growth of the cells, but also it rests upon a Kjeldahl analysis which is probably the easiest and most accurate of the determinations made.

As long as respiration of washed cells remains linear with time, assimilation of a portion of the substrate should not seriously interfere with interpretation of the results.

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Production of Anti-Bacterial Agglutinins by Carp and Trout at 10° C.

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In recent years costly losses to the salmon industries and trout hatcheries of middle Europe, Great Britain, and North America have been caused by a generalized bacteremia, the etiological agent of which is *Bacterium salmonicida.* These losses have raised the question as to the possibility of inducing immunity in brood stocks of these species by the use of bacterial vaccines.

There is no physiological characteristic of fish, amphibians, or reptiles known to the author that indicates that the reaction of these animals to the injection of a bacterial vaccine should differ from the response of the warm blooded vertebrates. Nevertheless, a search of the literature has failed to reveal a single attempt to immunize fish with vaccines. Indeed, there are very few studies reporting the production of any antibodies by cold-blooded animals upon the injection of foreign proteins, and most of these reports concern animals held at 20°C or above.

Five studies report the production of antibodies against red blood cells. Lazar[1] injected frogs with washed bovine erythrocytes and incited the development of agglutinin titers of 1:10 to 1:80 in some individuals. Many animals, however, were negative. Schwarzmann[2] also found that frogs developed hemoagglutinins on the injection of erythrocytes. Allen and McDaniel[3] held 2 groups of frogs at room temperature (22°C to 27°C) and 2 similar groups

*Most of this study was subsidized by and carried out in the laboratories of the Wisconsin Conservation Department, Madison.

1 Lazar, Erwin, *Wien. Klin. Wchnschr.*, 1904, 1057.

2 Schwarzmann, L., *Z. f. Immunitatsf.*, 1927, **51**, 138.

3 Allen, F. W., and McDaniel, C., *J. Immunol.*, 1937, **32**, 143.

in the refrigerator (8°C to 10°C). One group at each temperature was injected with human erythrocytes. The only one of the 4 groups that developed hemolysins was the one injected with erythrocytes and held at room temperature. These workers concluded that only when the frogs were kept above 20°C did they develop antibodies. Wollman[4] confirmed these results in experiments which also indicated that temperature is a determining factor as to whether or not frogs will produce antibodies. Using sand turtles, Downs[5] has shown that 15 of 50 animals developed anaphylactic response to foreign erythrocytes and 9 of 19 developed precipitin titers of not over 1:100 within 3 weeks. The temperature of the turtles is not given.

Five other reports concern the production of specific anti-bacterial agglutinins in cold blooded vertebrates. Widal and Siccard[6] used the typhoid bacterium as an antigen and induced agglutinins in several frogs of 3 species, *(Rana esculenta, Rana usca,* and *Hyla viridis)* at temperatures from 12° to 24°C, 21° to 23°C, 27° to 33°C, and at 37°C. More rapid antibody production occurred at the higher temperatures than at the lower. Babes and Riegler[7] report the production of specific agglutinins in crucian *(Carassius vulgaris)* and in tench *(Tinca vulgaris)* within 24 hours by the injection with *Proteus piscidus versicolor.* The maximum titer observed was 1:50. Pliska[8] induced *Pseudomonas punctata* agglutinins in carp *(Cyprinus carpio)* by the injection of the bacterium intraperitoneally. The fish were held at 20°C and titers of 1:10,240 were attained in 6 weeks. Indeed, a higher titer was obtained in carp than rabbits. Pliska, also, cites the production of antibodies against certain vibrio strains by Bergman and by Aaser each using northern pike *(Esox lucius).* He reports that Nybelin was able to induce antibody production at 17°C but not at 9°C in eels. Gee and Smith[9] subjected 3 painted turtles *(Chrysemys elegans)* and one snapping turtle *(Chelydra tricarinata)* to semi-weekly injections of *Bact. salmonicida* vaccine. These turtles were held at room temperature which fluctuated between 23°C and 28°C. No agglutinins were present in the blood when the experiment started. Each animal developed agglutinins within 6 months and the gradual increase of agglutinins was observed in 2 animals. The maximum titer observed was 1:2,560.

