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.

RUTH ALIDA THOMAS
DEPARTMENT OF HYGIENE AND BACTERIOLOGY,
SMITH COLLEGE

[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

perature was extended to the first 24 hours of incubation, there was a 170 per cent. rise in the frequency of survival of homozygous Creeper embryos as compared with the control group. This difference is clearly significant. When the time of incubation at reduced temperature was increased to the first 48 hours the advantage to the homozygous Creeper embryos, as reflected in survival, disappeared.

The fact that a lowered rate of development for the first 24 hours favors increased survival of homozygous Creeper embryos, but that the same did not hold when the reduced temperature lasted for as much as 48 hours, requires explanation. The reasons can only be surmised. When the temperature of incubation was brought down to 93° F. for as long as seven days, the majority of embryos died during the second week of development and hatchability was reduced to zero. Even after as short a period as four days at 93° F. only 37 per cent. of the fertile eggs hatched, as compared with 56 per cent. of the controls (eggs from Creeper matings). It is evident, then, that prolonged exposure to a reduced developmental rate is harmful. Although such a harmful effect can not be discerned after 48 hours of incubation at 96° F., as judged by hatching results, it seems reasonable to assume that the beneficial effect which a reduced developmenal rate has on survival of homozygous Creeper embryos is vitiated by harmful agencies which come into play if the duration of lowered developmental rate is extended beyond an optimum.

The fact that more homozygous Creeper embryos survive to late stages when the first 24 hours of their development proceed at a reduced developmental rate fixes the activity of the homozygous Creeper condition at a considerably earlier embryonic period than it has been possible to demonstrate by morphological means. It may also be concluded from these observations that the improved chances of survival of homozygous Creeper embryos under the conditions of our experiment indicate that the production or utilization of a critical substance or the occurrence of some chemical transformation has been aided.

WALTER LANDAUER

UNIVERSITY OF CONNECTICUT

SCIENTIFIC APPARATUS AND LABORATORY METHODS

FLUORESCENT STAINING OF INSECT TISSUES

THE staining of plant and animal tissues by means of fluorescent substances has come into use during recent years and numerous materials have been found suitable for this purpose. Known as fluorochromes, these act specifically to stain certain cellular structures, in a way comparable to the more commonly used microscopical stains like haematoxylin and various aniline dyes. While the latter are ordinarily viewed by transmitted white light or by certain other bands of the visible spectrum, fluorochromes show practically no color until irradiated by ultra-violet light. Thus illuminated, the stained structures fluoresce and appear as a brilliantly luminous pattern whose color (most commonly yellow) is that characteristic of the specific stain used. Fluorescence is usually most strongly activated by the longer wave-lengths of ultra-violet, especially those near λ3650 which are particularly strong in the light of mercury-vapor lamps. These, then, form a satisfactory source of illumination. The insertion of a "black" Corning glass filter (such as Wratten No. 18) between the lamp and the microscope removes the visible light (except a little red) and all the ultra-violet below about λ3100. Thus the light is perfectly safe for visual work, especially if a light yellow protective filter (such as Wratten No. 2a) is interposed between the microscope and the eyes.

The dyes earlier used by Haitinger and his associates[1] included a few plant extracts, alkaloids and dyes of various sorts whose staining properties these workers investigated briefly. More recently a number of other substances have been found to be satisfactory fluorochromes for certain purposes, for example, the use of auramin as a stain for bacteria, especially in the diagnosis of tuberculosis.

Following a simple procedure of fixing in 5 per cent. formalin followed by the ordinary methods of dehydration in alcohol, clearing in xylol, imbedding and cutting in paraffin, sections may be stained in aqueous solutions of the fluorochromes and examined under the microscope after mounting in glycerin. The latter is not fluorescent and must replace the ordinary balsam or other media which are highly fluorescent and "fog the picture," as will also the use of oil-immersion objectives, due to the natural blue fluorescence of immersion oils.

The application of several of these dyes to the staining of insects' tissues has been reported on by Metcalf and Patton,[2] who have used berberine extensively, finding it to be a powerful stain which differentiates nuclei very clearly as it causes the chromatin to fluoresce a brilliant light yellow.

A number of specific alkaloids, or these in mixture as obtained by extracting the roots, bark or other

[1] *Bot. Centralbl., Beihefte,* 50: pp. 432–444, 1933; *ibid.,* 53, pp. 378–396, 1935; *ibidem,* pp. 387–397; Abderhalden's Handb. d. Biol. Arbeitsmeth., Abt. II, Physik. Abt., Teil 3, Heft 5, Lief, 433, pp. 3307–3337, 1934.

[2] *Stain Technology,* 19: 11–27, 1944.