THE NATURE OF CERTAIN RED CELLS IN
DROSOPHILA MELANOGASTER

JACK COLVARD JONES 1 AND E. B. LEWIS 2

In a stock of the spineless (ss) mutant of Drosophila melanogaster, some of the
flies were observed to have bright red cells under the cuticle. The presence of
these pigmented cells has been found to depend upon a recessive mutant gene located
at 26.D ± in the second chromosome. The mutant has been named "red cells," symbol, rc. This paper is a brief account of the location, histology, and cytology
of the red-pigmented cells of the rc mutant.

Methods

A stock homozygous for rc and ss has been used for all studies unless otherwise
specified. The ss mutant serves merely as a marker to check on contamination of
the stock and does not have any obvious effect on the expression of rc. Under
crowded culture conditions rc may overlap wild type. To obtain maximum ex-
pression of the rc mutant, it is desirable to rear the larvae on an abundant supply
of yeast. In the present work, additional dried yeast or paper towelling saturated
with a thick fresh yeast suspension was added to the standard culture medium on
the fourth day after introducing the parents. To study the effect of trypan blue,
the rc mutant was grown on standard culture media containing 1.5% trypan blue.

Larvae of the third stage, pupae of various ages, and young adults of both sexes
were studied. Intact larvae were immersed in 0.85% NaCl or in water, covered
with a cover slip, and their various tissues examined in situ under high power
(970 X).

Pupae and anaesthetized adults were pinned to a paraffin dish and dissected
in Beadle-Ephrussi saline or in ethyl cellosolve. While a variety of fixatives were
employed, cellosolve-fixed specimens were chiefly used. Fixed specimens were
transferred to methyl benzoate and finally embedded in paraffin. Serial, cross,
and sagittal sections were stained principally with picric acid since this stain did
not interfere with the recognition of trypan blue uptake nor of red pigment dis-
tribution. Several series were stained with methylene blue. One series was
stained with hematoxylin and eosin, two series with Sudan III for fat, and two
series according to the Bauer test for polysaccharides.

Observations

Third-instars and early pupae of the rc strain, whether examined as whole
mounts or as sectioned material, do not show any pigmented cells. In older pupae,

Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of
Tropical Diseases, Bethesda, Maryland.

2 Division of Biology, California Institute of Technology, Pasadena, California.

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a pale yellow substance appears in the eye and in many of the cells destined to contain red pigment granules. Red granules first appear in scattered cells in the head *before* becoming visible in the facet cells of the eye (Fig. 3). Whether yellow material is itself converted directly into red pigment has not been determined. As pigment in the eye changes from pale brown to dark brown and then to red brown, the number of red granules in the cells of the head and thorax increases from one or two to many per cell. Before the granules actually form, or when only one or two are present, these thoracic cells are conspicuous by their yellow tinge. By the time the eye pigment is formed, pigment is present in the cells of the head, thorax, and abdomen.

Living *rc* flies of both sexes show red-pigmented cells under the cuticle in the thorax and head (Figs. 1 and 2). While red-pigmented cells may be widely distributed throughout the body of young adults, they are most numerous and conspicuous in the head and thorax. In the thorax, loose aggregations occur in two longitudinal rows along the dorsal midline of the mesonotum and scutellum (Fig. 1), and there are less conspicuous groups on either side of these. Small clusters of pigmented cells are sometimes seen in the legs (coxa and trochanter) and isolated pigmented cells occur within the abdomen (Fig. 5).

The red-pigmented cells occur either singly or in definite groups of four or five or more, but they do not form syncytia (Fig. 4). When the cells are in definite locations, as in the supra-aortal masses and alongside the anterior part of the gut (Fig. 6), they are not bounded by connective tissue membranes and hence are extremely difficult to examine in fresh dissections.

The pigment granules are round, ovoid, or irregular in shape (Fig. 4) and they vary in size from less than 0.5 to about 4 microns. The number of granules per cell ranges from a few to 50 or more. The cells bearing the granules are round or ovoid (Figs. 3–6), measure about 20 microns in diameter, and have a single
Figure 3. Horizontal section through head of young pupa before eye pigment has formed, showing a red pigmented fat cell (RC). Hemocyte is shown at H. Picric acid. ×940. Ref. no. 2173.

