present the rôle of carbonic anhydrase in the kidney is only the subject of speculation, but the effects on the kidney of sulfanilamide, a powerful inhibitor of carbonic anhydrase, offer a promising line of attack on the problem.

Summary. Carbonic anhydrase is present in significant concentrations in the cortex of cat, dog and rat kidneys.

13219

A Method for Detecting in Human Serum Protective Bodies Against Hemolytic Streptococci.*

H. W. DIEFENDORF. (Introduced by Grover F. Powers.)

From the Department of Pediatrics, Yale University School of Medicine, and the Children's Clinic of the New Haven Hospital.

The antigenic structure of the Group A hemolytic streptococci has been greatly clarified by the important work of Lancefield. Antistreptococcal rabbit serum has been used extensively in these studies for determining antigen-antibody relationships. There has been as yet no similar analysis of the antibodies in human serum from streptococcal disease. The passive protection of mice would be a useful approach to such a study. Neufeld[1] used this technic in human streptococcal disease but failed to obtain protection with the one scarlatinal serum tested. The work of Dochez, Avery, and Lancefield,[2] and of Hirst and Lancefield[3] indicates that in using mouse-protection it may be necessary to employ strains of streptococci which kill with 10^{-8} cc of culture. Unfortunately, freshly isolated strains of streptococci do not often kill in dilutions greater than 10^{-4} cc.[4, 5] Recently, however, the use of 50% endpoints in biological titrations has become more and more popular. This method of analysis has been made simple and practicable by Reed and Muench.[6] Accordingly, it appeared worth while to attempt to

* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

1 Neufeld, F., *Z. f. Hyg.*, 1903, **44**, 161.

2 Dochez, A. R., Avery, O. T., and Lancefield, R. C., *J. Exp. Med.*, 1919, **30**, 179.

3 Hirst, G. K., and Lancefield, R. C., *J. Exp. Med.*, 1939, **69**, 425.

4 Wadsworth, A., and Coffey, J. M., *J. Immunol.*, 1935, **29**, 505.

5 Wu, J. P., *J. Immunol.*, 1941, **40**, 179.

6 Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

use fresh human strains of streptococci of only moderate virulence in protection-tests, carried out in such a way as to make use of the 50% endpoint method.

Materials and Methods. 1. *Media.* The basic culture medium was beef-heart infusion-broth containing 1% neopeptone. Blood broth was prepared by adding 0.1 cc of defibrinated rabbit blood to 5 cc of broth.

2. *Organisms.* Hemolytic streptococci were obtained from children by throat cultures on blood-agar plates. Typical hemolytic colonies were picked and the organisms identified by stained smear, bile-insolubility, and the presence of soluble hemolysin for rabbit erythrocytes. The organisms were cultured in blood broth, and were used within one month after isolation for the virulence- and protection-tests.

The number of streptococci injected into mice was determined by pour-plates; 10^{-7} cc of the 5-hour cultures contained 30 to 70 organisms in the 14 protection-tests.

3. *Virulence-tests.* White mice weighing 18-22 g were used for both virulence- and protection-tests. Preceding the actual protection-experiment a virulence-test was done with the patient's organism. A young culture was prepared by adding 0.1 cc of an 18-hour blood-broth culture to 5 cc of blood broth and incubating for 5 hours. This culture was diluted serially in broth in multiples of ten. Five-tenths cc of each dilution was inoculated intraäbdominally into one mouse, and the approximate time of death recorded. The 3 highest dilutions of culture which killed mice were used for the protection-test.

4. *Sera.* Blood was drawn by venapuncture and the serum stored in the icebox without preservative. Serum from the 10 scarlet-fever patients was taken during the latter part of the third week of the disease, from the 4 rheumatic-fever patients approximately one month after the onset of the attack under study. Control serum was obtained from 3 normal persons without a history of recent streptococcal infection. Control serum from one individual was used for 12 tests, and 2 other persons contributed serum for the 2 remaining tests.

5. *Protection-tests.* Undiluted serum was inoculated intraabdominally 12 hours before the dose of streptococci. For the first few tests 0.5 cc serum was used. Later it was found that 0.25 cc gave protection also, so for economy this dosage was used for the remaining tests.

Hirst and Lancefield[3] showed that passive immunity with antistreptococcal rabbit serum was type-specific. Consequently the

serum from each patient was tested against the organism isolated from him, in order to take advantage of type-specific action if it were present.

Eight mice, 4 receiving patient's serum and 4 control serum, were used for each of the 3 dilutions of streptococci. Each mouse was injected intraäbdominally with 0.5 cc of the diluted culture of the patient's organism, prepared in the same manner as for the virulence-tests. Observations were terminated at the end of 4 days. In each test at least one mouse dying before 4 days was necropsied and heart-blood cultures were made on blood-agar plates. A pure growth of hemolytic streptococci was obtained in every case.

A few protection-tests were carried out by inoculating the organisms intracerebrally following the procedure suggested by Huang and Sia,[7] but the results were so irregular that the method was not used for further work.

Results. The results of the protection-tests are shown in Table I. For each dilution of hemolytic streptococci, the mice injected with the patient's serum and those receiving normal serum are represented side by side, and for simplicity will be referred to subsequently as 'serum-injected' and 'control' mice respectively. The numerator

TABLE I.
Protection of Mice Against Hemolytic Streptococci with Human Convalescent Serum.

