THE NATURE OF CERTAIN RED CELLS IN DROSOPHILA MELANOGASTER

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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 26.0 ± 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 expression 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 distribution. 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,

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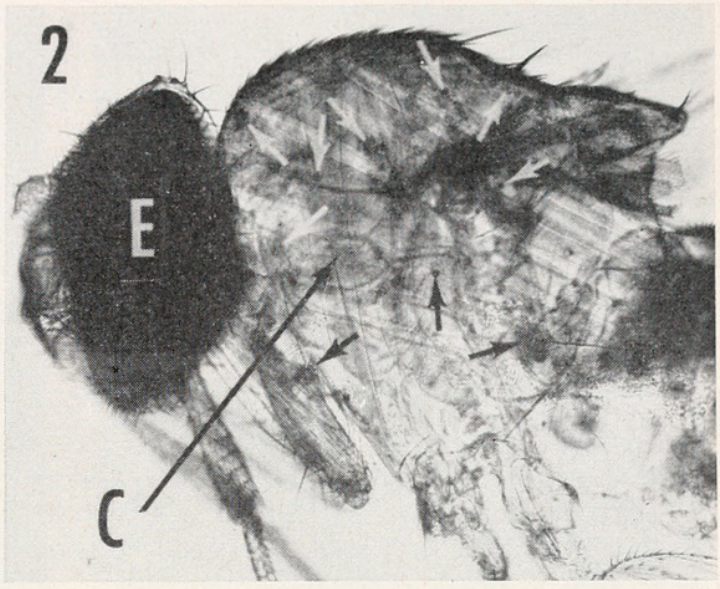


FIGURE 1. Dorsal view of thorax of adult *Drosophila rc* mutant showing alignment of red-pigmented cells. Unstained whole mount. × 87.

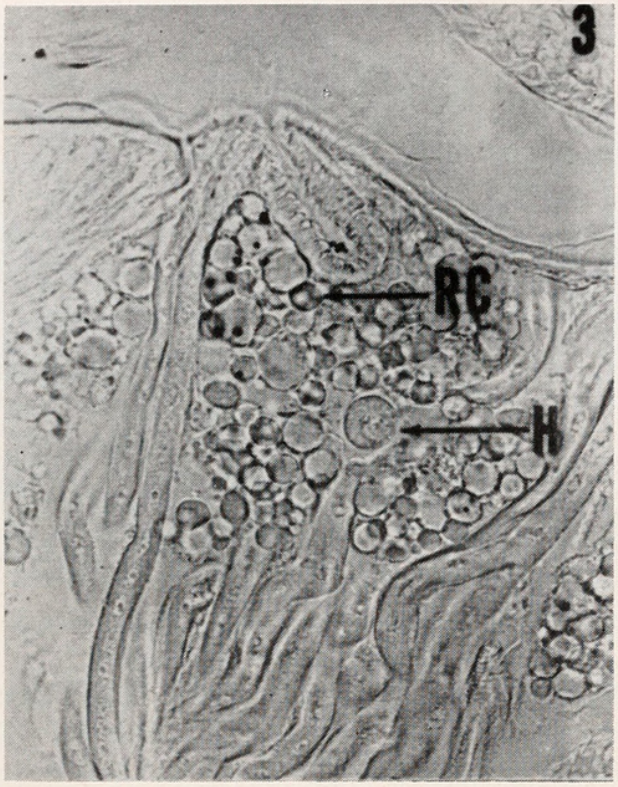
FIGURE 2. Lateral view of *rc* mutant showing clusters of pigmented cells. C = cardia, E = eye. Unstained whole mount. × 63.

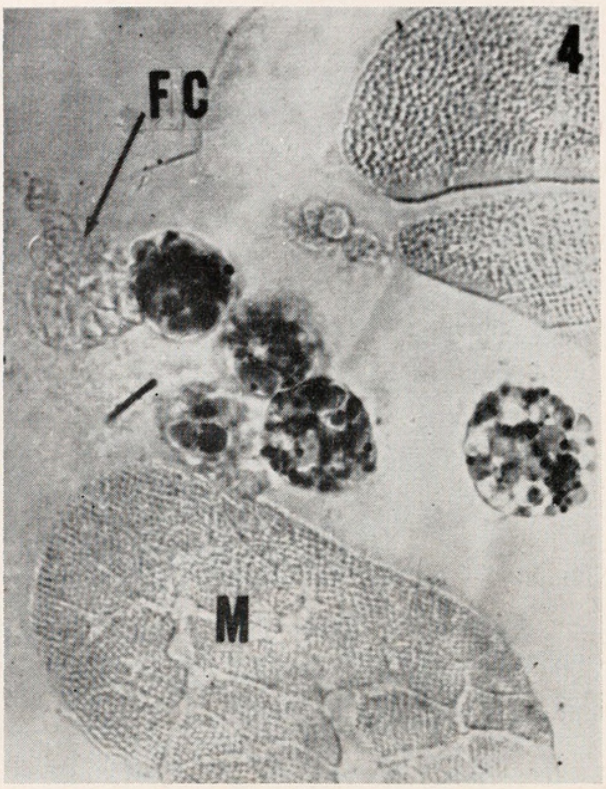
a pale yellow substance appears in the eye and in many of the cells destined to contain red pigment granules. Red granules first appear in scattered cells in the head *before* becoming visible in the facet cells of the eye (Fig. 3). Whether yellow material is itself converted directly into red pigment 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.

