My lifetime memory in Naples

Masatoshi MITA

It happened after about one month after I started living in Naples. I was in the arrival lobby for international flights at Fiumicino International Airport in Rome to meet my family from Japan. The arrival information board showed that Alitalia Airlines flight AZ 1781 from Tokyo (Narita) via Milano was delayed by two hours. When I felt like giving up on meeting them soon, I heard my dear old Japanese from one of the several telephone booths at the far right of the lobby. The voice belonged to a middle-aged lady. ".....The flight was delayed for two hours? I came here on time because this flight arrives early but it has never been delayed before. You called me at the office! You don't have to call me at the office. Because, I don't always stop by the office. Why don't you call me at my house?....." It seemed that she was not very calm. This lady was Miyoko Ito and a tour conductor of a travel company. She came to the airport to meet Japanese travelers who would come all the way from Japan to the country of sightseeing, Italy. Ms. Ito lives in Italy and has two children and an Italian husband. If Alitalia Airlines flight AZ 1781 had not been delayed, I would not have had a conversation like this with Ms. Ito. This might have been a once in a lifetime meeting. I felt that meeting Japanese in a lonely foreign country would become a lifetime memory.

Alitalia Airlines flight AZ 1781 that was scheduled to arrive at seven twenty p.m. arrived in Rome after ten p.m. after a three-hour delay. As a matter of course, my family missed a connecting flight to Naples and in the situation that we might have been left behind in the airport. However, since I had gotten used to the Italian way of living, I bargained with Alitalia Airlines and got a night's stay at a hotel in Rome and round-trip taxi fare from the airport to the hotel for my family.

Our house in Naples was on Vomero hill in an exclusive residential quarter. On Vomero hill, but the apartment was close to the junction of the express way (Tangenziale) that went halfway around Naples City and was pretty far from the downtown Naples. Because of that, we could rent a luxurious apartment at a low rent. Our apartment was on the eighth floor of a modern ten-story building. It was fully equipped with Italian furniture in two splendid bedrooms, a TV, a phone, a washing machine, and a fridge, and even an oven and a dishwasher in the kitchen. It was a beautiful lady (Napolitana), Adriana Mauriellu who accommodated us with such a life in Naples. The owner of this apartment lived on the first floor with her mother and we made all sorts of trouble for her.

There were three ways to commute to the Zoological Station. The first way was to commute there by cable car on the Chiaia line. This way, it was about ten minutes on the car but it took forty minutes for a one way trip due to the time lost in walking from one station to the other. The second way was to take bus No.128 that connected City Hall (Municipio) and Vomero. The walking distance was shortened this way, but possible strikes (sciopero) and an unreliable time schedule troubled me. The last way was to commute there on foot. This was well suited to those who ate too much Italian food and those who were short of exercise. Viewing Naples Bay, Mount Vesuvius, and Sorrento peninsula while going down the stairs was great. Going there was O.K., but I do not recommend doing this on the way home because it was a steep climb all the way.

The theme of my study at the Naples Zoological Station was "energy metabolism in sea urchin spermatozoa belonging to the order Arbacioida". Actually, it took a long time to come up with this theme. In Japan, as far as equipment was concerned, there was only a little research that had to be done in Italy, even though marine animals were the subjects of study. And study on the energy metabolism of sea urchin spermatozoa, that was the theme of my study, was already being studied at the Naples Zoological Station by Dr. Hideo Mohri, who is now

the President of Okazaki National Reserch Institute. In this situation, I considered what theme would be proper for research that had to be done in Italy. Let us focus on the species of sea urchins that appear in research papers. Sea urchins that are used frequently in Japan are Hemicentrotus pulcherrimus, Anthocidaris crassispina, and Pseudocentrotus depressus. They belong to the order Echinoida under taxonomic classification. In America, Strongylocentrotus purpuratus, Lytechinus pictus, and Arbacia punctulata are used, and in Italy, Paracentrotus lividus and Arbacia lixula are used for research. In short, the order Arbacioida is used for research in addition to the order Echinoida. However, it is hard to obtain Arbacia in Japan, and Glyptocidaris crenularis of the suborder Phymosomatoida is used to a small extent. It turned out fortunately for me. I had just found that the energy metabolism of Glyptocidaris spermatozoa is different from that of sea urchins of the order Echinoida. In short, sea urchin spermatozoa that belong to the order Echinoida, such as Hemicentrotus, obtain ATP for flagellar movement through oxidation of phospholipid, particularly phosphatidylcholine (PC)¹⁰. On the other hand, Glyptocidaris spermatozoa use triglyceride as a substrate for energy metabolism²⁰. In other words, it is possible that sea urchin spermatozoa of the order Arbaci oida utilize triglyceride for producing ATP for flagellar movement as Glyptocidaris spermatozoa do. Thus, it became the theme of my study at the Naples Zoological Station in Italy.

