Summary. The development of type-specific anti-M precipitins was studied in 2 groups of patients suffering from hemolytic streptococcal infections. Most of the patients in group I, none of whom developed rheumatic fever, showed relatively strong type-specific reactions in their serum by the 4th or 5th week after the onset of infection. The members of group II all developed rheumatic fever; some had similar strong antibodies early; but most rheumatic patients from whom hemolytic streptococci were obtained in significant numbers did not show strong anti-M precipitins until distinctly later than was the rule for the non-rheumatic group.

8819 C

Rate of Urinary Excretion of Test Doses of Ascorbic Acid.

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Studies of the urinary excretion of test doses of ascorbic acid have been concerned for the most part with the total amount excreted during the 24-hour period following the administration of the vitamin C preparation. A few isolated observations[1–5] have indicated that a large portion of the total amount excreted appears in the urine during the first few hours after the test dose is given. This report presents the results of a detailed study by the authors of the rate of excretion of test doses of ascorbic acid, administered by mouth and intravenously, under conditions of vitamin C depletion and saturation.

Test doses of 100 or 200 mg. of ascorbic acid were given in the form of orange juice or crystalline ascorbic acid† by mouth, and in the form of crystalline ascorbic acid intravenously, to subjects whose

* Aided by a grant from the California Fruit Growers' Exchange.

1 Harris, L. J., *Biochem. J.*, 1933, **27**, 2011.

2 Johnson, S. W., and Zilva, S. S., *Biochem. J.*, 1934, **28**, 1393.

3 Harris, L. J., and Ray, S. N., *Lancet*, 1935, **1**, 71.

4 Schroeder, H., *Klin. Wchnschr.*, 1935, **14**, 484.

5 Hawley, E. E., Stephens, D. J., and Anderson, G. K., *J. Nutrition*, 1936, **11**, 135.

† We are indebted to Merck & Co. for the crystalline ascorbic acid ("Cebione") used in these experiments.

states of vitamin C saturation were known. For the most part, states of vitamin C depletion or "unsaturation" followed the intentional administration of a diet low in anti-scorbutic foods for several days. States of vitamin C saturation were induced by the administration of large amounts of orange juice until a large part of the daily test dose appeared in the 24-hour urine specimens. For the experiments here reported, urine specimens were collected at hourly intervals for six hours after the administration of the test dose, then in 2 3-hour periods. Urine voided between the 12th and 24th hours was pooled in a single 12-hour sample. Each specimen of urine was placed immediately after voiding in a dark brown, stoppered bottle containing glacial acetic acid. It was then placed in the ice-box until titrated. The ascorbic acid content was determined by titration against a standardized solution of 2:6 dichlorophenol-indophenol according to the method described by Birch, Harris and Ray.[6] In all instances, the titrations were carried out on the same day, usually within one hour after voiding.

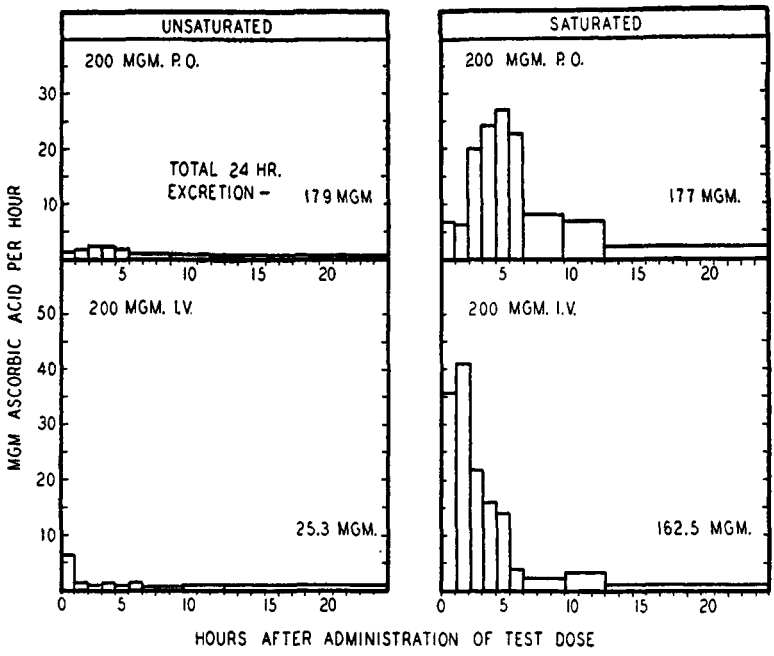

FIG. 1.

Hourly excretion of ascorbic acid after four comparable test doses given to subject L.B.

Fig. 1 presents typical urinary responses to 4 comparable test

[6] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

doses given to a single individual and shows in graphic form the hourly excretion of ascorbic acid given orally and intravenously, in the unsaturated state and after saturation with orange juice. The results of 50 such experiments in unsaturated and saturated subjects are summarized in Fig. 2 which shows the composite curves of the

