THE GENETICS AND BIOCHEMISTRY OF RED FAT CELLS IN DROSOPHILA MELANOGASTER[1]

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IN the strain of Drosophila known as "red fat cells" conspicuous pigmented cells are visible in the head and thorax of the flies (JONES and LEWIS 1957). Normally colorless fat cells acquire a pigment that is related to, or perhaps identical with, the brown ommochrome derived from tryptophan.

This report concerns some properties of the red fat cells (RFC) strain, two of which have been briefly reported (GRELL 1958). The larvae, pupae and adults of the RFC strain contain much more of the amino acid, lysine, than wild-type animals. The RFC phenotype is the result of an interaction between two mutants, lysine (*lys*) and red cells (*rc*). Each of these mutant loci seems to have the tendency to produce pigmented fat cells in the absence of the other, but this tendency is expressed only under special conditions.

BIOCHEMICAL ASPECTS

Accumulation of lysine: That Drosophila of the RFC strain contain more lysine than wild type was demonstrated largely by paper chromatography. The chromatographic techniques were essentially those first employed on Drosophila by HADORN and MITCHELL (1951). All chromatograms of larvae, pupae and adults of the RFC strain showed that one ninhydrin-positive spot was much larger and darker than the corresponding spot produced from wild-type Canton-S animals. Several solvent systems were used and in all cases the Rf values of the accumulated amino acid were equivalent to that of lysine.

Two dimensional patterns resulting from paper electrophoresis in one dimension and chromatography with butanol-1:acetic acid:water (11:3:4) in the second dimension clearly show the lysine accumulation in RFC animals (Figure 1).

A further indication that the accumulated amino acid is lysine was a test of its ability to act as a growth factor for a lysineless mutant (37811a) of Neurospora. The accumulation isolated from chromatograms of RFC animals contained more growth factor than material isolated from the same region of chromatograms of Canton-S animals.

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[2] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

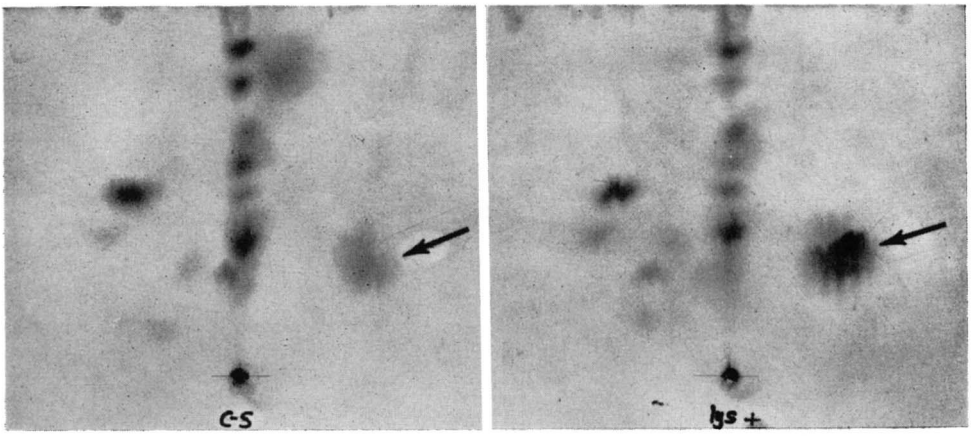

FIGURE 1.—Canton-S and the *lys* mutant compared by paper electrophoresis in horizontal direction and chromatography in butanol-acetic acid-water in the vertical direction. Paper sprayed with ninhydrin solution. Arrows point to the lysine spots.

Injections of L-lysine-C^{14}: In order to learn the cause of the abnormally large amounts of lysine in the RFC strain, C^{14}-labeled lysine was injected into RFC and wild-type adult flies. The amount of radioactivity was measured in respired CO_2 and other fractions of the flies.

Fractions of carbon dioxide were collected by precipitation as $BaCO_3$ for eight hours after injection of lysine. The flies were then ground with sand and extracted with solvents. In Experiment I the animals were extracted only with boiling water, but in Experiment II they were first extracted with ether and then with boiling water. The tube which had contained the injected flies for eight hours was rinsed with hot water to recover the excretory products. The residue was the material that was insoluble in ether and water or was precipitated by exposure to boiling water, as in the case of some proteins. Table 1 lists the results of Experiments I and II.

