Rearing of the Silkworm Under Aseptic Condition

By TOSHIO ITO

Head, Silkworm Physiology Division, Sericultural Experiment Station

Rearing of larvae of the silkworm, *Bombyx mori* L., entirely on an artificial diet has been achieved nine years ago (Fukuda *et al.*, 1960; Ito and Tanaka, 1960; Yoshida *et al.*, 1960). Subsequently, rearing under the aseptic condition has been reported, and the aseptically-grown larvae have made cocoons in the Erlenmeyer flasks (Ito and Horie, 1962; Ito and Tanaka, 1962).

At that time the dietary efficiency of the artificial diet was poor. Since then, many of information have been accumulating on the nutritional requirements of this insect (Ito, 1967), and the dietary efficiency has been increased by improving the composition of the diet.

The method of aseptic rearing of the silkworm was first applied for the nutritional studies. Aseptic larvae were successfully used for the determination of essential B vitamins (Horie and Ito, 1963).

Recently, the aseptic method has been highly appreciated in the field of silkworm pathology (Ito *et al.*, 1968). Most recently, the aseptic method has been used in a mass-culture of the silkworm, since there is possibly no chance of bacterial contamination of the diet and of bacterial infection of the larva.

In order to get aseptic larvae, the following procedures should be carried out carefully: (1) Sterilization of rearing apparatus, (2) formulation and preparation of the diet, (3) sterilization of the diet, (4) disinfection of surface of silkworm eggs, (5) putting the eggs into rearing apparatus, and (6) rearing.

Preparation of the diet

Since the first success of rearing of silkworm larvae in 1960, many compositions have been reported for this plant-feeding insect. At present it is entirely possible to maintain almost normal growth and to yield almost normal size of cocoon with artificial diet, which contains mulberry leaf powder as one of the ingredients.

For example, the composition of the diet, shown in Table 1, contains approximately fifty per cent of dried mulberry leaf powder. The growth on this diet is almost normal. Furthermore, so-called semi-synthetic diet, which does not contain any leaf powder, is available. However, on this diet the growth is somewhat reduced. Semi-synthetic diet or synthetic diet has been used for the basic nutritional studies.

The components of the diet, previously ground and mixed thoroughly, are transferred with water containing vitamins and antiseptic into a Petri dish, which is then covered with a lid, and the dish is heated to approximately 95°C in a water bath for 10 minutes, then the diet is allowed to cool to gel. The diet is cut into slices, and is placed into flasks or test-tubes, which have previously been heated to approximately 200°C.

Flasks or test-tubes are plugged with cotton.

Aseptic culture

In general, no elaborate apparatus is required in aseptic culture of the silkworm, and Erlenmeyer flasks or big test-tubes are re-

Table 1. Composition of artificial diet

Substance	Dry diet (g)
Dried mulberry leaf powder	50
Potato starch	20
Soybean meal, defatted	20
Mineral	1
Ascorbic acid	2
Citric acid	0.5
Cellulose powder	8
Agar	12
Total	113.5
Vitamin B mixture	Added
Antiseptic	Added
Dist. water	300 ml

* Vitamins μg per g of the dry diet: biotin, 2: choline chloride, 1,500; folic acid, 2; inositol, 2,000; niacin 100; Ca-pantothenate, 150; pyridoxine HCl, 30; riboflavin, 20; and thiamine-HCl, 20.

commended for a small-scale culture. The cotton-plugged flasks or test-tubes containing sliced media are autoclaved for 20 minutes at 10 lb. pressure.

The surface of silkworm eggs is disinfected by immersion in 2 per cent formalin for 15 minutes, then rinsed in sterile water. Previously, 0.1 per cent $HgCl_2$ has been used, but some toxic effect has been observed, perhaps because of possible trace of $HgCl_2$ remaining on egg surface, which in turn is swallowed by the larva on hatching. Thus, the use of $HgCl_2$ is not recommended. Usually, the eggs close to hatch, within one or two days before hatching, are placed carefully on sliced media in sterile flasks or test-tubes, which are then kept at 25°C.

When the larvae hatch, they start feeding. Since the silkworm grows and increases its body size quickly, enough room and diet must be supplied beforehand. When necessary, diet must be supplemented aseptically. Full-grown larvae make cocoons in the flasks (Fig. 1), and it is also possible to obtain aseptic pupae and moths.

A vinyl-isolator (Fig. 2), which has been used for aseptic rearing of higher animals, can be used for a mass-culture of the silkworm (Ito *et al.*, 1967; Matsuda and Matsuura, 1967).

