over a 4-day period into subcutaneous areas remote from the crop-sacs. Here again we find another striking example of the importance of route of injection. It is notable also that if subcutaneous instead of intramuscular injection were used in the "threshold" bioassay method of McShan and Turner[3] a unit of notably smaller size than that described by them would be expected, unless indeed seepage into the subcutaneous area normally occurs after their injections into very superficial parts of the pectoral muscles of the pigeon.

In our tests with the effect of route of administration on the potency of pituitary F.S.H. we have failed to obtain a significant difference (weights of testes of immature pigeons). And, again unlike prolactin, division of the daily dose of F.S.H. into 3 time-spaced injections failed to change the amount of the response of the testes. In the rat, however, Collip has found subcutaneous injection of gonadotropic hormone much more effective than intraperitoneal injection.

Summary. In young pigeons of the same age and race the response of the crop-glands to prolactin administered by 5 different routes has been studied and found to differ widely. Subcutaneous and intracutaneous injections are about 11 times as efficient as intravenous injections, about 5 times as efficient as intramuscular injections, and about 8 times as efficient as intraperitoneal injections at the dosage level used.

8818 C

Type-Specific Anti-M Precipitins in Rheumatic and Non-Rheumatic Patients With Hemolytic Streptococcal Infections.

HOMER F. SWIFT AND B. E. HODGE.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

The thesis concerning the relationship between streptococcal infections, rheumatic fever, and rheumatoid arthritis, rests in part on clinical observation and in part on a study of antibodies against streptolysins,[1] fibrinolysins,[2] and various chemical components of

[3] McShan, W. H., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 50.

[1] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

[2] Tillett, W. S., *J. Bacteriol.*, 1935, **29**, 111.

hemolytic streptococci. The chief of these components in respect of immunological classification have been described by Lancefield.[3] Under suitable conditions most group A hemolytic streptococci induce the formation of antistreptolysins, antifibrinolysins, anti-C precipitins, and anti-P precipitins; and these four antibodies have been demonstrated in the serum of patients with various streptococcal infections. Because they have also been found by some observers in a high proportion of patients with rheumatic fever,[1,4,5,6] it has been suggested that there is a close connection between streptococcal infection and this disease. The demonstration of agglutinins against hemolytic streptococci[7,8] and the existence of group A anti-C precipitins in the sera of patients with rheumatoid arthritis[9,6,10] has, furthermore, indicated that the etiology of this disease may rest in part, at least, upon a streptococcal basis.

None of these antibodies has a type-specific relationship; in other words, they may result from streptococcal infection with any member of group A of Lancefield. On the other hand, the protective action of an anti-streptococcal immune serum runs roughly parallel with its content of anti-M precipitin;[11] M appears to be the type-specific substance of group A hemolytic streptococci.[3] Until the present time, however, no reports have appeared dealing with the anti-M content of the serum of patients suffering from hemolytic streptococcal infection, nor of those with rheumatic fever.

This information is most desirable, for it seems probable that elimination of part of the pathogenic action of these microörganisms depends upon the development of a suitable degree of type-specific immunity.

This report deals with 2 groups of patients: (I) 24 suffering from streptococcal infections, without demonstrable rheumatic sequelæ; (II) 17 with streptococcal infections and rheumatic sequelæ. Group I consisted of 6 cases of otitis media, 4 of scarlet

3 Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91, 469, 481, 843, 857.

4 Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

5 Coburn, A. F., and Pauli, R. H., *J. Clin. Invest.*, 1935, **14**, 769.

6 McEwen, C., Bunim, J. J., and Alexander, R. C., *J. Lab. and Clin. Med.*, 1936, **21**, 465.

7 Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., *Am. J. Med. Sc.*, 1931, **181**, 12.

8 Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

9 Dawson, M. H., Olmstead, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 355.

10 Dawson, M. H., Olmstead, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 80.

11 Lancefield, R. C., and Todd, E. W., *J. Exp. Med.*, 1928, **48**, 769.

fever, 4 of tonsillitis, 5 of sore throat, and one of cervical adenitis.* The streptococci from 15 of these were obtained from throat-cultures; 6 were from pus from the middle ear, one from pleural exudate, one from a purulent lymph-node, and one from sputum. In group II the antecedent streptococcal infection was not always clear. There were 2 cases of otitis media, 2 of tonsillitis, 3 of sore throat, one of head cold, and in 8 the history of recent upper respiratory infection was negative. The streptococci in 6 patients were obtained from the throat, in one from the middle ear, and in 10 from the interior of excised tonsils. Throat cultures from these 10 patients yielded too few hemolytic streptococci to be of significance.

All the strains were shown by Dr. Lancefield to belong to group A; they grew in matt colonies.[12] The M precipitinogens were obtained from HCl extracts, which were neutralized and freed from group-specific carbohydrate, C, by 2 precipitations with alcohol.[13]

Sera were collected at irregular intervals from one week to 7 or 8 months following the onset of illness. The specimens from patients with uncomplicated streptococcal infections, as a rule, were obtained earlier than those from the rheumatic patients, for the latter were not usually seen until rheumatic symptoms had existed for a few days to a week or more. On the other hand, the rheumatic patients were sick longer; and hence specimens were obtained over a longer period. In addition to the test for homologous anti-M precipitins, tests were made to detect heterologous anti-M precipitins, anti-C, and anti-P precipitins, and anti-streptolysins. The titer of the latter ranged from 25 to 1,200 or 1,600 units per cc. of serum, with a median of 200 to 250 units; no significant difference was found between the rheumatic and non-rheumatic groups.

