FOOD CONSUMPTION, FEED EFFICIENCY, METABOLIC RATE AND UTILIZATION OF GLUCOSE IN LINES OF *TRIBOLIUM CASTANEUM* SELECTED FOR 21-DAY PUPA WEIGHT

JUAN F. MEDRANO AND G. A. E. GALL

Department of Animal Science, University of California, Davis, California 95616

Manuscript received October 17, 1975

ABSTRACT

Food consumption, feed efficiency, metabolic rate and glucose utilization were studied throughout development in one control (1C) and three selected lines (3, 9, 10) of *Tribolium castaneum* that had been subjected to long term selection for 21-day pupae weight. Growth rate, body composition, cellular growth and the activity of four dehydrogenase enzymes in the same lines have been reported (MEDRANO and GALL 1976).——Larva of selected lines consumed 1.2 times as much food as the control and gained an average of 2.9 times as much weight. The rapid growth of the selected lines was associated with a gross feed efficiency 20 to 30% above that for the control line. There was also a small but consistent improvement in the conversion of digested food. Average digestibility was higher for selected lines.——There was little apparent differentiation between the control and selected lines in metabolic rate/individual, but the rate measured on a per-unit weight basis was two- to three-fold greater for the control during the active growth stages. Respiratory quotients (R.Q.) of 1.0, indicative of carbohydrate oxidation, were observed through larval growth in all lines. Pupae at 21 days showed R.Q. values greater than 1.0, which were interpreted as resulting from a phenomenon in insects in which CO_2 is released by pupae, in large bursts at irregular intervals. The rate constant of glucose oxidation, measured as the rate of C^{14} labelled CO_2 respired during 2- to 6-hour incubation periods, was two- to three-fold higher in the control. In addition, the control line larvae expired 5% to 17% more of the ingested C^{14} as CO_2. It was apparent that control line individuals maintained a much more active turnover of metabolites but without an effective retention of carbon as body substances. The results are discussed in support of the hypothesis that selection for large body size resulted in improved control mechanisms that influence the biological efficiency of growth in Tribolium.

IN a preceding investigation (MEDRANO and GALL 1976) correlated changes in body composition, cellular development and enzyme activities to selection for large 21-day pupae weight in *Tribolium casaneum* were analyzed. Selected lines averaging a two- to three-fold larger size than the control line through development were found to have a higher fat percent content at all ages prior to pupation. No major differences in percent protein were detected and water content had an inverse relationship to fat. The increase in fat throughout the growing period was very closely correlated with developmental rate or the physiological age of the lines. The general pattern of the data indicated that all the lines

reached very similar levels of fat content at pupation. A marked increase in size and number of cells of the selected lines above the control line was revealed. Selected lines' activities of isocitric dehydrogenase and lactic acid dehydrogenase per mg-wt were 60% lower than those of the control.

The present study examines food consumption, feed efficiency, metabolic rates and glucose utilization in the same lines. Evidence is presented to support the contention that genetic selection has effectively changed the control mechanisms that influence the metabolic and physiological capabilities of the organism.

MATERIALS AND METHODS

The study was conducted with three lines of *Tribolium castaneum* selected for large 21-day pupa weight (lines 3, 9, and 10), and a randomly selected control line (line 1C) originating from the same base population (GALL 1970). Their response over the first 30 generations was described by GALL (1971). The expansion and sampling procedure of the lines were described in an earlier report (MEDRANO and GALL 1976). Food intake was assessed at generation 64 in lines 3, 10 and 1C, and generation 65 in line 9. The lines had been maintained to this point following the procedure for suspended selection described by GALL (1971). Selection was suspended at generations 31, 32 and 53 in lines 3, 10 and 9, respectively. The organisms were kept in 4 oz. Mason jars in a medium consisting of 90% unbleached white wheat flour and 10% dried brewers yeast at 33° and 70% relative humidity.

