dosage given early in experimental relapsing fever (*B. recurrentis* var. *turicatae*) infection will not only cure the blood stream involvement but will prevent brain involvement in a great majority of the cases. The fact that the 1 positive brain passage in this group of 8 animals occurred in a rat which received 45,000 units of penicillin per kg, whereas in two other instances 38,400 and 41,300 units per kg prevented brain involvement, indicates that even early treatment with apparently adequate dosage will not prevent brain involvement in all cases. Also the fact that brain passage was positive in the 7 late treatment rats of Groups I and II which received more than 40,000 units per kg is strong presumptive evidence that adequate dosage for early treatment is not adequate dosage for late treatment. The question of whether or not brain involvement can be cured with any dosage of penicillin remains to be solved.

This is a preliminary report, and complete details and additions will be published elsewhere. We believe that the question of brain cure or prevention of brain involvement in spirochetoses is a most important consideration and that the results of our experiments reemphasize the need for early treatment and adequate dosage in relapsing fever therapy.

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PRECIPITATION AND AGGLUTINATION TESTS WITH THE HEMOLYTIC STREPTOCOCCUS. TITRATION OF "M" AND "T" ANTI-BODIES IN HUMAN SERA

THE determination of the predominating "M" and "T" antigens by means of precipitation or agglutination tests is the accepted means of identifying hemolytic streptococci as to type in epidemiological studies of human infection. Since such investigations are frequently subject to error due to a multiplicity of antigens in the strains isolated or to the presence of cross infections, it seemed possible that the type implicated in any given infection might be more readily ascertained if it could be shown that type-specific antibodies are produced in the patient's serum in sufficient strength for identification by means of either precipitation or agglutination tests using antigens of known specificity.

In the course of studies at the U. S. Naval Training Station at Newport, R. I.[1] in 1941, blood sera from naval recruits were made available for the determination of antibody content. Forty samples of serum were tested for specific agglutinins by slide agglutination. Nine were from cases of upper respiratory infection—either sore throat or scarlet fever, and eleven were from cases of rheumatic fever. In each instance, the hemolytic streptococcus isolated from the throat was typed by agglutination at the time of hospitalization. Twenty sera from well recruits were included as controls. In a few cases hemolytic streptococci were isolated from the throat cultures in this group also, and the type established.

The sera were tested in dilutions ranging from 1–1 to 1–2,000, from six to eighteen dilutions being set up in each case as needed. All cultures for the agglutination tests were from the type collection of the Department of Preventive Medicine of the Harvard University Medical School. Since "T" antigen[2] is apparently constant and unrelated to colony morphology, it seemed justifiable to use these cultures without continuous mouse passage. Suspensions were prepared from 20- to 22-hour cultures grown at 37° C. in 5 cc broth prepared according to the formula of Swift and Hodge.[3] Before use, the supernatant fluid was pipetted off and the sedimented cells resuspended to a uniform density in a small volume of broth. The slide agglutination technique of Griffith[4] was followed, readings being made after a brief agitation of the serum dilution-suspension mixtures. Control suspensions were included on each slide to eliminate any possibility of error due to spontaneous agglutination. The type cultures used formed smooth suspensions with the exception of types 6 and 13, which frequently had to be prepared from cultures grown at room temperature to ensure stable suspensions.

Cross reactions were common in all the sera and the more sensitive suspensions showed agglutination in relatively high dilutions. Agglutination in dilutions 1–5, 1–10 and 1–20 were frequent. However, agglutinins of sufficient strength or specificity to indicate an antibody response related to either present or past infection could not be demonstrated. In no instance could correlation be shown between serum agglutinins and the streptococcus type isolated from the throat cultures. Heterologous agglutinins were present to the same or even higher titre than those of homologous type. Moreover, the control sera showed cross reactions of the same magnitude and complexity.

Although these experiments seemed to indicate that the presence or absence of "T" antibodies in human sera have little significance, it was hoped that comparable tests for "M" antibodies would show more conclusive evidence of correlation between antibody content

[1] Captain R. M. Lhamon (M.C.), U.S.N.; Commander R. W. Huntington (M.C.), U.S.N.R.; Lieutenant S. M. Wheeler (M.C.), U.S.N.R., and T. Duckett Jones, M.D. To be published.

[2] Rebecca C. Lancefield, *Jour. Exp. Med.*, 71: 521–550, 1940.

[3] Homer F. Swift and B. E. Hodge, *Proc. Soc. Exp. Biol. and Med.*, 30: 1022–1023, 1933.

