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.

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Assay of Sodium-Retaining Substances.*

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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.

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described.[1, 2] In this paper we give the results of 3 years' experience with the method of Hartman and Spoor.[2] We are proposing certain modifications including the use of desoxycorticosterone as a standard.

Methods. The preparation and care of the animals have been described previously.[2] Animals showing evidence of kidney damage were discarded. The diet was adjusted so that the weight of each animal was maintained at an essentially constant level. For the average 10 to 12 kg dog the diet consisted of 250 g of beef heart, 70 g of Purina Dog Chow, 1 yeast pill and 2 g of sodium chloride daily. Cod liver oil capsules were given once a week. They were allowed water *ad lib.* except on test days. The animals were fed at 11 p.m., 9 hours before the beginning of a test period. They were catheterized at the beginning and end of the experiment and kept in metabolism cages during the 6-hour test. At the beginning of the test they were given 100 cc of distilled water by stomach tube.

All injections were given subcutaneously in order to prolong the effect and to avoid the development of refractoriness.[3] The material to be injected was dissolved either in peanut oil or 10% ethyl alcohol. Standard solutions of desoxycorticosterone were prepared and the response of each test animal was determined following the injection of a given dose (usually 0.6 to 0.8 mg was used). If the dose injected gave less than 35% or more than 65% retention of sodium the test was repeated using a modified dose. If the unknown to be tested was dissolved in alcohol, then the standardization dose of desoxycorticosterone was given in alcohol; if in oil, both materials were dissolved in oil. From time to time, injections of 10% alcohol or oil alone were made in order to determine any possible effect that the solvent might have on electrolyte excretion.

Sodium was determined by the method of Butler and Tuthill,[4] potassium by Consolazio and Talbott's modification[5] of the Shohl and Bennett method and chloride by the Volhard-Harvey method.[6]

The normal electrolyte excretion (control level) of the dogs was determined for the 6-hour test period. Control tests were run daily until it was established that the control level was essentially

1 Harrop, G. A., and Thorn, G. W., *J. Exp. Med.*, 1937, **65**, 757.

2 Hartman, F. A., and Spoor, H. J., *Endocrinology*, 1940, **26**, 871.

3 Hartman, F. A., Lewis, L. A., and McConnell, K. P., *Endocrinology*, 1939, **24**, 197.

4 Butler, A. M., and Tuthill, E. J., *J. Biol. Chem.*, 1931, **93**, 171.

5 Consolazio, W. V., and Talbott, J. H., *J. Biol. Chem.*, 1938, **126**, 55.

6 Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, **2**, 834, Williams and Wilkins, Baltimore, 1932.

constant. A control was then run the day before each test day. At least one day was allowed to intervene between an injection and the following control period. The percentage of sodium retention was calculated using the average of the 2 controls immediately preceding the test as the normal level.

Results. In our experience to date we have used twelve dogs for sodium retention assay. These were selected from 48 healthy animals. As high as 100 assays have been made in a single dog during 2.5 years. Assays could not be made during hot weather because the results were so variable. With air conditioned quarters this might be possible.

The sodium retention of different dogs when injected with desoxycorticosterone is summarized in Table I. So far we have had but one dog (No. 48) which gave little or no response to the usual dose of desoxycorticosterone. The response in different animals varies somewhat. However, the important consideration is whether the same animal gives a constant response to the same dose. Some individuals are better than others in this respect. On this account they were used most frequently for assay. The responses of Dogs 32 and 42 were reasonably constant when it was realized that the

TABLE I.
Sodium Retention Caused by Desoxycorticosterone in Different Dogs.

Dog No.	Weight, kg	Date	Dose, mg	Retention %	Retention per mg	
37	7.8	3/31/40	.60 intrav.	41	68	
44	8.0	1/30	.60 "	61	100	
45	8.2	4/2	.60 "	40	67	
5	7.0	1/30	.75 "	51	69	
32	10.0	12/2/39	.75 "	49	66	
	10.0	1/12/40	.75 "	49	66	
	9.1	10/11	.80 subcu.	43	54	
	9.9	12/3	.80 "	53	66	Avg 63
42	10.2	1/16/40	.75 "	46	61	
	10.5	10/11	.80 "	51	64	
	10.2	12/6	.80 "	60	73	
	9.9	4/18/41	.75 "	51	69	Avg 66
31	7.5	1/30/40	.80 "	45	56	
	7.1	10/11	.80 "	35	44	
	7.0	12/3	.80 "	50	62	Avg 54
48	18.7	2/28/41	.80 "	0		
		3/4	1.00 "	19	19	

first injections of desoxycorticosterone were given many months before the final injection.

In specific sodium retention no other substances except water, sufficient to maintain isotonicity, should be retained. Any additional retention can be attributed to an anti-diuretic effect. If, for example, potassium is also retained the latter effect may be suspected.

We define the unit for sodium retention as one-tenth of that amount of material which will cause the same sodium retention as 0.7 mg of desoxycorticosterone in the same dog. This quantity of desoxycorticosterone will cause retention within the sensitive range (35 to 65%) in most dogs. Calculation can be made by the following formula:

$$\text{No. of units} = \frac{\text{\% retention by unknown}}{\text{\% retention by 0.7 mg desoxycorticosterone}} \times 10$$

If a different amount of desoxycorticosterone is used the results are calculated on a basis of 0.7 mg.

For example, 5 cc of an extract gave 40% retention. In the same dog 0.6 mg of desoxycorticosterone gave 36% retention or 0.7 mg should have given 42% retention.

$$\text{No. of units} = \frac{40}{42} \times 10 = 9.5$$

or 1.9 units per cc.

Table II gives examples of the assay of different extracts. If a

TABLE II.
Assay of Different Extracts.

Extract	Dog No.	% Na retention	Desoxycorticosterone (0.7 mg) % Na retention	Units per cc
Commercial A	5	45	48	1.8
	37	46	48	2.0
	42	50	45	2.2
" B	31	37	38	2.0
	37	32	48	1.4
Experimental I	31	37	38	1.95
	32	33	44	1.50
" II	5	49	48	2.04
	31	35	38	1.84
	42	46	46	2.0
" III	32	33	44	.74
	42	31	46	.67

larger number of animals were used for each preparation the assay could be improved.

Discussion. We prefer female dogs because they are easier to catheterize and one is more certain of complete evacuation of the bladder. However, they can not be used when they become pseudo pregnant. The sodium retention produced by adrenal extract or its fractions is essentially reproducible in any one dog. When compared with desoxycorticosterone the results elicited by a given preparation in different dogs may be similar. Why an occasional animal departs from this is not understood. There seems to be little correlation between size of animal or period of year and response.

It is realized that the sodium retention alone may not give the entire picture of the effect of a fraction on the electrolyte balance. It is felt, however, that assays are satisfactory if there is no decrease in the potassium excretion from the control level and if the water retention is not more than that necessary to maintain isotonicity. Under these conditions the retention produced by the extract may be considered specific sodium retention.

We believe that the method described can be employed to assay various substances which cause sodium retention. All commercial adrenal extracts should be assayed for this property because these sodium-retaining substances reduce the amount of extract required.[7]

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Summary. A method is described for the assay of sodium-retaining substances in which desoxycorticosterone is employed as a standard. The sodium retention produced by the unknown is compared with that of desoxycorticosterone in a normal dog on a constant diet, over a 6-hour period.

[7] Hartman, F. A., Lewis, L. A., Gabriel, J. E., Spoor, H. J., and Brownell, K. A., *Endocrinology*, 1940, **27**, 287.