Analytical Procedures

Food intake and utilization indices were measured in 10 replicate samples of each line for the following 2-day time intervals: 8 to 10, 10 to 12, 12 to 14, and 14 to 16 days of age. Data were collected utilizing a fecal uric acid method proposed by BHATTACHARYA and WALDBAUER (1969a,b, 1970). The method provides an indirect estimate of the amount of fecal material in the media.

In each replicate, 25 larvae were allowed to feed on approximately 100 mg of medium for a 48-hour period. Amount of food consumed and weight gained were determined by difference after drying the samples at 105° for 24 hours. Dry weight at the beginning of the feeding trial was determined using an equivalent sample of larvae. Small samples of feces were collected by allowing samples of larvae to sit in a clean aluminum dish for 2 hours.

The leftover medium and the feces were extracted with 10 ml of a 0.6% solution of lithium carbonate. An aliquot was then analyzed for uric acid by an automated procedure with a Technicon Auto-Analyzer following a method adapted from that described by HAWK, OSER and SUMMERSON (1926). In a separate trial it was determined that the method extracted and assessed uric acid with an 88% efficiency; consequently, the data were corrected for this value.

Since Tribolium medium contains no uric acid, the weight of feces present was calculated as:

$$\text{Wt feces} = \frac{\text{mg uric acid in medium}}{\text{mg uric acid/mg feces}}$$

and the weight of food consumed was estimated as:

Food intake (mg) = initial wt medium — (final wt medium — wt feces). A more objective assessment of food intake adjusted for the differences in body size among the lines was calculated as a consumption index (C.I.) suggested by WALDBAUER (1968):

C.I. = daily dry wt food intake/mean wet wt of animals during feeding period.

Three parameters based on food intake and weight gain were utilized to describe efficiency of feed conversion and digestibility (WALDBAUER 1968): Efficiency of conversion of ingested food to body substance (E.C.I.):

E.C.I. = 100 × wt gained/wt food intake;

efficiency of conversion of digested food to body substance (E.C.D.):

E.C.D. = 100 × wt gained/(wt food intake —wt feces);

and approximate digestibility (A.D.):

A.D. = 100 × (wt food intake — wt feces)/wt food intake.

Metabolic rate was evaluated from 10 to 25 days of age by the direct measurement of oxygen consumption and CO_2 production with a Warburg respirometer following the procedure outlined by UMBREIT, BURRIS and STAUFFER (1964). All incubations were performed at 33° in 25 ml flasks containing approximately 150 mg of medium and 30, 25, 15 and 15 larvae at ages 10, 12, 14, and 16 days, respectively; 15 larvae in lines 10 and 1C, and 15 pupae in lines 3 and 9 at 19 days; 25 pupae at 21 days and 15 adults at 25 days of age. To measure oxygen consumption and CO_2 production, 0.2 ml of 20% KOH was utilized to absorb the CO_2. Total oxygen consumption and CO_2 production were expressed in ul/hr/animal, and in ul/hr/mg wet wt. Respiratory quotients were calculated as the ratio of CO_2 produced to oxygen consumed.

Rate of glucose utilization and percent of ingested carbon expired as CO_2 were assessed by feeding radioactive [U-C^{14}] glucose labelled flour to larvae and measuring the radioactivity in respired CO_2 and in body tissue after a period of incubation.

The test diet contained 0.43 uCi of [U-C^{14}] glucose per 100 mg of medium. Ten grams of diet were prepared by making a slurry of 1.0 g of flour in 1.0 ml of 95% ethanol and 0.05 ml of 25% ethanol solution containing 1.0 mCi of [U-C^{14}] glucose per ml. The radioactive slurry was dried in an oven at 56° for 90 minutes, then gently powdered with a spatula and passed through a 60-mesh sieve. The remainder of the diet, 8.0 g of flour and 1.0 g of yeast, was added and thoroughly mixed by tumbling in a glass jar.