[4] F. Griffith, *Jour. Hyg.*, 34: 542–583, 1935.

and infection type. Eleven sera from rheumatic fever patients were subsequently tested, using crude extracts of "M" antigen supplied by Dr. Lancefield's laboratory in connection with another study.[5] Precipitation tests were carried out by the capillary technique of Swift, Wilson and Lancefield.[6] The sera employed were obtained from the same rheumatic fever patients but represented different bleedings. No precipitation could be demonstrated at 37° C although reactions did occur in several of the sera after exposure overnight at ice-box temperature. This, together with the fact that cross reactions were numerous, would seem to indicate an indefinite antibody response of an unspecified nature between the unabsorbed sera and the "crude" extracts of "M" antigen employed. None of the sera showed the prompt precipitation with homologous extract which might logically be expected if true anti-"M" were present. As in the case of the agglutinins, correlation between antibodies produced, either as regards amount or specificity, and infection could not be demonstrated.

More conclusive evidence of the absence of relationship between "M" antibody and infection type in rheumatic fever was shown by the results of precipitation tests on sera from a rheumatic fever patient under treatment in the House of the Good Samaritan in Boston, Massachusetts. Three bleedings were obtained over a six-week period, during which throat cultures were taken at frequent intervals. From these, hemolytic streptococci were isolated on six occasions which, in each instance, agglutinated as type 12/28. In the precipitation tests on these sera, the crude "M" extracts and the technique employed were the same as those used in the tests on the Newport cases. Complete absence in reaction at 37° C and a multiplicity of cross reactions at ice-box temperature were again demonstrated.

SUMMARY

While "M" and "T" antibodies can be demonstrated in low titre in human sera, an almost complete absence of specificity seems to indicate an apparent lack of correlation with current or past streptococcal illness. In the present study, agglutination and precipitation tests on patient's sera, using known "T" and "M" antigens, appear to have little value in determining the antigenic relationship of the streptococci involved in infection. Moreover, no correlation could be shown between the amounts of "M" antibody and of "T" antibody present in the different sera.

It is obvious that further work is needed. With a greater degree of purification of "M" and "T" and the elimination of non-specific substances, it may ultimately be possible to demonstrate the development of significant type-specific antibodies in human sera.

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[5] Obtained from Br. B. F. Massell, House of the Good Samaritan, through the courtesy of Rebecca C. Lancefield.

[6] Homer F. Swift, Armine T. Wilson and Rebecca C. Lancefield, *Jour. Exp. Med.*, 78: 127–133, 1943.

LENGTH OF SURVIVAL OF HOMOZYGOUS CREEPER FOWL EMBRYOS

EXTENSIVE studies on Creeper fowl have established that the Creeper factor has a lethal effect when homozygous. The homozygous Creeper embryos generally die soon after the end of the third day of incubation, but a few survive to later stages. These late homozygous Creeper embryos are readily recognizable by complex deformities of the extremities (phokomelia), the eyes and other parts.

It was observed repeatedly that a higher percentage of homozygous Creeper embryos survived beyond the early lethal stage when, prior to incubation, the temperature of the egg storage room had risen considerably above physiological zero, *i.e.*, above the temperature below which no development occurs in chicken eggs. This suggested that initial development at a lower rate than that prevailing at standard incubation temperatures favors the survival of larger than usual numbers of homozygous Creeper embryos to late stages. Systematic tests have now been made to verify the correctness of this assumption.

Our routine incubation is done in a forced-draft incubator running between 99° and 100° F. The standard temperature of still-air incubators is 103° F. Our tests consisted in starting eggs from Creeper matings in a still-air incubator running at 96° F. and to transfer them to standard conditions of a forced-draft incubator after 12, 24 and 48 hours, respectively. The control eggs were throughout incubated in the same forced-draft incubator. Eggs of the same hens were distributed evenly into test and control groups.

The results of these experiments are shown in Table 1. There was no difference in the frequency of late homozygous Creeper embryos when the eggs had been incubated for only twelve hours at reduced temperature. When, however, the duration of lowered tem-

TABLE 1

Duration of reduced initial incubation temperature (96° F.) in hours		12	24	48
Test group	N	310	627	574
	Number of late CpCp embryos	8	27	13
	Hatch per cent.	42.4 ± 2.85	54.0 ± 2.03	58.9 ± 2.04
Control group	N	309	609	570
	Number of late CpCp embryos	6	10	7
	Hatch per cent.	52.0 ± 2.88	55.9 ± 2.03	55.0 ± 2.09
Significance of differences in frequency of late CpCp embryos	χ^2	0.034	7.495	1.832
	P	> .80	< .01	> .10