summary. An investigation was conducted to determine the urinary oxalate excretion of normal males on their regular diet, and to determine to what extent extra-dietary ascorbic acid would increase this excretion. The mean 24 hour urinary oxalic acid excretion of

51 healthy adult males on their regular diets was 38.3 ± 1.7 mg. The 24 hour excretion of any individual was remarkably constant over a three week period. The daily ingestion of 4 or more g of ascorbic acid increased urinary excretion of oxalic acid; whereas the daily ingestion of less than 4 g of ascorbic acid produced no significant increase in oxalate excretion.

1. Herbert, R. W., Hirst, E. L., Percival, E. G. V., Reynolds, R. J. W., and Smith, F., *J. Chem. Soc.*, 1933, 1270.

2. Flaschenträger, B., and Müller, P. B., *Z. Physiol. Chem.*, 1938, v251, 52.

3. Ghosh, J. C., and Rakshit, P. C., *Biochem. Z.*, 1938, v299, 394.

4. Jurist, A. E., and Christiansen, W. J., *Am. J. Pharm.*, 1939, v111, 347.

5. Rosenfeld, B., *J. Biol. Chem.*, 1943, v150, 281.

6. Scheinkman, E. A., *Biochem. J.*, Ukraine, 1940, v16, 397.

7. Burns, J. J., Burch, H. B., and King, C. G., *J. Biol. Chem.*, 1951, v191, 501.

8. Lowry, O. H., Bessey, O. A., and Burch, H. B., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 361.

9. Massell, B. F., Warren, J. E., Patterson, P. R., and Lehmus, H. J., *New Eng. J. Med.*, 1950, v242, 614.

10. Powers, H. H., and Levatin, P., *J. Biol. Chem.*, 1944, v154, 207.

11. Jeghers, H., and Murphy, R., *New Eng. J. Med.*, 1945, v233, 208, 238.

Received October 7, 1953. P.S.E.B.M., 1954, v85.

An Antipyretic Action of Aureomycin. Inhibition of Febrile Response to Influenza Virus in Rabbits.* (20828)

I. GRAY TILLOTSON AND HAROLD S. GINSBERG.

From the Departments of Preventive Medicine and Medicine, School of Medicine, Western Reserve University, and the University Hospitals, Cleveland, O.

Aureomycin has been reported to influence favorably the course of certain viral infections in man(1-3). Except for the psittacosis-lymphogranuloma group(4), however, there is no evidence that this drug either limits viral multiplication(5) or prevents production of lesions by these agents(6). It seemed possible, therefore, that the effect of aureomycin in these instances might be non-specific; that is, its action might be mediated through a mechanism other than one which influences the pathogenesis of the disease. For example, in those circumstances in which the major objective evidence for the effectiveness of aureomycin is provided by an early return of temperature to normal, the question of an antipyretic action may be raised. As an approach to this problem, an investigation was carried out to determine whether aureomycin could inhibit the pyrogenic response to a viral agent in a situation where infection was not concerned. The experimental model chosen for study was the febrile reaction produced in rabbits by intravenous inoculation of influenza virus(7).

Materials and methods. Virus. The virus employed was influenza B, Lee strain, which was propagated in the allantoic sac of chick embryos 9 to 11 days of age. Methods of cultivation were identical to those previously described(8). Infected allantoic fluids were stored at -30°C(9). Fluids were clarified by centrifugation at 3000 RPM for 10 minutes at 4°C and tested for bacteriological sterility on blood agar plates at 37°C for 24 hours. All virus infected allantoic fluids employed had hemagglutination titers of 1:512, using methods previously described(8). *Normal allantoic fluid.* Allantoic fluid was obtained from chick embryos handled in the same manner as described above except that they were not inoculated with virus. *Aureomycin.* Each injection of aureomycin consisted of 100

* This investigation was conducted under the sponsorship of the Commission on Acute Respiratory Diseases, Armed Forces Epidemiological Board, and was supported in part by the Office of The Surgeon General, Department of the Army, and by grants from the Brush Foundation and Mr. Philip R. Mather.