At 10, 12, 14, 16 and 25 days of age, animals were weighed in groups of 30, 25, 15, 15 and 15 individuals, respectively, and placed in 25 ml Erlenmeyer flasks containing 100 to 150 mg of labelled diet which had been equilibrated for at least 12 hours in an incubator at 33° and 70% relative humidity. Each flask was then returned to the incubator after being sealed with a self-sealing rubber serum cap fitted with a plastic center well containing a 1.5 cm^2 folded piece of Whatman 1 filter paper. The incubation was continued for periods of 1, 2, 3, 4, 5, and 6 hours with four replicate samples for each line, age and time period.

Fifteen minutes before the end of an incubation period, 0.2 ml of hydroxide of hyamine-10X were added to the plastic center well to collect the CO_2 produced. The incubation was terminated and CO_2 liberated from the medium by injecting 0.2 ml of chloroform in 1.0 ml of 1N sulfuric acid into the flask. The flasks were allowed to sit at room temperature for 1 hour with occasional slow shaking to ensure complete uptake of CO_2. The larvae were thoroughly washed with distilled water and allowed to air dry before being placed in scintillation vials containing 2.0 ml of a 3:1 mixture of hydroxide of hyamine-10X and 30% KOH. Atfer a 24-hour period of initial digestion at room temperature, the samples were heated with occasional shaking for 45 minutes at 70°, allowed to cool, five drops of 30% hydrogen peroxide were added and the samples reheated for 30 minutes. After cooling to room temperature, 1.0 ml of glacial acetic acid and 10 ml of scintillation fluid for aqueous samples (5.0 g of omniflour in 500 ml of Triton-X-100 and 1000 ml of toluene) were added. The samples were then allowed to sit for 48 hours before counting with a liquid scintillation spectrometer. Internal standards were used to correct for quenching effects.

The rate constant of glucose oxidation was estimated by the slope of the linear regression of CO_2 production on incubation time, and the percent of total ingested C^{14} expired as CO_2 as: $100 \times$ total cpm CO_2/(total cpm CO_2 + total cpm in animal body).

RESULTS

Food intake and utilization: The results of comparisons made of food intake, weight gain, efficiencies of feed conversion and digestibility are presented in Table 1. Analyses of variance are presented in Table 2.

The dry weight-fresh weight consumption index (C.I.) calculated from dry weight of food eaten and fresh weight of animal is an indicator of relative intake of nutrients. The index as calculated underestimates absolute amount of food consumed due to the water content of the medium (11.6% water after equilibrating for 72 hours at 33° and 70% relative humidity).

TABLE 1

Consumption index (C.I.), average initial dry body weight, average daily dry weight gain, efficiency of conversion of ingested (E.C.I.), and digested (E.C.D.) food calculated on dry weight basis, and approximate digestibility (A.D.)

Trait	Line	Age interval (days)			
		8 to 10	10 to 12	12 to 14	14 to 16
C.I.	9	1.07	1.06	1.00	0.56
	10	1.08	1.16	0.89	0.49
	3	1.07	1.08	0.94	0.47
	1C	0.84	0.93	0.74	0.80
Average initial dry body weight (mg)	9	0.06	0.18	0.47	1.19
	10	0.06	0.18	0.43	1.25
	3	0.05	0.15	0.40	1.12
	1C	0.04	0.10	0.20	0.42
Average daily dry body weight gain (mg)	9	0.05	0.15	0.37	0.30
	10	0.05	0.15	0.27	0.20
	3	0.04	0.10	0.28	0.24
	1C	0.02	0.04	0.07	0.13
E.C.I. (%)	9	13.3	13.6	16.6	14.6
	10	12.5	13.1	15.4	11.9
	3	12.6	12.0	15.8	15.7
	1C	13.5	10.4	12.3	11.8
E.C.D. (%)	9	18.1	17.8	24.1	22.8
	10	18.1	18.5	22.4	21.1
	3	19.9	17.3	23.4	27.9
	1C	23.4	16.7	18.8	22.3
A.D. (%)	9	74	77	69	64
	10	69	71	69	57
	3	64	69	67	57
	1C	58	63	66	54