Figure 4. Typical red-pigmented cells in the thorax. Muscles are shown at M and an unpigmented fat cell at FC. Picric acid. ×1120. Ref. no. 2174.

Figure 5. Fat cells in the abdomen of an adult fly, showing a single cell with red granules at R. Picric acid. ×1120. Ref. no. 2172-5.
round nucleus. They are thus considerably larger than hemocytes which measure between 5 and 10 microns in diameter. The red cells, when examined in wet mounts, generally have large non-refringent droplets and other inclusions in their cytoplasm, in addition to pigment. Isolated pigment cells in saline do not send out lamellar extensions, and thus differ from certain kinds of hemocytes.

When larvae are reared on trypan blue media enriched with added yeast, the resulting pupae and adults have blue dye in the gut lumen, hemocytes, scattered thoracic fat cells, garland cells, and thoracic and abdominal nephrocytes. In the nephrocytes the dye appears in irregular diffuse granular masses and/or in dense aggregates. Sometimes large dye droplets are seen free in the hemocoele. Only occasionally do cells having red pigment take up the dye. Abdominal fat cells do not take up trypan blue.

Red-pigmented cells of the rc strain have large fat droplets (Sudan III) and smaller deposits of polysaccharide (Bauer-positive material), and are in the same size range as typical fat cells in the head and thorax. Fat cells in the abdomen of adults are distinctly larger (25–35 microns) than those in the head or thorax (14–21 microns).

**COMBINATIONS OF rc WITH OTHER MUTANT GENES**

The rc mutant was combined with mutants which affect the eye color in order to determine whether the red granules in the fat cells are related to the eye pigments. When rc is combined singly with mutants which remove the brown component of the eye pigment, namely, vermilion, cinnabar or scarlet, the homozygous double mutant combination has no pigment in the fat cells. When rc is combined with the brown mutant, which removes the red component and leaves the brown component of the eye pigment, the homozygous double mutant has typical red fat cells. It should be added that rc does not modify the eye color of wild-type nor of any of the above mutant types.

The rc mutant was combined with Microcephalus, a dominant mutant, locus 60.0 in the third chromosome which frequently results in a completely eyeless fly. Eyeless specimens thus obtained show the same degree of pigment development in the fat cells as do the cells of rc flies with normal eyes.

**DISCUSSION**

The red-pigmented cells in *Drosophila* adults resemble typical peripheral fat body cells of the thorax and head in size, shape, general distribution, and possession of deposits of fat and polysaccharide. However, they do not usually stain vitally with trypan blue, while some other typical head and thoracic fat cells without red pigment often incorporate the dye. Those relatively few red-pigmented cells present in the abdomen are usually peripheral in location and of about the same

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3 Dr. M. T. M. Rizki, who has examined our rc mutant, independently concludes from his observation of the red-pigmented cells that they are fat cells (personal communication).

**Figure 6.** Sagittal section through thorax of adult, showing clusters of cells lateral to the gut (G), some with trypan blue and others with red granules (RC). Picric acid. × 940. Ref. no. 2174–10.
size as the thoracic fat cells. Red fat cells are larger than typical hemocytes and do not send out lamellar extensions ("pseudopodia") in fresh dissections as many hemocytes do in vitro. None of the sessile or circulating hemocytes examined had red pigment. Indeed, so far as the authors are aware, there is no report of hemocytes with colored pigments among the insects.

Red pigment in fat cells first appears in the pupal stage at about the same time as pigment forms in the eyes. Combinations of re with eye color mutants strongly suggest that the pigment in the fat cells is closely related to, or identical with, the brown eye pigment. Microscopically, brown eye pigment is red in color. That the pigment has not diffused out of the eyes and been secondarily taken up by fat cells is shown by the presence of abundant red fat cells in eyeless re flies.

**Summary**

A mutant of *Drosophila melanogaster* possesses pigmented, stationary cells in the body cavity of the pupal and adult stages. The pigment is present as numerous red granules in the cytoplasm. By a number of criteria, the pigmented cells are a type of fat cell. The evidence suggests that the pigment is related to, or identical with, the brown component of the eye pigment and that it develops in the fat cells autonomously.