		Dilutions of Hem. Strep.									
		10–1		10–2		10–3		10–4		10–5	
		Injected Mice									
Patients	Disease	Serum	Control	Serum	Control	Serum	Control	Serum	Control	Serum	Control
W.B.	Scarlet fever					4/4	1/4	4/4	0/4	4/4	4/4
J.M.	" "			0/4	0/4	2/4	0/4	3/4	3/4		
J.P.	" "	1/4	0/4	4/4	0/4	4/4	1/4				
D.W.	" "	0/4	0/4	0/4	0/4	4/4	0/4				
R.F.	" "			4/4	0/4	3/4	1/4	4/4	3/4		
R.W.	" "	0/4	0/4	1/4	0/4	3/4	0/4				
G.V.	" "			0/4	0/4	2/4	1/4	4/4	4/4		
M.N.	" "			1/4	0/4	3/4	0/4	4/4	2/4		
P.R.	" "			0/4	0/4	0/4	0/4	2/4	2/4		
A.J.	" "			0/4	0/4	1/4	1/4	3/4	2/4		
B.B.	Rheum. fever	0/4	0/4	4/4	0/4	4/4	1/4				
B.H.	" "					0/4	0/4	3/4	0/4	3/4	1/4
J.M.	" "	0/4	0/4	0/4	0/4	3/4	0/4				
W.T.	" "			2/4	0/4	3/4	1/4	4/4	4/4		

Serum—Mice receiving patients' serum.
Control—Mice receiving normal serum.
Fraction—No. mice survived 4 days/No. injected.

[7] Huang, C. H., and Sia, R. H. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 87.

of each fraction signifies the number of mice surviving 4 days, and the denominator, the number of mice injected. It will be noted that the surviving mice in most cases were distributed over 2, and sometimes over all 3 dilutions, which shows how difficult it is to choose the MLD and to express protection in terms of it.

However, the results can be analyzed quite readily by using the method of 50% endpoints recommended by Reed and Muench.[6] By calculating for each test the dosage of organisms required to kill half the serum-injected mice, and by comparing this figure with the dosage killing half the controls, some indication of the protective power of convalescent serum is obtained.

The results of the analyses of the 14 tests are listed in Table II. The calculated dilutions killing half the serum-injected and control animals (50% endpoints) are given for each test. The last figure on the right, the 'factor', is the number obtained by dividing the dilution killing half the serum-animals by that killing half the controls. For example, in the test on the serum from patient W.B. in Table II, 1/100 cc of 5-hour culture killed half the serum-animals, but the control animals were killed by 1/40,000 cc. In other words, 400 times as many organisms were required to kill the serum-injected mice as the controls. In one instance, the killing dosage for the serum-animals was 500 times that for the corresponding controls. In another case the 50% endpoints for the 2 groups

TABLE II.
Demonstration of Protection with Human Convalescent Serum Using the 50% Endpoint Method.

Patients	Disease	Dilutions of Hem. Strep.*		Factor
		Serum-injected mice	Control mice	
W.B.	Scarlet fever	1:100	1:40,000	400.0
J.M.	" "	1:2,000	1:7,000	3.5
J.P.	" "	1:30	1:4,000	133.3
D.W.	" "	1:500	1:10,000	20.0
R.F.	" "	1:10	1:5,000	500.0
R.W.	" "	1:500	1:10,000	20.0
G.V.	" "	1:1,000	1:3,000	3.0
M.N.	" "	1:500	1:10,000	20.0
P.R.	" "	1:10,000	1:10,000	1.0
A.J.	" "	1:5,000	1:8,000	1.6
B.B.	Rheum. fever	1:50	1:4,000	80.0
B.H.	" "	1:8,500	1:400,000	47.1
J.M.	" "	1:700	1:10,000	14.3
W.T.	" "	1:200	1:3,000	15.0

Serum-injected mice—Mice receiving patients' serum.
Control mice—Mice receiving normal serum.
*Dilution of broth culture of hemolytic streptococci calculated to give 50% endpoint.

coincided, but in no case was the protective power of the control serum greater than that of serum from patients.

For purposes of convenience, a purely arbitrary criterion for protection is used: sera with a 'factor' below 10 are considered non-protective; a 'factor' above 10 is assumed to indicate that protection was demonstrated. By this criterion, of the 10 scarlet-fever sera, 6 were protective and 4 were not. All 4 of the rheumatic-fever sera gave protection. Grouping all the tests together, protection was demonstrated in 10 out of 14 sera.

It may be added that the totalled results were subjected to the *chi*-square test and found to be statistically significant.

Discussion. Lancefield has described 2 type-specific antigens, M and T, in Group A hemolytic streptococci.[8] She showed that the M-anti-M complex was largely responsible for mouse-protection, and that the T-anti-T complex was not concerned with protection. However, antisera prepared with a glossy strain and devoid of anti-M detectable by the precipitin-test did give protection against 10 to 100 MLD of the homologous matt organisms. This observation led Mrs. Lancefield to suggest the possibility of another type-specific protective body, which is as yet poorly defined; this is another reason why the serological analysis of human streptococcal blood may prove valuable.

Summary. A method has been described to demonstrate that serum of patients convalescing from rheumatic fever and scarlet fever will protect mice against hemolytic streptococci.

13220

Assay of Sodium-Retaining Substances.*

FRANK A. HARTMAN, LENA A. LEWIS AND JONATHAN S. THATCHER.

From the Department of Physiology, The Ohio State University, Columbus.

Sodium retention is an important function of the adrenal gland. The efficacy of adrenal preparations depends in part on this property. Methods for the assay of sodium-retaining substances have been

[8] Lancefield, R. C., *J. Exp. Med.*, 1940, **71**, 521.

* Aided by grants from the National Research Council Committee on Research in Endocrinology and the Comly Fund of the Ohio State University.