The C.I. value for the control line was consistently and significantly ($P < 0.01$) lower than for the selected lines for the periods from 8 to 14 days of age when the selected lines realized their highest weight gain. Comparing dry weight gain in relation to the initial dry body weight from 8 to 10, 10 to 12 and 12 to 14 days, the relative weight gain of the three selected lines averaged 1.6, 1.9 and 2.1 times that of the control during the three periods and consumed an average of only 1.3, 1.2 and 1.3 times as much food. But in the period from 14 to 16 days when the selected lines were growing at a decreasing rate and approaching pupation (MEDRANO and GALL 1976), the control line consumed 1.6 times more food per unit weight ($P < 0.01$) then the average of the selected lines and realized a relative weight gain only 1.5 times as much as the selected lines. These comparisons indicated a superior efficiency of the selected lines. Among the selected lines, significant ($P < 0.05$) differences in consumption were observed in the

TABLE 2

Mean squares showing the linear contrast of control (ctrl.) versus *selected (sel.) lines and a linear contrast among the three selected lines on consumption index (C.I.), average daily dry weight gain, efficiency of conversion of ingested (E.C.I.), and digested (E.C.D.) food, and approximate digestibility (A.D.)*

Source	df	Mean squares				
		C.I.	Dry wt. gain	E.C.I.	E.C.D.	A.D.
8 to 10 days						
Ctrl. *vs.* sel.	1	0.2166**	0.0025**	1.62	78.75*	463**
Among selected	2	0.0001	0.0004**	1.63	10.60	207**
Error	28	0.0205	0.00001	2.98	12.54	12
10 to 12 days						
Ctrl. *vs.* sel.	1	0.2100**	0.0635**	45.89**	11.01*	662**
Among selected	2	0.0275	0.0083**	6.87**	3.81	145**
Error	36	0.0123	0.0001	1.50	3.34	17
12 to 14 days						
Ctrl. *vs.* sel.	1	0.2970**	0.4225**	97.58**	155.27**	43**
Among selected	2	0.0280*	0.0315**	4.18**	7.35	9
Error	36	0.0068	0.0008	0.90	3.21	4
14 to 16 days						
Ctrl. *vs. sel.*	1	0.6381**	0.1062**	37.80**	19.68	225**
Among selected	2	0.0227*	0.0243**	36.86**	126.21**	168**
Error	35	0.0065	0.0027	4.96	23.73	22

* $P < 0.05$.
** $P < 0.01$.

two periods from 12 to 16 days. The difference was due mainly to a higher value of line 9, the line that also realized the highest weight gains during this stage.

No differences in efficiency of conversion of ingested food (E.C.I.) were detected among the lines in the initial period from 8 to 10 days. The control line showed an unexpectedly high value that does not seem compatible with the gain, food intake, and digestibility observed during that period or the E.C.I. observed in later periods. The result may be due to sampling error since experimental loss reduced the sample size to four replicates in the control line as compared to 10 for all other determinations. Therefore, limited confidence can be placed in the parameters estimated for the 8 to 10 day interval in line 1C.

In the three periods from 10 to 16 days the selected lines differed significantly ($P < 0.01$) among themselves in E.C.I. values, and were consistently more efficient than the control line. Line 9 had the highest E.C.I. values over the periods from 10 to 12 days and 12 to 14 days, and also gained the most weight. In contrast, from 14 to 16 days line 10 dropped in both efficiency (E.C.I.) and rate of gain. In general, E.C.I. and rate of gain showed a strong positive relationship. A similar relationship has been reported in mice (Falconer 1960; Fowler 1962; Rahnefeld et al. 1965; Lang and Legates 1969; Timon and Eisen 1970; Sutherland *et al.* 1970; Meyer and Bradford 1974).

The selected lines differed from the control in efficiency of conversion of digested food (E.C.D.) in the three periods from 8 to 14 days. Line 1C had a higher E.C.D. from 8 to 10 days but, as mentioned earlier, this value seems incompatible with the rest of the data. In the two periods from 10 to 14 days the selected lines excelled in E.C.D. with the most significant ($P < 0.01$) differences occurring at 12 to 14 days when the lines realized very high gains.