Table I gives the cumulative totals by weeks, of the various strengths of homologous anti-M precipitins developed by the two groups of patients. It is arranged so that any given degree of reaction is indicated in the same category as that in which it was originally detected until there was a change to another category. No attempt was made to continue the tests until the reactions became negative. This arrangement of the table permits a comparison of the time the stronger degrees of reaction appeared.

There is an obvious difference in the 2 groups. From relatively

* We are indebted to Dr. Currier McEwen for the cultures and serum from some of the patients with tonsillitis; and to the Medical Board of Willard Parker Hospital for the privilege of studying the scarlatinal patients.

12 Todd, E. W., *Brit. J. Exp. Path.*, 1928, **9**, 1.

13 Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91.

few + or ++ reactions in group I during the first 2 weeks the ratio shifted so that 70% were of this strength by the 4th week and over 80% by the 5th week.

In group II the period indicated was dated from the onset of rheumatic symptoms rather than from the onset of streptococcal infection, as was done in group I. This was necessary because the patients were not seen until after rheumatic symptoms had developed. To make the timing in the 2 groups comparable, from 1 to 3 weeks should probably be added to each period charted in group II.

Four patients in group II had strong reactions when examined the second week. One of them had otitis media, one tonsillitis, and 2 rather severe sore throats. These 4 had relatively mild rheumatic fever, with persistently anti-M reactions. Among the remaining 13 patients, strong reactions did not appear until distinctly later than was the case in group I; and not until the 3rd month were over 80% included in the + and ++ categories. In some patients anti-M reactions did not become even + until the 4th or 5th month, at a time when recovery was taking place. Other patients with persistent ± reactions had prolonged low-grade rheumatic infection.

As the readings recorded were made after 48- or 72-hour standing in the ice-box in addition to 2-hour incubation at 37°, *and not after centrifugalization,* it is felt that the ++ reactions have real significance. Incidentally it may be noted that readings not recorded here were also made after centrifugalizations, and that they were usually stronger than those charted. When a reaction no stronger than + was detected it is possible that this reaction might have been due to some other antibody of unknown nature. Such a reading indicated, however, that the patient had not developed strong type-specific immune bodies. On the other hand, because the appearance and intensity of these reactions was not paralleled by the presence of other types of precipitins, we felt that they were due to specific anti-M antibodies. The full significance of their occurrence cannot be definitely determined with the data at hand. Their presence in non-rheumatic patients indicates that they were probably not responsible for rheumatic manifestations. In fact, the relatively mild rheumatic symptoms among rheumatic patients who early showed strong anti-M reactions, and the tendency, in some instances, towards recovery at the time of the appearance or increase in strength of the type-specific antibody, indicate that the presence of this antibody had a favorable implication.

TABLE I.
Anti-M Reactions in Patients with Hemolytic Streptococcal Infections.

Degree of anti-M reaction	Week→ 1 No.	2 No.	3 No.	4 No.	5 No.	6 No.	7 and 8 No.			
				Period after onset of streptococcal infection.						
Group I—Non-Rheumatic										
—	8 = *66%*	5 = *25%*	2 = *9%*	1 = *4%*	1 = *4%*	1 = *4%*	1 = *4%*			
±	3 = *25%*	10 = *50%*	11 = 48%	6 = *25%*	3 = *12%*	3 = *12%*	3 = *12%*			
+	1 = *8%*	3 = *15%*	8 = *35%*	9 = *38%*	9 = *38%*	8 = *33%*	8 = *33%*			
++ }										
+++ }	....	2 = *10%*	2 = *9%*	8 = *33%*	11 = *46%*	12 = *50%*	12 = *50%*			
Total	12	20	23	24	24	24	24			
				Period after onset of rheumatic fever.						
Group II—Rheumatic										
	Week→ 1	2	3	4	5	6	7 and 8	Month→ 3	4	5
—	....	3 = *33%*	4 = *33%*	5 = *35%*	5 = *35%*	2 = *13%*	1 = *6%*	1 = *6%*	3 = *17%*	4 = *23%*
±	....	1 = *11%*	1 = *8%*	1 = *7%*	1 = *7%*	4 = *27%*	5 = *29%*	2 = *13%*	1 = *6%*	1 = *6%*
+	....	1 = *11%*	3 = *25%*	4 = *28%*	3 = *21%*	4 = *27%*	6 = *35%*	6 = *35%*	5 = *29%*	3 = *17%*
++ }										
+++ }	1	4 = *45%*	4 = *33%*	4 = *28%*	5 = *35%*	5 = *33%*	5 = *29%*	8 = *47%*	8 = *47%*	9 = *53%*
Total	1	9	12	14	14	15	17	17	17	17

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.

ESTELLE E. HAWLEY* AND DORAN J. STEPHENS.

From the Departments of Pediatrics and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

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.