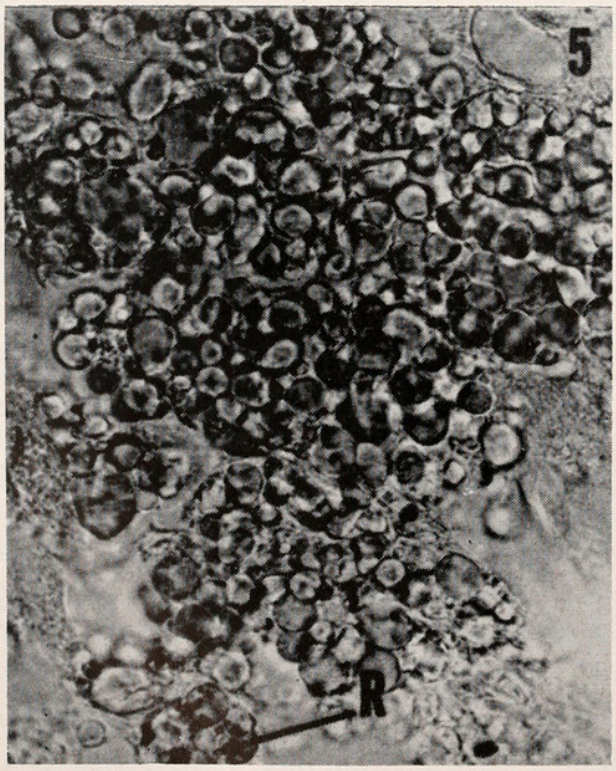
Living *rc* flies of both sexes show red-pigmented cells under the cuticle in the thorax and head (Figs. 1 and 2). While red-pigmented cells may be widely distributed throughout the body of young adults, they are most numerous and conspicuous in the head and thorax. In the thorax, loose aggregations 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 membrances 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







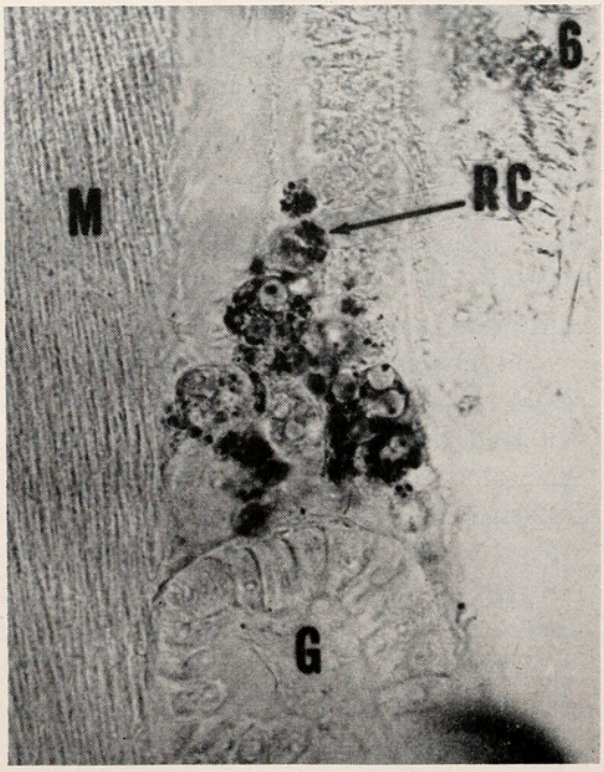


FIGURE 3. Horizontal section through head of young pupa before eye pigment has formed, showing a red pigmented fat cell (RC). Hemocyte is shown at H. Picric acid. × 940. Ref. no. 2173.

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.[3] 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

[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 *rc* 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 *rc* 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.