Since materials for this research were Paracentrotus lividus and Arbacia lixula which were of Mediterranean origin, my time of stay was limited to their breeding period and determined as 128 days starting from November 30th, 1992 to April 10th, 1993. The person who supported my research at the Naples Zoological Station was Dr. Rosaria De Santis of the Cell Developmental Biology Laboratory. By the way, I had heard a lot about research in Naples from my senior associates and acquaintances, but I think that I actually managed to conduct experiments fairly well. In experiments, I planned in Arbacia and Paracentrotus spermatozoa to analyze lipid quantities, to measure oxygen consumption rate and to conduct morphological observations with an electron microscope. For measuring the oxygen consumption, I could use an oxigraph of Dr. Palumbo of the Naples Zoological Station. Samples for morphological study were fixed and observation was planned to conduct with electron microscopy in Japan. I also planned to measure biochemical study, such as enzyme assay, in Japan after they were lyophilized. Furthermore, I brought necessary reagents and thin layer chromatography (TLC) plates for analyzing lipid from Japan. So I flattered myself that trouble could never happen. In fact, I can not say there were not any problems. This was research at an unfamiliar place. An unexpected situation occurred when I was about to measure determinate quantity of lipid due to lack of sufficient information on a measuring instrument. Basically, determinate quantity of lipid is expanded on a TLC plate, spotted, and then measured by a densitometer. However, a densitometer I found at the Naples Zoological Station was old, and it was almost unusable for measuring determinate quantity with a TLC plate. I asked Rosaria to check and see if the laboratories of the universities in Naples had a densitometer I needed, but she could not find one. Reluctantly, I decided to send TLC plates with expanded lipid on them to Japan and to have someone to measure them for me. But, anyone who understood the circumstances of postal services in Italy would hesitate to send several plates that were memorized as precious data to Japan. Also express package services were not reliable at that time, either. Yes, there is the best way. If you visit the Naples Zoological Station in the future and have occasion to send samples to Japan urgently, use the CAI Post (business mail). According to my actual experience, a package will be delivered to Japan by the weekend if you send it off on the previous Monday and you can receive the analyzed data of lipid by fax from Japan on the following week.

I acquired following results from my study in Naples³⁾. The substrate that is utilized for energy metabolism of *Paracentrotus* spermatozoa as well as the Japanese sea urchins of the order Echinoida is phospholipid, particularly PC. However, in the case of *Arbacia* spermatozoa of the order Arbacioida, triglyceride is expectedly utilized for energy metabolism⁴⁾. From morphological observation, the lipid bodies were found the intramembrane space of mitochondria of *Paracentrotus* spermatozoa and, on the other hand, lipid globules were contained in the midpieces of *Arbacia* spermatozoa. It is believed that both play a role to preserve phospholipid or triglyceride that are utilized for energy metabolism⁵⁾. In addition, both *Paracentrotus* and *Arbacia* utilized creatine phosphate as

phosphagen. The quantity of creatine phosphate as well as ATP decreased noticeably with the initiation of flagellar movement⁶⁾. These results and other results from experiments conducted later proved that sorts of energy metabolism of sea urchin spermatozoa are different from those classified in taxonomic classification. More specifically, the substrate that is utilized for energy metabolism by sea urchins of the orders Diadematoida, Arbacioida, and Clypeasteroida is triglyceride. On the other hand, phospholipid, which was considered to be utilized according to the accepted theory, is only utilized by sea urchins of the order Echinoida for energy metabolism⁷⁾.

Finally, I would like to present part of a letter I received after I returned from Italy from Ms. Ito who I met at Fiumicino international airport, and to show my appreciation to the Italian people. I could not write it down due to limited space, but I visited Professor G. Giudice of Palermo University and Professor B. Baccetti of Siena University and gave seminars on energy metabolism of sea urchin spermatozoa. I could also hold meaningful debates with many researchers, students, and graduate students on their subjects of research. I deeply and sincerely appreciate what Dr. Rosaria De Santis and the staffs at the Naples Zoological Station have done for me.

"Italian people live smiling heroically wishing that Italy could be theirs alone. Yet, they tolerate foreign people, hide their annoyance and frustration, and..." (quoted from a letter from Ms. Ito, who lives in Italy.)

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