cies in the F_1 population, viz. (1) carriers of *a* only, (2) carries of *b* only, (3) carriers of both *a* and *b* and (4) carriers of neither. If linkage exists, classes (3) and (4) will be in excess of classes (1) and (2).

This method was used in rats in testing for linkage between the recessive lethal anemia[1] and an undescribed sublethal ("wobbly"), neither of which at the time was known to be linked with any previously discovered rat gene.

Back-cross individuals to the number of 161 were tested individually by matings with individuals doubly heterozygous for anemia and wobbly. They were found to be distributed as follows:

CARRIERS OF ANEMIA ONLY	CARRIERS OF WOBBLY ONLY	CARRIERS OF BOTH	CARRIERS OF NEITHER
40	42	43	36

The sum of the first two classes (non-crossovers) is 82, of the last two classes (recombinations or crossovers) is 79. The deviation from equality is 1.50 ± 4.28, or less than might be expected from random sampling. Anemia and wobbly are beyond question independent.

Later anemia was found to be closely linked with curly, the crossover percentage being 5.2. An independent test showed that wobbly was, as expected, *not* linked with curly, thus confirming the previously reached conclusion that anemia and wobbly are borne in different chromosomes.

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THE GAS EXCHANGE OF DROSOPHILA LARVAE

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Following a suggestion from Sturtevant's[2] work on mosaics, Beadle, Ephrussi[3] and others have shown that a diffusible substance, v^+ hormone, is concerned with the expression of the mutant character vermilion (*v*) in *Drosophila melanogaster*. The substance is normally absent in larvae and pupae homozygous for vermilion and present in those carrying its wild type allele. It can be extracted from young wild type pupae[4,5] and when injected into vermilion brown test larvae shifts the eye color of the adult fly toward brown, i.e., non-vermilion. Transplanted fat-bodies of wild-type larvae will produce an eye-color change in vermilion brown hosts, but v^+ substance cannot be extracted from them.[6] Wild-type Malpighian tubes yield v^+ hormone both on transplantation and extraction.[6] Recent work by Khouvine, Ephrussi and Chevais[7] and by Beadle, Tatum and Clancy[8] shows that v^+ hormone production is affected by diet. Vermilion

brown larvae grown on an intermediate food level (small amount of yeast) such that their pupation time is delayed two to six days produce flies with an eye-color modified toward brown, i.e., v^+ hormone has been produced. The addition of sugar to low yeast media was found to inhibit this starvation effect. In view of the fact that the hormone is amino acid-like in nature,[5,9] these authors suggest that an alteration in protein and amino acid metabolism is concerned with its formation in larvae on low food. This work is an attempt to study the metabolism of such larvae by respiratory measurements in order to test this hypothesis.

Materials and Methods.—Four different diets, each having different effects, were used in the experiments. Full food consisted of standard Drosophila culture medium[10] seeded with an excess of live yeast. Low yeast consisted of a suspension of 0.25 per cent of dried brewer's yeast in 1.0 per cent agar. Sixty larvae were transferred at 28 hours after hatching from full food to 100 cc. of low yeast in a dish having an area of about 40 cm.^{2}. This treatment gave a strong eye-color change and a delay in puparium formation of from one to four days. Preliminary experiments with media containing sugar were unsuccessful because of bacterial and mold contamination, so an aseptic method (Tatum, unpub.) was employed. Two series consisted of 0.5 per cent and 2.0 per cent sucrose added to a basic low yeast level of 0.5 per cent dried yeast in 1.0 per cent agar. Ten cc. of a given food were placed in each of ten 35-cc. vials and autoclaved. Eggs were collected on autoclaved spoons, removed in groups of thirty with sterile glass rods, immersed in 80 per cent alcohol for ten minutes and placed thirty per vial. No contamination occurred in any unopened vials, although after pupation growth was occasionally observed in vials from which larvae had been removed for measurement and returned. Later tests showed that the 0.5 per cent yeast diet alone, provided from hatching, had the same effects on eye color, pupation delay and gas exchange as did the low food level described above. Also, a 3.0 per cent dried yeast suspension has been found to be equivalent to full food (living yeast) used here, showing that the use of the two techniques did not introduce complications. All cultures were kept at 25°C.