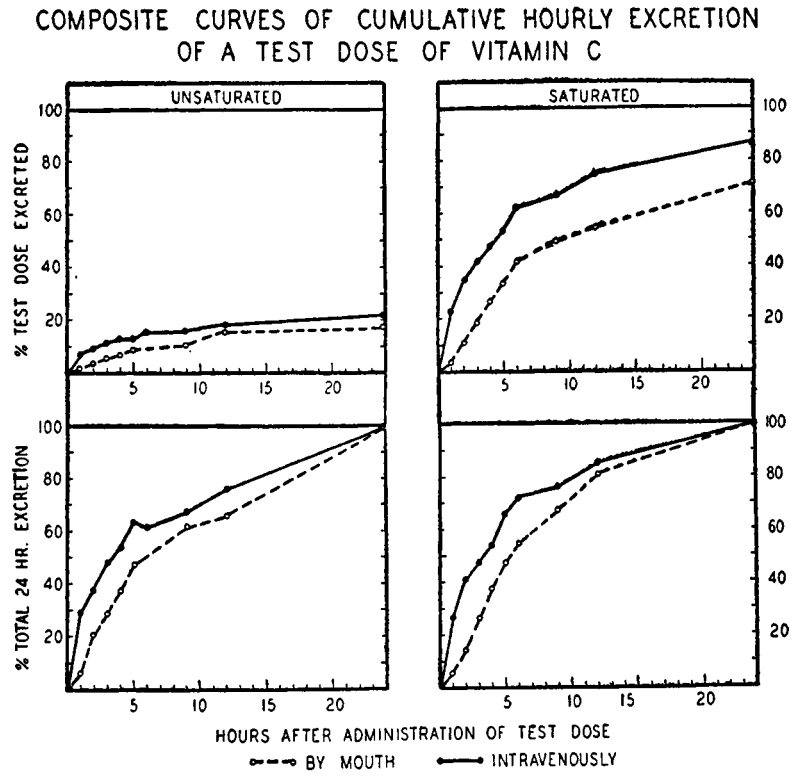

FIG. 2.

Summary of 50 experiments, showing the rate of excretion of test doses of ascorbic acid administered by mouth and intravenously to saturated and unsaturated subjects.

cumulative hourly excretion of test doses of ascorbic acid expressed as % of the test dose which was excreted and as % of the total 24-hour excretion.

It has been repeatedly shown that the administration of a large test dose of ascorbic acid to a subject whose stores have been depleted of vitamin C results in the excretion of an amount of ascorbic acid but little, if any, larger than that excreted during a control period without the test dose. In such subjects only a very slight increase in the rate of excretion occurred during the first few hours

after the test dose was given (Fig. 2). After intravenous administration, the rate of excretion was only slightly greater than when the vitamin C was given by mouth. Even after the introduction of the ascorbic acid directly into the blood stream it was apparently removed from the circulation so rapidly that only a very small portion of the test dose found its way into the urine.

In the subjects who had been taking large amounts of orange juice, from 40 to more than 100% of the test dose appeared in the urine within 24 hours. These individuals excreted a large portion of the ascorbic acid within the first few hours after it was given. After intravenous administration, maximum excretion occurred during the 1st and 2nd hours. When the ascorbic acid was given by mouth, maximum excretion occurred during the 3rd, 4th, 5th, and 6th hours after administration. Delay occasioned by the absorption of the ascorbic acid from the gastro-intestinal tract was apparently responsible for the differences in the rate of excretion observed after oral and parenteral administration.

The composite curves of cumulative hourly excretion calculated as % of the total 24-hour excretion are of particular interest. When the test dose was given orally to subjects who had been partially depleted of vitamin C, approximately 47% of the total 24-hour excretion appeared in the urine by the end of the 6th hour, 67% by the end of the 12th hour. When the test dose was given intravenously to similar subjects, 60% of the total 24-hour excretion appeared in the first 6 hours, 75% in 12 hours. In the saturated subjects, 40% of the total amount excreted in 24 hours appeared in the urine within 2 hours after intravenous administration of the test dose, 72% by the end of the 6th hour and 85% by the end of the 12th hour. When the test dose was given by mouth, the corresponding percentages for the 2nd, 6th and 12th hours were 14, 55 and 81 respectively. It is apparent that the first 12 hours after the administration of a test dose of ascorbic acid are the most important from the point of view of urinary excretion, regardless of the state of saturation or the method of administration of the test dose.

Summary. The rate of excretion of ascorbic acid was determined after the oral and intravenous administration of test doses to unsaturated and saturated subjects. In those subjects whose reserves of vitamin C had been depleted, there was only a slight increase in the rate of excretion during the first few hours, regardless of the method of administration. In saturated subjects, an

average of 80 to 85% of the total 24-hour excretion occurred during the first 12 hours after administration of the test dose. When the latter was given intravenously, maximum excretion occurred during the 1st and 2nd hours. After oral administration, maximum excretion occurred during the 3rd, 4th, 5th, and 6th hours.

8820 C

Properties of Different Fibrinolysins Produced by Streptococci.

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Tillett and Garner[1] reported that hemolytic streptococci and some strains of staphylococci produce a fibrinolytic substance; this observation was corroborated by Schmidt.[2] But, Tunnicliff[3] found some strains of *S. viridans* to be fibrinolytic, and Madison[4] reported fibrinolysin-production by *B. pestis*. In a previous paper[5] the production of fibrinolysin by *S. hemolyticus* and *viridans, enterococcus, pneumococcus* and others was described. The discrepancy in these results may be explained by differences in the culture media employed: When 0.05% glucose-broth or 5% serum-broth was used, hemolytic streptococci only produced the fibrinolysin; when cultivated in 2% glucose-broth, however, various kinds of microörganisms proved to be fibrinolytic. The experiments to be reported are concerned with the question whether the fibrinolysins obtained in these media have different properties.

Technic. Infusion broths, containing 1% peptone, 0.05% and 2% glucose, respectively, with pH 7.4, were employed. Strains from human sources were incubated for 18 hours at 37° C. The supernatant fluid was tested for fibrinolytic activity according to the usual technic.[1]

Plasma was obtained by mixing 10 cc. of normal human blood with 1 cc. of 2% solution of potassium oxalate; it was stored in refrigeration not longer than 48 hours; supernatant fluid of a cul-

1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

2 Schmidt, H., *Zeitschrift für Immunitaetsforschung*, 1936, **87**, 1.

3 Tunnicliff, R., *J. Infect. Dis.*, 1936, **58**, 92.

4 Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 301.

5 Neter, E., and Witebsky, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 549.