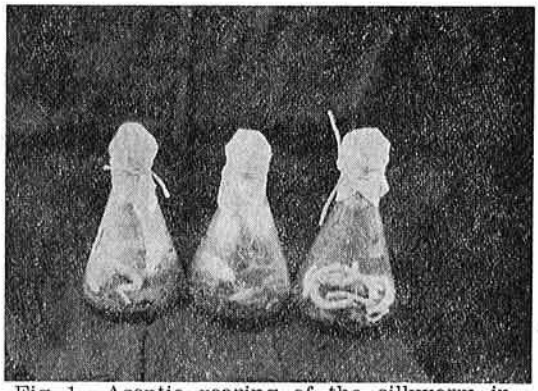

Fig. 1. Aseptic rearing of the silkworm in Erlenmeyer flasks

Fig. 2. Vinyl-isolator used for aseptic mass-culture of the silkworm

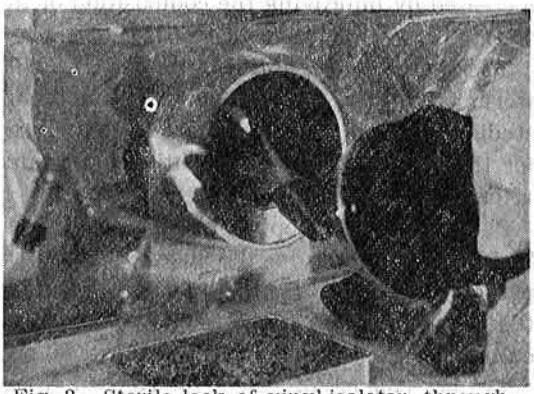

Fig. 3. Sterile lock of vinyl-isolator, through which the diet or other necessary apparatus are brought into the isolator aseptically

Peracetic acid is sprayed for the disinfection of the inside of the vinyl-isolator. With this isolator, it is possible to rear several thousands of larvae. The supplementing diets are brought into the isolator aseptically by means of a sterile-lock (Fig. 3).

Application of aseptic silkworm

In the aseptic rearing it is, of course, unnecessary to replace the diet daily, and there is no bacterial contamination. Thus, the aseptic larvae as well as aseptic methods have been used successfully in various fields of silkworm sciences.

1) As mentioned above, aseptic method has been used in the field of nutritional studies. This method will further enable to conduct more precise nutritional research.

2) The occurrence of D-serine in the blood of the silkworm and its biosynthesis have been reported (Srinivasan *et al.*, 1962, 1965). Subsequently, it was demonstrated that the large amount of D-serine had their origin in biosynthetic mechanisms, by using aseptic silkworm pupae (Ennor, A. H., personal communication). Furthermore, by means of adding or subtracting fatty acid(s) to synthetic diets, effect of dietary fatty acid on the composition of silkworm larvae has been investigated. In this study aseptic larvae have been used (Ito and Nakasone, 1969).

3) There are already more than several papers on the application of the aseptic method to silkworm pathology. For instance, as one example of the application of this method to pathology, polyhedrosis viruses were fed to aseptic larvae. No sign of the induction of polyhedrosis virus diseases was seen in aseptic larvae. When those larvae were cold treated, polyhedrosis occurred only in those which had been previously inoculated with the virus (Ito *et al.*, 1968)

4) In the laboratory, it is easy to supply the larvae of the silkworm for research program, if we keep larvae aseptically, any time when we need. Very recently, an aseptic laboratory for silkworm rearing has been built in order to consider the problems, which are not solved for rearing on artificial diets, perhaps done on a practical scale.

References

1) Fukuda, T., Suto, M., and Higuchi, Y.: Silkworm Raising on Artificial Food. J. Seric. Sci. Japan, 29: 1-3, 1960.

2) Horie, Y. and Ito, T.: Vitamin Requirements of the Silkworm. Nature, 197: 98-99, 1963.

3) Ito, T.: Nutritional Requirements of the Silkworm, *Bombyx mori* L. Proc. Japan Acad., 43: 57-61, 1967.

4) Ito, T. and Horie, Y.: Nutrition of the Silkworm, *Bombyx mori*. VII. An Aseptic Culture of Larvae on Semi-Synthetic Diets. J. Insect Physiol., 8: 569-578, 1962.

5) Ito, T., Horie, Y., and Kurata, K.: Rearing of the Silkworm, *Bombyx mori* L., by the Germfree Method and Its Application to Silkworm Pathology. In Adv. Germfree Research & Gnotobiol. (ed. by Miyakawa, M. and Luckey, T. D.), pp. 424-429. CRC Press, Ohio, 1968.

6) Ito, T., Horie, Y., and Watanabe, K.: Rearing of Germfree Silkworm by means of Vinyl-Isolator. J. Seric. Sci. Japan, 36: 49-412, 1967.

7) Ito, T. and Nakasone, S.: Effects of Dietary Fatty Acids on the Fatty Acid Composition of Larvae of the Silkworm, *Bombyx mori* L. Bull. Seric. Exp. Sta., 23. 295-311, 1969.

8) Ito, T. and Tanaka, M.: Rearing of the Silkworm on an Artificial Diet and the Segregation of Pentamolters. J. Seric. Sci. Japan, 29: 191-196, 1960.

9) Ito, T. and Tanaka, M.: Rearing of the Silkworm by Means of Aseptic Technique: J. Seric. Sci: Japan, 31: 7-10, 1962.

10) Matsuda, M. and Matsuura, Y.: The Germ-Free Reading of the Silkworm *(Bombyx mori* L.) in the Flexible Plastic Isolator. J. Seric. Sci. Japan, 36: 403-408, 1967.

11) Srinivasan, N. G., Corrigan, J. J., and Meister, A.: D-Serine in the Blood of the Silkworm *Bombyx mori.* and other Lepidoptera. J. Biol. Chem., 237: PC3844, 1962.

12) Srinivasan, N: G., Corrigan, J. J., and Meister, A.: Biosynthesis of D-Serine in the Silkworm, *Bombyx mori.* J. Biol. Chem.. 240: 796-800, 1965.

13) Yoshida, T., Matsuoka, M., and Kimura, K.: On the Rearing of Silkworm Larvae with an Artificial Diet Containing Dried Mulberry Leaf Powder as Its Basic Material. Bull. Seric. Exp. Sta., 15. 543,586, 1960.