The medium we used is similar to that described for egg collection (Elgin & Miller 1978). Water (270 ml) and grape juice (Welch’s; 230 ml) were heated to boiling. After removing the liquid from heat, the following ingredients were added while stirring: Bacto-agar (Difco), 11 g; glucose, 30 g; sucrose, 10 g; fructose, 5 g; yeast (Fleischmann’s dry) 10 g. Yeast dissolved more readily if first mixed with approx. 25-50 ml of heated water-juice and then the paste is added to the mixture. The mixture was then autoclaved for 15 min. After cooling the mixture to approx. 60°C, the remaining ingredients were added while stirring; 1 M NaOH, 10 ml; propionic acid, 2 ml; phosphoric acid, 0.2 ml. The medium was poured into sterilized containers, covered with autoclaved tops, e.g., Kaputs (Carolina Biological Supplies), and stored at either room temperature or 4°C.

To produce vitamin A deprived flies, adult flies were introduced into vials containing the vitamin A deficient medium, the vials were cleared of the flies after 1-3 days, and the eggs laid on the deficient medium were allowed to develop into adults. Vitamin A deprivation can be maintained over many generations simply by repeating the above procedure at each generation, i.e., by allowing previously deprived adult flies to lay eggs on a fresh, deficient medium and letting the eggs develop into a new generation of deprived flies. We have maintained flies on this medium for over fifty generations with no obvious ill effects on flies.

Principal effect of vitamin A deprivation on flies is a drastic reduction in visual pigment concentration. There are at least two methods of determining the amount of visual pigment in intact, white-eyed flies: microspectrophotometry of the deep pseudopupil (Stavenga et al. 1973) and the electrophysiological measurement of the amplitude of the M-potential (Stephenson & Pak 1980). In addition, the amplitude and waveform of the prolonged depolarizing after potential (PDA) of the electroretinogram (ERG) give a semiquantitative measure of the amount of pigment (Stark & Zitzmann 1976; Larrivee et al. 1981), and the distinctness of the deep pseudopupil is in itself a qualitative measure of the amount of pigment. All these tests were carried out on the flies grown on the described medium. Results showed consistently that the visual pigment concentration of the deprived flies were less than a few % of undeprived flies.


Flies were reared in culture boxes (Fig. 1) on cornmeal-agar medium. About 30-40 mg of Drosophila embryos were uniformly spread with small volume of water on the food surface of each culture box. This assured optimal density of the developing larvae as well as a population of flies which is uniform in age within two or three days. We needed about 50 to 60 culture boxes to obtain a population of 50 to 70000 flies which were transferred into the population cage. Two cages containing 25 to 35000 flies each, maintained in a constant temperature and humidity room (25°C, 70%), provided an excellent egg supply within four weeks.

Figure 1. Drosophila culture box.
Figure 2. *Drosophila* population cage.

Figure 3. *Drosophila* embryo harvesting tray: (a) scheme, (b) photograph:
1: polyester material;
2: yeast suspension;
3: nylon gauze;
4: eggs.
Figure 2 shows the structure of a cage. The ceiling was wrapped with cheese-cloth to provide ventilation. The food was provided daily on trays (20 x 13.5 x 2 cm). New trays were placed through the openings on the bottom of the cage, pushing the old ones into an anaesthetizing box in order to collect flies from the medium surface. The anaesthetized flies were put back into the cage through the cheese-cloth sleeve of the side opening.

All equipment mentioned above was made of transparent plexiglass.

The embryo harvesting trays were placed and removed in the same way. Each harvesting tray (Fig. 3) consisted of a flat piece of silon sponge, a piece of polyester material and some fine nylon gauze. The sponge piece (20 x 13.5 x 1.5 cm) was wetted with a solution containing 1.5% acetic acid, 2.5% ethanol and covered with the polyester material whose colour resembled the food medium colour. A thick suspension of Baker’s yeast was layered and the nylon gauze was placed over it. In this way the desired rough medium surface which is the preferable place of egg deposition was obtained (Spencer 1950; Elgin & Miller 1978). In addition the nylon gauze prevented flies from sticking to the yeast suspension. The embryos were collected within two hours during the oviposition activity of the flies.

Egg harvesting was carried out by washing the nylon gauze in water and filtering the washing water through a sintered glass funnel S1.

The method described above allowed us to collect 10-15 g of synchronous Drosophila embryos for a laying period of two hours, employing a population of 50 to 70000 flies.

References:


Quantitative estimation of the red (drosopterins) and brown (xanthommatins) eye pigments of Drosophila melanogaster in eye color mutants has normally been carried out following Clancy's "double extraction" procedure (1942). This method is based in the extraction of the red pigment in AEA (30% ethyl alcohol acidified with HCl to pH 2.0) by placing the heads in this solvent for 24 hr. Then, the brown pigment is extracted by transferring the heads into a solution of AMA (absolute methyl alcohol containing 1% by volume of dry HCl).

Ephrussi & Herold (1944) systematically studied Clancy's procedure and found it inappropriate for the analysis of the brown pigment in mutants having also drosopterins. They reported that the "double extraction" had two main problems: the AEA seems to render insoluble a part of the brown pigment, and second, some brown pigment is extracted by the AEA.

We have revised the procedures reported in the literature for selective extraction of the eye pigments. In mutants containing both eye pigments, an accurate quantitative estimation can be made using the following methods:

Quantitation of the red pigment ("drosopterins"): Fly heads (around 40, 20 from males and 20 from females, nine-day-old adults) split longitudinally into halves, are placed in AEA (3 ml) for 24 hr (Ephrussi & Herold 1944). Then, the extract is filtered through an inert filter (glass fiber) and the absorbance measured at 480 nm. The absorbance is linear versus the number of heads at least up to values around 0.900.

In order to test the above procedure, the following mutants were chosen: scarlet (it does not have "xanthommatins"), sepia (it has no "drosopterins" and it is the mutant known to accumulate the highest amount of the yellow pigment sepiapterin) and brown (it only has "xanthommatins"). Table 1 shows that the extraction of the red pigment is higher when the heads are split longitudinally or when homogenized with AEA. Interferences due to the yellow and brown pigments were higher when heads are homogenized than when split into halves (Table 1). Only in the case of the sepia mutant was the yellow pigment contribution important. In all the other eye color mutants, the "drosopterins"/sepiapterin ratio is high enough to minimize sepiapterin contribution to "drosopterins" quantitative estimation.

The stability of the red pigment in AEA has been compared with its stability in 0.1 M NaOH and 0.1 M HCl. After 72 hr, the loss of absorbance at 480 nm of a homogenized extract of scarlet heads is: 2% in AEA, 9% in HCl and 90% in NaOH.