# THERMAL PROPERTIES OF THE COLLAGEN OF JELLYFISH (*AURELIA COERULEA*) AND THEIR RELATION TO ITS THERMAL BEHAVIOUR*

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It has been reported elsewhere (Rigby 1968*a*, 1968*b*) that for a number of poikilotherms sudden changes occur in physiological behaviour at temperatures which are the same as the melting temperature of their molecular collagen. A more general statement, which includes the above observations, is that the upper limit of the environmental temperature for an animal corresponds with the melting temperature of its molecular collagen. A number of workers have contributed to this idea and details may be found in Rigby (1968*a*). It is not suggested that collagen is uniquely involved in these events, although this may be so. No doubt other body proteins are altered at the same time. The useful point is that collagen is one of the few easily prepared body proteins with a characteristic melting point, and as such can serve as an indicator in studies concerned with the temperature relation of poikilotherms. For example, animals, which can be adapted to reproduce at higher or lower temperatures than is usual for them, may produce a collagen which shows a parallel alteration in its melting temperature. Such an experiment would afford a test of the general assumption that the constitution and properties of a protein are determined only by genetic coding (see also Rudall 1968).

It has been known for many years that Medusae (jellyfish) pulsate at a rate which varies with temperature (Thill 1938) so this seemed to be an appropriate animal with which to further test the idea that the upper limit of the environmental temperature for an animal corresponds with the melting temperature of its molecular collagen, provided collagen could be isolated from the animal.

Although there has been some controversy about the presence of collagen in Medusae, we have isolated from a species found in Sydney waters, *Aurelia coerulea*, a tissue which has a number of properties characteristic of a collagen (see below). We therefore performed tests on the pulsation rates of jellyfish as a function of temperature and determined the melting point of its molecular collagen.

To examine the rate of pulsation, a jellyfish was placed in sea-water in a temperature-controlled bath. The temperature was slowly reduced to 10°C and the time measured for a regular sequence of pulsations. The rate of pulsation at any temperature was found to be independent of the number of pulsations observed in a sequence. Over the range 18–27°C, regular sequences of 100 or more pulsations were usual, but outside this range the number in a sequence was much reduced. At 15°C the jellyfish was sluggish, i.e. the rest period between pulsations was long, and at 10°C there was little movement at all. Figure 1 shows how the number of pulsations per

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minute depends upon temperature. They are mean values calculated from sequences of pulsations, which were erratic in number outside the range 18–27°C as mentioned above. The top curve is the first run and the lower curve the second run, obtained one day later after resting the jellyfish at 20°C. Over the temperature range 15–25°C the pulsation varies directly with temperature as others have found (Thill 1938); further, this range agrees quite well with the mean limits of surface waters around Sydney of 17–23°C (Dakin 1953). The lower curve suggests that the animal has been adversely affected by being heated beyond the linear region of the pulse rate–temperature curve.

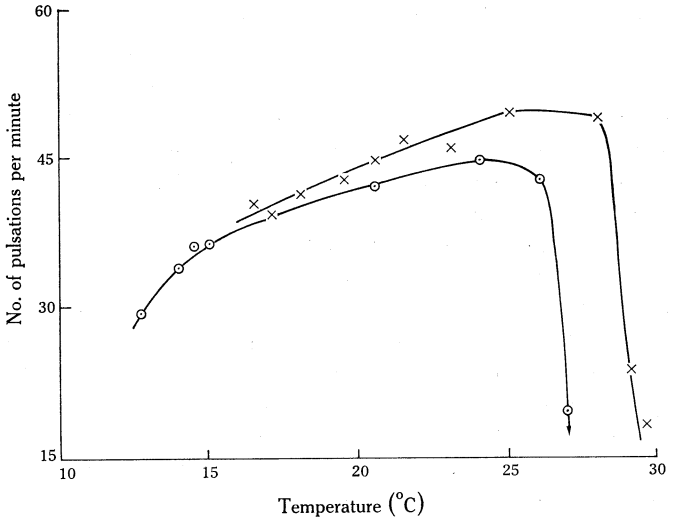


Fig. 1.—Pulsation rate of the bell of the jellyfish *A. coerulea* in sea-water at various temperatures. × First experiment. ⊙ Same specimen after resting overnight at 20°C.

We then isolated collagenous tissue from the animal. A jellyfish about 10 cm in diameter was killed by freezing, and after thawing was dehydrated as much as possible by successive soakings in acetone. Material appeared to be dissolved and leached away and eventually a thin skin remained. After washing in distilled water and drying in air this skin weighed 0·43 g from an animal of original fresh weight 71 g. This tissue (stretched and dried) gave a high-angle X-ray diagram, characteristic of collagen, i.e. strong meridional or near-meridional arcs at approximately 2·9, 4, and 9·5 Å together with an equatorial spot at approximately 12 Å and a diffuse spot about 4·5 Å. When wet out in sea-water it had a shrinkage temperature, $T_S$, of 52–54°C. Our estimate of the melting temperature of the molecular collagen, $T_D$, was 28–30°C. $T_S$ and $T_D$ determinations were made by observation of the force developed in normal and swollen samples as they contract during the melting process (Rigby 1961, 1967*a*).

The dried skin of *A. coerulea* was prepared for chemical analysis by boiling for 30 min in 0·1N HCl and centrifuging. The resulting clear solution was vacuum-dried and then hydrolysed for 24 hr in boiling 6N HCl under reflux. The acid was removed

under vacuum and the residue redissolved in water and filtered. The composition of the filtrate was determined using a Beckman Spinco amino acid analyser.

The amino acid composition is shown in the following tabulation, in which results are expressed as residues per 1000 residues:

| | | | | | |
|---|---|---|---|---|---|
| Ala | 87 | Tyr | 5 | Asp | 88 |
| Gly | 321 | Ser | 44 | Glu | 93 |
| Val | 30 | Thr | 31 | Hyp | 42 |
| Leu | 33 | Met | 6 | Hyl | 25 |
| Ile | 24 | Arg | 48 | Pro+Hyp | 120 |
| Pro | 78 | His | 2 | Pro : Hyp | 1·86 |
| Phe | 8 | Lys | 35 | | |

This composition fits the usual pattern for collagens in respect of glycine (1 out of 3 residues), no cystine, low amounts of phenylalanine and tyrosine; no tryptophan. However, it is low in total imino acid residues (120), and this amount is not sufficient to correlate with either $T_S$ or $T_D$ in the well-known plot between these and total imino acid (Rigby 1968*a*). On the other hand the number of serine residues (44) fits a correlation between $T_D$ and serine shown to occur for a wide range of collagens from both vertebrate and invertebrate animals (Rigby 1967*b*).

To summarize, the jellyfish *A. coerulea* contains collagen or a collagen-like material which melts (at the molecular level) at a temperature which is close to the upper limit temperature of its environment (27°C). Its physiological behaviour as measured by its bell pulsation rate changes abruptly in the vicinity of this same temperature. These results augment earlier work where it was shown that collagen has thermal properties that reflect properties of the whole animal (Rigby 1968*a*, 1968*b*).

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