The distribution of radioactivity recovered from Canton-S flies was quite different from the distribution from the RFC strain. Canton-S animals converted on the average 37.9 percent of the recovered radioactivity into CO_2, but RFC animals converted only 3.0 percent into CO_2. In other words, Canton-S flies converted 12.6 times more lysine into CO_2 in an eight-hour period than did RFC flies.

Most of the radioactive lysine injected into RFC flies was recovered in the water soluble fraction. Chromatography of this material indicated that most of it was unaltered lysine.

The amount of radioactivity in the ether soluble fractions is consistent with the results of the CO_2 tests. If in the Canton-S strain more lysine were degraded to CO_2 than in mutant flies, then more carbon atoms from lysine also would be expected in fats and other ether-soluble materials. In confirmation of this expectation, Canton-S flies had about five times more radioactivity in the ether soluble fraction than did the RFC flies (Table 1).

The greater amount of radioactivity in the excretion products of the RFC flies

TABLE 1

Results of experiments in which 25 Canton-S and 25 RFC males were injected with randomly labeled 0.1 M L-lysine-C^{14}

Fraction	Experiment I Time (min.)	Counts per minute Canton	RFC	Experiment II Time (min.)	Counts per minute Canton	RFC
CO_2	0–75	680	51	0–120	1800	120
CO_2	75–170	460	52	120–270	2200	230
CO_2	170–325	1400	150	270–480	1500	240
CO_2	325–480	1500	23	....	....	....
Total CO_2	0–480	4040	276	0–480	5500	590
Percent	....	36	2	....	40	4
Ether soluble	480	....	....	480	1300	330
Percent	....	....	....	....	10	2
Water soluble	480	5300	15000	480	3200	8330
Percent	....	47	80	....	23	61
Residue	480	1900	3400	480	2600	1700
Percent	....	17	18	....	19	13
Excretion	480	....	....	480	1100	2670
Percent	....	....	....	....	8	20
Total	....	11240	18676	....	13700	13620

may reflect a real difference although this category is subject to error. Since free lysine remains at a high concentration in RFC animals, more of it might be excreted than in normal animals where a large proportion is degraded to carbon dioxide.

The radioactivity in the residue is probably an index of the lysine incorporated into protein. In Canton-S animals 18.2 percent, and in RFC animals 15.6 percent, of the recovered counts were in the residue. This probably indicates no real difference. The amount of radioactive lysine incorporated into protein might be expected to be slightly lower in RFC flies because at the time of injection there is a larger pool of unlabeled lysine in RFC flies and this unlabeled lysine would tend to dilute the radioactive material.

GENETICS OF RED FAT CELLS

The recessive factor responsible for the RFC phenotype has been located by LEWIS (1950) between Sternopleural (*Sp*) and Jammed (*J*) at approximately 26 on the second chromosome. In experiments to locate the factor more accurately, the two distinct abnormal phenotypes, red fat cells and accumulation of lysine, were classified independently.

Females of the constitution, *Sp* RFC *J*/+ + +, were produced and crossed to Canton-S males. In the first generation the offspring could not be scored for the recessive RFC factor or lysine; however they were scored for crossing over between *Sp* and *J*. Males possessing a crossover chromosome were mated to yellow forked double X; RFC virgin females (*y f*:=; RFC) and the *Sp* or *J* progeny were scored for red fat cells. In addition, a sample of flies bearing each crossover was

tested for lysine accumulation by chromatography. In another cross *Sp* RFC *J*/*d* females (*d* = dachs) were crossed to Canton-S males and the crossover chromosomes tested as above.

