later and gradually increased in size. The clumps were usually irregular in shape and 20 to 50 μ in diameter.

In electron micrographs of the hemolymph of SR.B-3 and Neb.SR females and of the mixed hemolymphs of the two strains, typical SR spirochetes with knoblike granules along the filaments were found in the hemolymph of SR.B-3 and Neb.SR females (Fig. 3, *A* and *B*). In the mixed hemolymphs, however, clumps appeared approximately 1 hour after the hemolymphs were mixed. Individual spirochetes and the knoblike bodies could be distinguished within the clumps after 1 hour (Fig. 3*C*), but after 6 hours neither individual spirochetes nor knoblike bodies could be seen clearly (Fig. 3*D*). When hemolymphs of different SR.B-3 females, or of Neb.SR females, were combined, clumps did not form. Thus a means is provided whereby populations of SR.B-3 and Neb.SR spirochetes can be distinguished from one another by the formation of clumps in vivo and in vitro.

As shown in Figs. 1 and 2, the proportion of males in progenies of SR females carrying both *D. willistoni* and *D. nebulosa* spirochetes increased in the third or fourth broods but gradually decreased in later broods, and the typical SR condition was restored. To clarify the mechanism of recovery of the SR condition in these later broods, hemolymph from doubly infected females was taken on different days following the initial injection and mixed with hemolymph containing SR.B-3 or Neb.SR spirochetes as testers. The results are summarized in Table 2 from which it is evident that the two kinds of spirochetes brought together artificially interfere with each other. Experiment 1 suggests that the SR.B-3 spirochete is the weaker of the two and was ultimately replaced in this experiment by the SR.Neb spirochete. Thus, when Neb.SR spirochetes are injected into an OR.SR.B-3 host, Neb.SR spirochetes interfere and finally replace SR.B-3 spirochetes in the host females. Similarly, when SR.B-3 spirochetes are introduced into an OR.Neb.SR host (experiment 2) there is interference, and SR.B-3 spirochetes are eliminated.

In an attempt to gain a better understanding of the mechanism of interference between SR.B-3 and Neb.SR spirochetes, approximately three hundred OR.Neb.SR females were homogenized in 0.24*M* sucrose and the homogenate then centrifuged at 30,000 rev/min for 1 hour. The supernatant, which was almost free of spirochetes, was injected into OR.SR.B-3 females and the proportion of males and females in successive broods from injected flies was tabulated. The proportion of females was 100 percent 6 to 9 days after injection but gradually decreased to approximately 50 percent in the fourth and subsequent broods; the SR condition was never restored. Moreover, no spirochetes were found in the hemolymph of injected OR.SR.B-3 females in the later periods following the injection. These results indicate that the supernatant contains some substance which can inactivate or destroy SR.B-3 spirochetes. The nature of this substance is not yet known.

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Activation Heat in Muscle: Method for Determination

Abstract. *By varying the interval between two stimuli it is possible to measure the activation heat in a skeletal muscle twitch. The method depends upon finding a range of stimulus intervals where complete mechanical fusion exists and where there is a plateau in the heat production. At 0°C, and when muscles are at normal body length, activation heat represents about 40 percent of the heat in an isometric twitch.*

The heat liberated in an isometric muscle twitch consists of the activation heat and the heat effects accompanying the internal shortening of the contractile element against the series elastic component. In the investigation described here, we measured the heat liberated in two twitches and studied the effect of varying the interval between the two stimuli. The results have shown a simple way of determining the activation heat separately and directly. The method depends on finding a stimulus interval such that the second contraction involves a complete activation, while the internal shortening events from the first stimulus still persist.

The experiments were conducted at 0°C in Ringer solution, *p*H 7.2, on the sartorius muscle of *Rana pipiens*. The thermopile itself was made up of 44 silver constantan junctions of which 29 were active and 15 were protective. The pile was 40 μ thick and its sensitivity was 732 μv/°C. The experimental records were corrected for heat loss and the total heat was generally read 3 seconds after the last stimulus.

The intervals between stimuli ranged from 10 msec to 2 seconds, and the total heat production in response to the two stimuli was plotted against the stimulus interval as shown in Fig. 1*A*. The dotted horizontal base line in Fig. 1*A* is the amount of heat produced in one single twitch. Heat production above this line rises in two stages separated either by a marked inflection point or by a small plateau. This inflection point or plateau occurred at a time when mechanical fusion was starting to occur as the stimulus interval was diminished. In fact, at stimulus intervals below 200 msec these two stimuli could represent the early part of an isometric tetanus. Hill (*1*) has made the suggestion that activation heats in a tetanus are summed to produce "maintenance heat" described by

him (*2*). It seems likely, therefore, that the first stage of Fig. 1*A*, shown on an expanded time scale in Fig. 1*B*, represents the activation heat in a twitch.

