



Invertebrate Tissue Culture Methods (Springer Lab Manuals)

Jun Mitsuhashi

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I started insect cell culture work in 1962, when T. D. C. Grace reported the first establishment of invertebrate continuous cell lines. He obtained growing cells from pupal ovaries of the emperor gum moth, *Antheraea euclipti*. At that time, I was trying to obtain growing cells from leafhoppers. Grace's method could not be applied directly to my culture because of the differences in species, the size of the insects, and the tissue to be cultured. The vertebrate tissue culture methods gave me some ideas for preparing cultures from leafhoppers, but those could not be used directly either. There were no textbooks and no manuals for invertebrate tissue culture, so I had to develop a method by myself. First, I considered what type and what size of vessels are suitable for insect tissue culture. Also, I had to look for suitable materials to construct the culture vessels. Second, I had to examine various culture media, especially growth-promoting substances, such as sera. Then I had to improve culture media by trial and error. The procedure to set up a primary culture was also a problem. How could I sterilize materials? How could I remove tissues from a tiny insect? How many tissues should I pool in order to set up one culture? I had to find out the answers. Naturally, it took a lot of time.

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