In the first generation the progeny was distributed as follows:

Parental:	Recombinant:
2441 *Sp J*	259 *Sp* +
2532 + +	263 + *J*

There is 9.5 percent recombination between *Sp* and *J*. Results of progeny tests of male recombinants are as follows:

109 + + + *J*	52 *Sp* lysine RFC +
10 + lysine RFC *J*	54 *Sp* lysine + +
0 + lysine + *J*	11 *Sp* + + +

The data shows that 54 of the crossovers accumulated lysine but did not have the RFC phenotype. On the other hand, all flies with the RFC phenotype accumulated lysine. The accumulation of lysine appears to be due to a single factor and the RFC phenotype is produced by an interaction between the factor for lysine accumulation and some other factor. The factor for lysine accumulation is called lysine (*lys*) and the other factor, red cells (*rc*).

From females heterozygous for dachs, 173 crossovers were scored for lysine and red fat cells. The results are given in Table 2.

TABLE 2

Genotypes of crossovers from $\frac{\text{Sp lys + rc J}}{\text{+ + d + +}}$ *females*

Region 1	Region 2	Region 3	Region 4
6 *Sp* + *d* + +	16 *Sp lys d* + +	26 *Sp lys* + + +	37 *Sp lys* + *rc* +
8 + *lys* + *J*	21 + + + *rc J*	+ + *d rc J**	+ + *d* + *J**

* 59 total in regions 3 and 4.

Combining all of the data the following map may be constructed: *Sp*—.85—*lys*—1.7—*d*—2.7—*rc*—4.2—*J*. Using the standard map location of *Sp* as 22.0, *lys* is located at 22.85 and *rc* at 27.25. It should be pointed out that there is a discrepancy between the standard distance between *Sp* and *J* (19 units) given in BRIDGES and BREHME (1944) and the one (10.5 units) that was obtained in these experiments.

The distance between *Sp* and *J* was measured in two separate experiments with females also containing *lys* and *rc* and found to be 10.5 and 9.5 percent. To test the possibility that *lys*, *rc* or another factor in that region was reducing crossing over, the region was measured in flies without *lys* or *rc*. The results of the cross of *Sp* +/+ J^{34e} females and + + males are as follows:

Parental:	Crossover:
1167 *Sp* +	135 *Sp J*
1083 + J^{34e}	155 + +

The crossing over amounts to 11.4 percent. Thus using *Sp* and *J* from different sources, the crossing over is always less than the standard distance. Combining the data from all of the crosses gives a crossover value of 10.2 percent from a total of 12,048 flies.

OTHER ALLELES AT THE *rc* AND *lys* LOCI

Red cells-2: Drosophila from the singed-4 stock kept in the California Institute of Technology collection were discovered by R. F. GRELL also to possess red fat cells. The pigment is located in the same cells as in *lys rc* and resembles that phenotype. There are important differences between the red cells-2 and *lys rc* phenotypes. At 17°C pigmented cells are present in rc^2 flies, but if the animals are cultured at 25°C on a usual Drosophila medium no red cells can be observed. At 25°C red cells may be detected if the rc^2 larvae have been starved. The enhancement of the RFC phenotype by starvation will be discussed later.

No biochemical difference (except the pigmentation of the fat cells) has been observed between rc^2 and wild type. There is no accumulation of lysine. The red cells phenotype in the case of rc^2 appears to be dependent upon a single gene.

By several genetic criteria, rc^2 is allelic to *rc*. Like *rc*, in crossover tests rc^2 localized to a position between *Sp* and *J*. The double homozygote, *lys* rc^2, resembles *lys rc* in that there are numerous red fat cells present at 25°C in flies cultured on a regular medium. The combination, *lys rc/lys* rc^2, has abundant red fat cells and no recombination occurs to give *lys* + from *lys rc/lys* rc^2 females.

Lysine-2: An allele of *lys* was induced by X-rays. Males carrying rc^2 on their second chromosomes were irradiated with 4000r and mated to *y; dp lys rc pr* (yellow; dumpy, lysine, red cells, purple) virgin females. From among 35,000 progeny of this cross one male with a RFC phenotype was found. He was mated to *Sp lys pr/Cy, Ins05,* dp^{tx1} *pr* cn^2 (OSTER 1956). The *Cy* dp^+ pr^+ progeny were selected to make a balanced stock which was heterozygous for the radiation-induced mutation and the balancer, *Cy, Ins05*.