A differential volumeter of the type described by Fenn[11] was used for the measurement of gas exchange. The vessels had a capacity of approximately 3.5 cc. each, the capillary a diameter of 0.687 mm. Triple distilled kerosene was used for the index drop. A hairline was mounted in a tube fixed to a mechanical stage so placed that the movement of the drop could be followed. Readings were taken to 0.1 mm. using the vernier of the stage. During use the respirometer was kept at 25.00 ± 0.02°C. in a well-stirred water bath. When a run was to be started, five to ten larvae were removed from food, washed thoroughly in sterile Ringer's solution, dried on filter paper, weighed to the nearest 0.1 mg. and placed in the experi-

mental vessel on 0.5 cc. of the same type of food as that from which they were removed (fully fed larvae were placed on a five per cent suspension of dried yeast in one per cent agar to avoid the obvious complications involved in using live yeast). After a half hour equilibration period in the water bath, readings were taken at five minute intervals for a period of one hour. The respirometer was then removed from the water bath, alkali introduced into the central cups of both vessels and readings again taken for an hour after equilibration. The control vessel contained 0.5 cc. of the same food used in the experimental vessel. Carbon dioxide production was calculated by the volume difference method, oxygen consumption directly from the second set of readings. Although the two were not measured simultaneously, tests showed that no appreciable change occurred in either during the time intervals used. Larvae left in the respirometer for two hours showed during that time a constant oxygen consumption when alkali was present, or a constant difference between oxygen consumption and carbon dioxide production when no alkali was present.

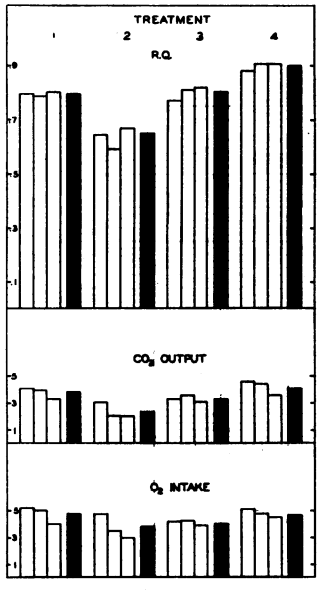

FIGURE 1

Gas exchange of larvae fed on (1) full food, (2) low yeast level, (3) low yeast plus 0.5 per cent sucrose, (4) low yeast plus 2.0 per cent sucrose. Oxygen consumption and carbon dioxide production are given in mm.^{3} per mg. hr. Solid columns represent mean values.

Eye-color change is given according to the set of standards used by Tatum and Beadle.[5] Vermilion brown (nearly white eye) is taken as 0.0, brown (brown eye) as 5.0, intermediate color values being determined by comparison with a graded series of inbred mutant stocks. Eye-color value is a measure of the quantity of v^+ hormone used by the fly. Pupation delay is the increase in larval lifetime over that of the fully fed controls (120 hours at 25°C.). Specimens of each experimental series were dissected just before puparium formation and the relative sizes of fat bodies with respect to larval size were noted.

Discussion of Results.—The results of the experiments are summarized in table 1 and in figure 1. The respiratory quotient (R.Q.) of larvae on a given diet is fairly constant over the periods measured in spite of fluctuations in CO_2 production and O_2 consumption and is different from that of larvae on any other diet. The lower R.Q. and rate of gas exchange of larvae on low yeast is in accord with previous work on starved and partially starved insects. The increase in R.Q. observed in larvae given sugar

in addition to low yeast level is to be expected from the fact that a purely carbohydrate diet usually results in an R.Q. of 1.0 (see Dreyer[12] and Cook[13] for insect data), while the higher rate of gas exchange is probably due to the greater quantity of food available. In order to interpret respiratory measurements of this kind the exact composition of the diets should be

TABLE 1

TREATMENT OF LARVAE	AGE HRS.	NO. OF LARVAE	WEIGHT IN MG./ LARVA	CO_2 OUTPUT MM.^{3}/MG.^{3}	O_2 UPTAKE HR.	R.Q.	EYE-COLOR VALUE*	PUPATION DELAY IN DAYS	RELATIVE SIZE OF FAT BODY
1. Fully fed	74	10	0.35	4.13	5.23	0.79			
	95	10	0.82	3.92	5.01	0.78	0.0	0.0	Large
	97	5	1.62	3.23	4.04	0.80			
Mean				3.76	4.76	0.79			
2. 0.25% dried yeast after 28 hours	76	10	0.15	3.04	4.76	0.64			
	93	10	0.77	2.08	3.52	0.59	2–4	1–3	Small
	121	7	1.31	2.03	3.03	0.67			
Mean				2.36	3.77	0.65			
3. 0.5% dried yeast plus 0.5% sugar from hatching	96	10	0.27	3.23	4.20	0.77			
	120	10	0.83	3.41	4.24	0.81	0.5–2	1–3	Medium
	144	5	1.10	3.11	3.81	0.82			
Mean				3.25	4.06	0.80			
4. 0.5% dried yeast plus 2% sugar from hatching	104	10	0.27	4.52	5.13	0.88			
	127	10	0.82	4.32	4.77	0.91	0–0.5	2–5	Medium
	153	5	1.12	3.64	4.01	0.91			
Mean				4.16	4.64	0.90			

* Tatum and Beadle[5] scale of eye-color values.

known. A completely synthetic diet for Drosophila has not been devised and so could not be used, but an idea of the proportions of the three principal foodstuffs can be had from analyses of yeast. Yeast is high in protein, about 60 per cent, low in fat, about 7 per cent, while carbohydrate makes up about 20 per cent, all expressed in dry weight. Fully fed larvae had an ample supply of all these. Larvae on low yeast without sugar had a limited supply of food containing about three times as much protein as carbohydrate. Those on low yeast with 0.5 per cent and 2.0 per cent sugar added had more food (but still not enough to allow normal development) with three and seven times as much carbohydrate as protein, respectively. The fat content of the last three diets was low, five per cent or less.