4 Wollman, E., *Rev. Immunol.* (Paris), 1938, **4**, 101.

5 Downs, M., *J. Immunol.*, 1928, **15**, 77.

6 Widal and Siccard, *Compt. rend. de la Soc. de Biol.*, 1897, **49**, 1047.

7 Babes, V., and Riegler, P., *Centralbl. Bakt.* (etc.), 1903, Abt. II, Orig., **33**, 438.

8 Pliska, F., *Centralbl. Bakt.* (etc.), 1938-39, Abt. I, Orig., **143**, 262.

9 Gee, L. L., and Smith, W. W., *J. Bact.*, 1941, **41**, in press.

Twenty-seven adult carp *(Cyprinus carpio)* were placed in water having a temperature varying from 20°C to 23°C by Gee and Smith. The fish were given semi-weekly intraperitoneal injections of 1 ml of *Bact. salmonicida* vaccine (5 billion bacteria.). After 7 weeks these fish were removed to running water, the temperature of which was constant at ca. 10°C. Twenty-six of these carp developed agglutinins. Furthermore, 21 carp were tested more than once and, with one exception, each had an increase in titer as the injections continued. This increase also took place after the animals were removed to 10°C water. Scores of control-carp were shown to have no natural agglutinins for *Bact. salmonicida.* Incidentally, there was slight indication that these animals were immune to trout furunculosis, the bacteremia caused by *Bact. salmonicida.*

In all these experiments reporting antibody production, the temperature of the animals has been either not recorded or has been near room temperature. It will be noted that some of the workers report that no antibodies are formed at temperatures as low as 10°C, the temperature of good trout water.

To determine if antibodies can be formed by carp and trout at temperatures lower than those reported, 74 animals were subjected to strenuous courses of vaccination. The bacterial vaccine consisted of a physiological saline solution suspension of 24 to 72 hour carp-infusion, peptone, agar culture of *Bact. salmonicida,* grown at 20°C and killed by heating at 60°C for 5 minutes. Each milliliter contained approximately 5 billion bacteria.

Five adult carp were placed in each of 3 tanks of 10°C running water. Five were injected bi-weekly, 5 weekly, and 5 semi-weekly, though all the fish received the same amount of vaccine, 2 ml, within a 2-week period. Most of the fish died within 12 weeks, because of confinement. Post mortem blood samples were titrated

TABLE I.

Titers of agglutinins of the blood of 74 fish held at 10°C and subjected to injections of suspensions of heat-killed *Bacterium salmonicida.* Each number in table represents the number of fish having the given titer at the time of death.

	Agglutinin titers							
Species	0	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
Carp	9*	2	1	2	—	—	1	—
Brown Trout	9*	8	2	5	1	2	1	—
Rainbow Trout	6*	4	9	9	1	—	1	1

*Nineteen of the 24 fish that died without showing agglutinin production died within 2 weeks of the start of the experiment.

for agglutinins. Of the 8 carp that survived until the tenth week, at death, 2 had titers of 1:20, 2 had titers of 1:40, one had a titer of 1:80, and one of 1:640 (see table). The remaining fish died during the first 2 weeks of the experiment and had no agglutinins. In earlier experiments it has been shown that carp in nature do not have these agglutinins.

The other fish, adult rainbow and brown trout, held in 10°C running water, were subjected to weekly injections of 1 ml of *Bact. salmonicida* vaccine containing approximately 5 billion heat-killed virulent bacteria. Because of the conditions under which they were held, few trout survived longer than 5 weeks, and most of them died during the second and third weeks. Post mortem blood samples taken after the first week showed agglutinins, the results being recorded in the table. It will be noted that a number of trout had no agglutinins; but most of these died early in the study. Three of 74 control animals were shown to have titers as high as 1:80. This does not necessarily indicate the presence of natural agglutinins. The presence of agglutinins for *Bact. salmonicida* in these trout, which had received no vaccine injections, is more readily explained by the fact that these animals have been exposed to trout furunculosis throughout most of their several years of life. It is very possible that the antibody has been developed in response to this natural exposure of the trout to the infection.

These experiments indicate that agglutinin production takes place at 10°C. It is believed that earlier investigators failed to detect this phenomenon because their animals were not given the antigen over a long enough period of time.

Experiments are now in progress with gold fish, which tolerate extremes of temperature, to determine the effect of temperature on the rate of agglutinin production.

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Comparison of Growth of *Trichophyton interdigitale* on Wool Fabric With and Without Additional Nutritive Media.

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In connection with the Department of Agriculture's investiga-