Toxic Substance in Blood of Scalded Crayfish (*Orconectes virilus* and *Procambarus clarkii*).* (27561)

D. H. GRIFFIN AND A. B. CHAET (Introduced by W. S. Root)
American University, Washington, D.C. and Marine Biological Laboratory, Woods Hole, Mass.

The toxic factor theory of heat death states that a toxic substance released at the area injured by heat circulates throughout the organism and kills the animal. Chaet has described such a toxic substance(s) in marine invertebrates(1,2,3,4). The present study was undertaken to determine whether the crayfish possesses the capacity to produce such a toxic substance when scalded.

Methods. Male and female crayfish of the *Orconectes* and *Procambarus* species weighing 5 to 50 g were used. When scalding was necessary, the crayfish were secured ventral side up to a plastic holder and the tail submerged to the base of the carapace in a fresh water constant temperature bath at 76°C ± 1° for 3 minutes. After such scalding, the vascular fluid was removed immediately from the donors by inserting a hypodermic needle between the base of the carapace and the first abdominal segment. Blood was slowly drawn into a 5 cc syringe and pooled with fluid taken from similarly treated animals. This fluid was centrifuged at 3,000 RCF for 20 minutes. A dose of .003 cc per gram of body weight of the supernatant fluid was injected into normal recipient crayfish by inserting the hypodermic needle into the claw musculature division between the chela and chaeliped. The recipient crayfish as well as the normal control crayfish were maintained in water at 12°C ± 3° for at least 7 days before terminating the experiment. Control fluid, injected into normal recipient crayfish, was drawn and prepared in the same way except for the fact that donor animals had not been previously scalded.

To determine whether the active components taken from scalded crayfish were heat labile and/or dialyzable, the experimental fluid (toxic fluid) as well as the control fluid was divided into 2 portions. One portion was autoclaved for 20 minutes at 15 lb pressure per square inch, whereas the second was kept untreated. The supernatant was removed from the autoclaved sample by centrifuging. Samples of 5-10 ml of the autoclaved fluid were then placed in commercial dialysis tubing (¾ inch diameter) and dialyzed against 150 ml of isotonic solutions for 12 hours at 5°C.

When recipient groups of experimental and control animals were to be injected, care was taken that each group contained animals of approximately the same weight.

Results. When the vascular fluid from scalded donor crayfish was removed and injected into normal recipient crayfish, 91.1% of the recipient animals died in an average of 30.6 ± 11.4 hours following injection (Table I). On the other hand, only 24.1% of the normal crayfish died following injection of blood serum or whole blood from normal crayfish. Average time of death was 40.8 ± 15.7 hours.

When control and experimental solutions were first autoclaved, centrifuged, dialyzed, and then injected into recipient animals, 35.0% of the controls died within 39.2 (± 23.4) hours, whereas 69.0% of the animals injected with fluid of scalded crayfish died within 36.7 (± 20.3) hours (Table II). The Chi Square Test gives the frequency for these independent control and experimental groups as 0.01.

TABLE I. Percentage of Deaths and Average Time of Death (± S.D.) of Normal Recipient Crayfish Injected with Blood from Normal (Control) Crayfish or from Scalded (Experimental) Crayfish. (Tails of crayfish scalded at 76°C. for 1½ min.)

	Control	Experimental
	Vascular fluid from normal crayfish (76 injected)	Vascular fluid from scalded crayfish (76 injected)
% deaths	24.1%	91.1%
Avg death time (± S.D.)	40.8 ± 15.7 hr	30.6 ± 11.4 hr

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TABLE II. Percentage of Deaths and Average Survival Time (Hours ± S.D.) of Normal Crayfish Injected with Autoclaved-dialyzed Control Solutions or Autoclaved-dialyzed Toxic Substance.

	Control		Toxin	
	Autoclaved-dialyzed (22)	Untreated (16)	Autoclaved-dialyzed (21)	Untreated (15)
% deaths	35.0	12.5	69.0	100.0
Survival time in hr (± S.D.)	39.2 ± 23.8	38.7 ± 12.4	36.7 ± 20.3	24.4 ± 13.1

Discussion. These experiments show that a toxic factor (or factors) was present in the blood of the scalded crayfish. Normal crayfish injected with blood of normal animals lived longer than those injected with blood of scalded crayfish.

Summary. Crayfish (*Orconectes virilus* and *Procambarus clarkii*) were scalded at 76°C for one to 3 minutes. A toxin was found in the blood of scalded crayfish, which, upon injection in normal crayfish, killed 91.1% of the recipients. The toxin taken from a scalded crayfish is a heat stable, nondialyzable substance.

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Amino Acid Analyses of Rheumatoid Factors and Normal Gammaglobulins.* (27562)

RALPH HEIMER, KENNETH R. WOODS, AND RALPH L. ENGLE, JR.
Hospital for Special Surgery and Department of Medicine, Cornell University Medical College, New York City

Recently available methods for preparing highly purified rheumatoid factors(1,2) facilitated separation of rheumatoid factors of different serological behavior in individual sera. These factors were found essentially to be of two types. One reacted with indicator particles coated with human as well as rabbit gammaglobulin, and the other reacted with indicator particles coated with human but not with rabbit gammaglobulin. In the ultracentrifuge both types were mainly 19.5 S, although moieties sedimenting at slightly lower as well as somewhat higher sedimentation constants were also encountered.

To obtain further evidence for differences in rheumatoid factors, we compared the amino acid composition of rheumatoid factor preparations of both serological types and again compared it to that of normal 7 S gammaglobulin as well as highly purified 19 S gammaglobulin. The amino acid compositions of normal 19 S gammaglobulin and rheumatoid factors have not been reported previously.

Materials and methods. Preparation of 19.5 S rheumatoid factors. Three samples of serum, approximately 80 to 100 ml each, obtained from patients with rheumatoid arthritis, and stored in a frozen state for varying periods of time, were used in the preparation of rheumatoid factors A, B, and C. The sera were absorbed with sheep erythrocyte stroma previously exposed to homologous rabbit antiserum. The rheumatoid factors so absorbed onto the immune complex were eluted from it by suspension in pH 5.5 phosphate buffer (0.15 M). The eluate was further purified by density gradient ultracentri-

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