On the basis of combustion *in vitro*, R.Q.'s of 1.0, 0.8 and 0.7 are to be expected from the complete oxidation of carbohydrate, protein and fat, respectively. The value of 0.78 observed in fully fed larvae would be expected on a high protein diet. The low value of 0.65 observed in larvae on low yeast level is too low to be accounted for by the complete oxidation of any food. It may be more readily understood if the tendency of insect larvae to store food material is taken into consideration. A substantial amount of fat is stored in the fat bodies and, in later stages, a rapid increase in glucose and glycogen content has been observed.[14] It is generally believed that stored material is necessary for the completion of metamor-

phosis.[14] The starved larvae, therefore, had to build up fat and carbohydrate reserves from a diet high in protein. Their relatively greater decrease in CO_2 production than in O_2 consumption indicates that some carbon normally excreted is retained, perhaps by incomplete oxidation of protein. Fink[15] using the potato beetle, Cook[13] using termites and Dreyer[12] using ants, all found that starvation or partial starvation caused insects to have a low R.Q. and all offered as the most probable explanation incomplete oxidation of protein and fat.

Since a large part of the stored tissue of insects is located in the fat body, it might be expected on the assumptions just presented that some change could be observed in them. As the table shows, gross changes do take place. Lack of food results in a smaller fat body than is present under conditions of maximal feeding—a result generally obtained in insects and analogous to that found in higher animals, within certain limits. Kollmann[16] working with *Tenebrio molitor* and Fink[15] with the potato beetle observed that albuminoid granules normally present in fat bodies of fed insects disappeared after a period of starvation. Schmeider[17] came to the conclusion that in Hymenoptera there is a relation between nitrogenous inclusions and fat, and suggested intracellular synthesis of protein. Apparently it can be reasonably assumed that starvation does upset the protein and fat metabolism, probably in such a manner and degree as to be a major factor in lowering the R.Q.

The effect of sugar in raising the R.Q. would be expected from the fact that insects fed on a pure carbohydrate diet exhibit an R.Q. of 1.0.[12,13] It might be suspected that its effects are solely due to complete oxidation in catabolic processes, but it seems more probable that, especially in the case of 2.0 per cent added sucrose, there is an effect on the fat and protein metabolisms themselves. In view of the observation mentioned above[14] that a marked increase in glucose and glycogen content takes place in late larval stages, it may be suggested that the sugar supplies the necessary carbohydrate, its oxidation replacing that of protein otherwise resorted to for the synthesis of these substances. A critical test of this hypothesis would require a much more detailed analysis of starvation metabolism than has been presented here.

It remains to point out the general parallelism between the effects of diet on R.Q. and on hormone production. Limiting the amount of yeast available to larvae causes them to produce more v^+ hormone as measured by eye-color value as well as exhibit a relatively low R.Q. as compared with fully fed controls. The addition of sugar to low yeast, while not completely remedying dietary deficiencies, as indicated by pupation delay and medium-sized fat bodies, nevertheless inhibits hormone production and causes a higher R.Q. than is observed for larvae on low yeast alone. Further, the decrease in eye-color value and increase in R.Q. are roughly proportional

to the amount of sugar added. It may be that the incomplete oxidation of protein postulated to account for the low R.Q.'s is also concerned with the increase in hormone production and that the inhibition of hormone production by sugar is secondary to its restorative action on total metabolism. In the low food series with added sugar a further parallel may be drawn if one remembers Beadle's[6] demonstration that wild type fat bodies yield v^+ hormone when transplanted into vermilion brown hosts. The larvae on low yeast only, having the smallest and hence most drastically affected fat bodies, showed the greatest eye-color change while those with added sugar, having the least reduction in fat body size, also showed less v^+ hormone production. As pointed out by Khouvine, Ephrussi and Chevais[7] and by Beadle, Tatum and Clancy,[8] the effect of partial starvation on eye color may be interpreted as a restoration of a reaction chain leading to the formation of v^+ hormone normally broken in the presence of the vermilion gene homozygous. The present discussion is in harmony with their suggestion that protein metabolism may be concerned.

Summary.—It was found that in vermilion brown *Drosophila melanogaster* larvae a low yeast level is associated with a low R.Q. (0.65) and that the addition of sugar is capable of raising the R.Q. up to or above that of fully fed controls (0.79) while still not supplying an optimum amount of nutrient materials.

The production of v^+ hormone was found to be associated with a low R.Q., non-production with a high R.Q. under conditions of the reported experiments.

Changes in protein metabolism were suggested to explain both phenomena, although their interrelationship has not been definitely established.

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