

NOTES

Silkworms, A Tool for the Preparation of Chloroplasts

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The random separation of chloroplasts from the excreta of silkworms (*Eri* and *Bombyx mori*) was carried out at different stages. The same has been reported herewith.

When leaves are mixed mechanically for example by means of a blender or a pestle in a mortar, the resulting mesh can be separated by means of a centrifuge into fractions, some of which contain mostly or exclusively whole or broken chloroplasts. They are capable of giving Hill reaction. Arnon and coworkers at Berkley found in fact, that illuminated chloroplasts in presence of proper cofactors can transfer C^{14} carbon from labelled carbonate to organic compounds previously found to occur as intermediates in the reduction of carbon dioxide in living cells.

It is known that in castor leaves chloroplasts are abundant. Presence of free chloroplasts could be observed by Limaye in excreta of silkworms *Bombyx mori*¹. Green bodies were regularly found in cells lining the digestive tract of some marine Sarcoglossam which were identified as chloroplasts. The chloroplasts could still carry out photosynthesis even after several weeks and products of photosynthesis detected in various regions of the animal within the digestive cells, the chloroplasts retain their morphological integrity, the plastid envelope remains intact and the thylakoid membrane, starch grains etc. all appear identical to the plastids in seaweeds from which they were derived².

Time of chloroplasts exposure to the digestive enzymes is only 45 minutes³. The separation of chloroplasts from excreta (*Attacus ricini* and *Bombyx mori*) has been reported herewith. It is being attempted to study the separated chloroplasts from fresh excreta or fresh excreta as such, as possible means for semi artificial photosynthesis in this laboratory.

Bembyx mori silkworm—mulberry leaves. *Eri* silkworm—*Attacus ricini*—castor leaves. *Bombyx mori* obtained from Silk Centre of Khadi and Village Industry, Pune.

Eri silkworm—*Attacus ricini* obtained from Central Sericultural Board, Titabar, Assam. 0.25% hypochlorite solution. S. M. Buffer containing 50 mM tris-HCl, pH 8.0., 5 mM sucrose, 0.5 mM $MgCl_2$, 10 mM mercaptoethanol and cheese cloth. Sucrose gradient containing 10 ml of 60%.

10 ml of 45% and 5 ml of 25% sucrose. Hypochlorite, $MgCl_2$, sucrose, mercaptoethanol bairum sulphate—BDH.

Tris buffer—Tris (hydroxymethyl)—aminomethane, $C_4H_{11}NO_3$, mol. wt, 121.14, Loba Chemie, Bombay.

Bombyx mori larvae feed almost exclusively on mulberry leaves, while the larvae of the Eri type of silk producing insects (*Attacus ricini*) species are less specific in their nutritional requirements and feed on leaves of castor oil plant⁴.

Both of these silkworm's race were used as experimental material. The surfaces of the silkworm eggs were sterilized using 0.25% hypochlorite solution one or two days before hatching (Isamu Shimizu, 1981). Newly hatched larvae were reared on young, fresh and tender leaves of mulberry plantation and castor oil plant from the school campus, respectively. The number of leaves and feeding adjusted according to the instars of larvae. Excreta from these 2 lots of healthy silkworms at various instars were collected in different plastic bags after separating from leaves and debris. Such collected excreta was used for the experiment.

500 ml of SM buffer of pH 8 was prepared with 3.5 g tris (50 mM) HCl—BDH, pH 8 + 171.15 g sucrose (0.5 M) + 0.50825 g $MgCl_2$ (5 mM) + 0.39060 g mercaptoethanol (10 mM). 100 ml of SM buffer was mixed with 20 g excreta and kept on stirring for 4–5 hrs. Then it was filtered through 4 layers of cheese cloth and then centrifuged at 1000 g for 10 min. The pellet was resuspended in SM buffer (50 ml) and centrifuged again at 1000 g for 10 min. The chloroplast fraction again was suspended in SM Buffer 25 ml and 10 ml of the suspension was layered on top of sucrose gradient SM buffer. After centrifugation for 1 hr at 27000 rpm, chloroplasts were collected according to the method described by Tewari and Goel⁵.

The chloroplasts were observed under binocular microscope for their physical characters like shape, size and structure.

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