The second stage of heat production shown in Fig. 1*A* occurs when the intervals between stimuli are greater than those necessary to obtain a smooth mechanical fusion. Indeed, at stimulus intervals of 1.2 seconds or more, there is no tension overlap at all. We interpret this second stage of heat production as being caused by three effects: (i) elastic heat released during relaxation, (ii) internal shortening heat, and (iii) positive feedback heat (*3*). These three effects must be small while there is complete mechanical fusion, as the additional tension above the normal twitch tension is about 10 percent. However, as soon as tension due to the first stimulus starts to decline before the second stimulus arrives, these effects become progressively greater as the stimulus interval is increased until they reach a maximum at a stimulus interval close to 1.2 seconds.

This method of determining the total activation heat is contingent upon finding a time interval in which the rate of production of activation heat has declined sufficiently to allow complete reactivation of the heat cycle, yet in which the mechanical effects of the first cycle persist fully. The occurrence of a plateau or a marked inflection in the heat records, while the tension record was fused, strongly suggests that this condition is fulfilled, but this point requires further study. The height of the first stage in Fig. 1*A*, measured from the dotted line to the plateau, should represent the total activation heat in a single twitch. In those curves with an inflection rather than a plateau, an unbiased estimate of the activation heat could be obtained by subtracting the base line heat from the heat produced at the fusion frequency as determined from the mechanical records.

In a series of experiments the value of activation heat as determined in these ways, comprised 0.39 ± 0.3 (standard error) of the total heat in an isometric twitch. This compares favorably with the values reported by Hill, who generally states that activation heat represents one half to one third of the isometric twitch heat.

It should be mentioned that the experimental procedure described in this paper is the same as that used by Hartree and Hill (*4*). At that time instrumentation was such that the time course of heat production in a twitch, and hence the components of it, were unknown. When allowance is made for the fact that these authors were working at 10°C, there is a close similarity between Fig. 6*b* of their paper and Fig. 1*A* of this paper.

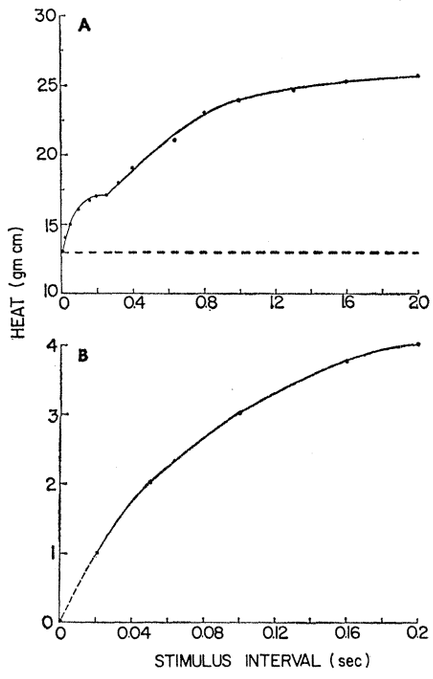

Fig. 1. (*A*) The total heat production in response to two stimuli in a pair of frog's sartorii, weight 118 mg and length 3.1 cm. The abscissa is the interval between the two stimuli and the dotted line represents the heat produced in a single twitch. (*B*) The first 0.2 second of (*A*) shown on an expanded scale. The dotted line indicates that intervals of less than 20 msec between stimuli were not used.

In 1949, Hill (*1*) obtained the time course of activation in a twitch. He used two methods: in one, he shortened a muscle on the thermopile until it could not shorten any further and then he stimulated it and obtained the heat of activation (Hill also obtained activation heat indirectly by subtracting the shortening heat from the total heat in an isotonic twitch). In the second method, the activation heat could be found by stretching the muscle, about 1.5 times its normal length, until the muscle did not develop tension when stimulated. The heat produced then, when the muscle is stimulated, is the activation heat (*5*). To verify our results, we also used this method and good agreement was obtained. We also found that at several lengths ranging from l_o to 4/3 l_o (where l_o is the normal body length) the magnitude of activation heat was unchanged, as Hill has already shown.

The method described in this paper has the advantage that the activation heat can be determined directly on a muscle maintained at l_o or any other length. Thus, the dependence upon length or tension can be studied experimentally without assumptions and without the disadvantages attributed by Hill himself (*1*) to two of his methods. These drawbacks are: when the muscle is allowed to shorten as far as possible, it is still possible that some concealed internal shortening occurs; at maximum extension, the muscle is easily damaged; at intermediate lengths, the estimation of the internal work is difficult and uncertain. Our method has a possible error related to the increase in tension consequent upon the two stimuli. This error will be examined further, but appears to be less than 10 percent. Within the limits of this uncertainty, we have obtained the indicated amount of activation heat, and we have found it to be independent of the length or tension of the muscle.

This independence of the activation heat on the degree of overlapping of the sliding filaments makes it unlikely that it is directly involved in the interaction between myosin and actin components. Instead, the activation heat might tentatively be ascribed to either a reaction of all the myosin molecules so as to bind adenosine triphosphate (*6*), or to the thermal effects of the release and movement of calcium ions (*7*).

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