A sample of the *Sp* pr^+ (*lys*^{2} rc^2/*Sp lys pr*) progeny were chromatogrammed and found to have an accumulation of lysine similar to homozygous *lys*. This constitutes an allele test, and the X-ray induced allele has been designated lysine-2 (*lys*^{2}). In addition, *lys*^{2} rc^2/*lys* + flies show some faintly pigmented fat cells. The production of red cells was unexpected since *lys* rc^2/*lys* + very rarely produces a red cell. (Under special starvation conditions, discussed later, it has been found that either homozygous rc^2 or *lys* is capable of showing a RFC phenotype in the absence of the other mutant.)

Homozygous *lys*^{2} rc^2 is poorly viable and the flies have heavy textured, slightly drooping wings. They have a RFC phenotype, but it is less extreme than *lys*^{2} rc^2/*lys rc* or *lys rc* homozygous. Attempts to separate *lys*^{2} from rc^2 were unsuccessful because *lys*^{2} is associated with a chromosomal rearrangement. There is an inversion from salivary chromosome map sections (BRIDGES 1935) 22F to 40 and a 2–3 translocation inside of the inversion with breaks at 33B in the second chromosome and 64A in the left arm of the third chromosome. The new order of

the two chromosomes is: 20 to 22F/40 to 33B/64A to 100, and 61 to 64/33B to 22F/40 to 60. The rearrangement is designated: T(2;3)lysine-2.

EFFECT OF LARVAL STARVATION ON THE RED CELL PHENOTYPE

RIZKI (1960) reported that the RFC phenotype in a combination red cells-tumor strain is expressed only if the animals are starved as larvae. It seemed desirable to determine if starvation also enhances *lys rc*, *lys* +, + *rc* and various heterozygous combinations. Experiments were performed in which larvae at the age of about 72 hours were removed from the regular Drosophila medium, washed several times in distilled water, and placed in quarter-pint milk bottles containing one inch of five percent glucose in two percent agar. The larvae worked into the agar and most of them began pupation after two days. Late pupae and young adults were examined for the presence and extent of red cells.

It was found that the homozygous *lys-rc* which had an RFC phenotype under normal culture conditions is enhanced by the starvation treatment, and some genotypes that never, or rarely, have red cells may then have some if they develop from starved larvae. The enhancement of *lys rc* by starvation was unexpected since addition of extra yeast to culture media also enhances the intensity of the phenotype (JONES and LEWIS 1957). A summary of the results is presented in Table 3. Two cases should be given special attention: (1) *lys* +/*lys* + flies

TABLE 3

Phenotypes of combinations of lys, lys^2, rc *and* rc^2

	Genotype	Phenotype	Phenotype after larval starvation
1.	*lys rc/lys rc*	lysine and RFC	lysine and RFC
2.	*lys rc/+ +*	wild type	wild type
3.	*lys rc/+ rc*	wild type	wild type
4.	*lys rc/lys +*	lysine	lysine and RFC
5.	*+ rc/+ rc*	wild type	wild type
6.	*lys +/lys +*	lysine	lysine and RFC
7.	*+ rc^2/+ rc^2*	wild type at 25°C, RFC at 17°C	RFC
8.	*+ rc^2/+ +*	wild type	wild type
9.	*lys rc^2/+ rc^2*	wild type at 25°C, RFC at 17°C	RFC
10.	*lys rc/+ rc^2*	wild type	RFC
11.	*lys rc^2/lys rc^2*	lysine and RFC	lysine and RFC
12.	*lys rc^2/lys rc*	lysine and RFC	lysine and RFC
13.	*+ rc^2/+ rc*	wild type	RFC
14.	*lys^2 rc^2/lys +*	lysine and RFC	lysine and RFC
15.	*lys^2 rc^2/lys^2 rc^2*	lysine and RFC	lysine and RFC

have red cells after starvation and (2) + rc^2/+rc^2 also have red cells at 25°C if they have been starved as larvae. E. B. LEWIS (personal communication) has found that *cho* (chocolate) also enhances the red fat cell phenotype and the combination, *cho*; *lys* +, produces red fat cells.

DISCUSSION

Lysine is an essential amino acid. It must be supplied in the diet for Drosophila growth and development (HINTON, NOYES and ELLIS 1951). Drosophila which accumulate lysine as the result of being homozygous for *lys* must acquire the lysine from an external source. Drosophila grown on a regular medium (brewer's yeast, corn meal, sucrose, glucose and agar) probably ingest more lysine than is required for synthesis of proteins. In normal flies the excess lysine is broken down and metabolized.

It is believed that the effect of *lys* is, in principle, similar to some other mutations of Drosophila. GREEN (1949) showed that nonprotein tryptophan is accumulated in the vermilion mutants of both *D. melanogaster* and *D. virilis*. KIKKAWA (1941) showed that kynurenine is probably accumulated in cinnabar flies. The two mutations block different steps in the formation of brown eye pigment from tryptophan. Vermilion prevents the formation of kynurenine from tryptophan and cinnabar prevents the formation of 3-hydroxykynurenine from kynurenine.

The xanthine dehydrogenase-deficient mutants, rosy and maroon-like, also demonstrate this principle in Drosophila. The substrates of the missing enzyme, 2-amino-4-hydroxypteridine and hypoxanthine, tend to accumulate (FORREST, GLASSMAN and MITCHELL 1956; HADORN and SCHWINCK 1956; MITCHELL, GLASSMAN and HADORN 1959).

In the case of lysine accumulation, it is postulated that the gene lys^+, is necessary for rapid degradation of lysine. Since lysine itself accumulates, one might expect that it is the first step in the degradation path that is affected in flies with the *lys* mutation. ROTHSTEIN and MILLER (1954) proposed that this first step is an oxidative deamination of the alpha carbon of lysine.

There is no known biochemical reason for the relationship between lysine and the pigmentation of the fat cell. Tryptophan is the precursor of the pigment and the injection of radioactive lysine into larvae does not lead to the incorporation of radioactivity into the brown eye pigment. On the other hand, no effect on the RFC phenotype is observed if trytophan is injected into *lys rc* larvae. (GRELL, unpublished.)

As stated by JONES and LEWIS (1957) RFC flies homozygous for vermilion do not have pigment in fat cells. However, *v*; *lys rc* animals do have pigmented fat cells (as well as brown eye pigment) if they have been fed kynurenine as larvae (LINDA SMITH RILES, personal communication; GRELL, unpublished). Therefore, kynurenine is a precursor of the fat cell pigment. The enhancement of RFC by starvation may be related to the formation of kynurenine (v^+ substance) when animals with certain alleles of vermilion are starved (BEADLE, TATUM and CLANCY 1938). It is reasonable that nonvermilion animals form more kynurenine when they are starved and therefore more precursor is available for fat cell pigment.

The interaction between the two loci *lys* and *rc* is expected if one considers that each mutation produces a tendency for a RFC phenotype. In single mutants

the tendency must be reinforced by certain environmental conditions, in order to be detectable, but the double mutant expresses the red cells phenotype under all conditions of nutrition and temperature that were tested.

SUMMARY

1. The red fat cells (RFC) phenotype of *Drosophila melanogaster* was found to be a digenic trait. The two recessive mutations are called lysine (*lys*) and red cells (*rc*) and are located at 22.85 and 27.25, respectively, on the second chromosome. These mutations may be separated, maintained in separate stocks, and recombined. When they are recombined, the red cells phenotype is produced again as it was in the original stock.

2. The mutation *lys*, with or without *rc*, causes the accumulation of the amino acid lysine in larvae, pupae and adults. The biochemical basis for the action of *lys* is postulated to be a disturbance in the degradation of lysine. Data to support this hypothesis were obtained from the injection of C^{14}-labeled lysine into *lys* and wild-type flies.

3. Environmental factors alter the RFC phenotype. If the flies develop from starved larvae, *lys* (without *rc*) causes red fat cells. The allele rc^2 likewise produces pigmented cells if the flies were cultured at low temperature or starved.

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