The approximate digestibility (A.D.) of food showed a marked decline beyond the 10 to 12 day period. However, the selected lines had consistently higher values than the control at all ages. Differences among selected lines resulted from the tendency of line 9 to show a higher digestibility than the other two lines. Digestibility directly affects E.C.I. since it is one of the processes involved in food utilization. For example, the apparent differences in E.C.I. among the selected lines at 10 to 12 days may be a reflection of differences in digestibility.

Metabolic rate: There was no consistent differentiation between control and selected lines in metabolic rate measured as average O_2 consumption per individual (Figure 1). During the active growth period from 10 to 14 days, metabolic rate in the selected lines paralleled body size. Line 10, a slower developing line, maintained an active respiration as well as an increasing growth rate through day 16, whereas lines 3 and 9 reached peak growth rates and metabolic rates at day 14. Thereafter, the lines declined to a low metabolic rate at day 21. The control line, although sustaining active growth through day 16, also declined in metabolic rate after day 14 but at a slower rate than lines 3 and 9. The 19-day pupae assessed in lines 3 and 9, as expected due to their reduced activity, consumed less oxygen than the 19-day larvae assessed in lines 10 and 1C. At 21 days significant differences were detected among the lines although their ranking

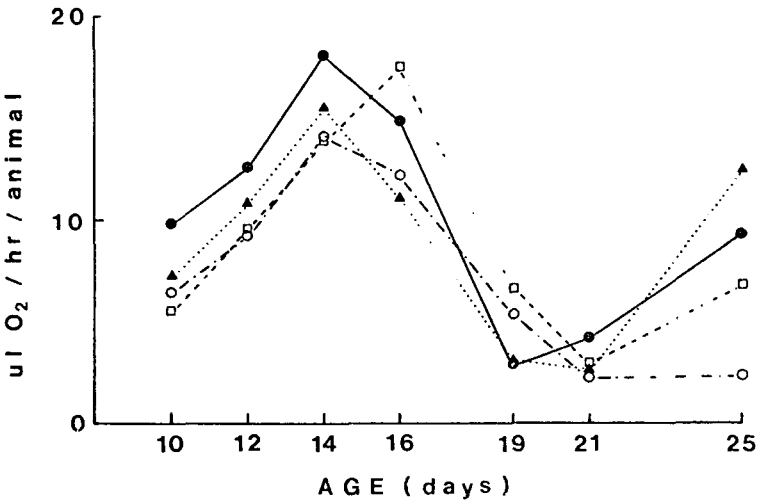

FIGURE 1.—Metabolic rate per animal measured as μl of oxygen consumed/hr/animal in Tribolium selected lines 9 (●——●), 10 (□---□), and 3 (▲···▲), and control line 1C (○-·-○).

in metabolic rate was directly correlated to body size. The apparent differentiation of the lines as adults at 25 days, on the other hand, showed no relationship to body size.

In contrast to the metabolic rate per animal, metabolic rate expressed per unit body size (Figure 2) decreased throughout the larval stage. On the average, O_2 consumption/mg was two- to three-fold greater for the control line than for the large selected line individuals during the active growth period. No differentiation was apparent among the lines beyond 16 days of age.

Respiratory quotients: The R.Q. is useful as a general indicator of the nature of animal metabolism (KLEIBER 1961). Average R.Q. values from 10 to 25 days in Tribolium are presented in Table 3. Although variable, values were similar in both control and selected lines from 10 to 16 days. All R.Q. values exceeded 0.9 with some exceeeding 1.0 at 14 and 16 days. The general observation of values near 1.0 is indicative of carbohydrate oxidation during active growth. The R.Q. values close to 0.8 observed in larvae of lines 10 and 1C at day 19 reflected their approach to pupation and suggested oxidation of protein and fat as energy sources. At the same age, a similar R.Q. value of 0.76 was observed for pupae of line 3, whereas the value of 0.68 in pupae of line 9 seemed to indicate reliance on fat as a main energy source.

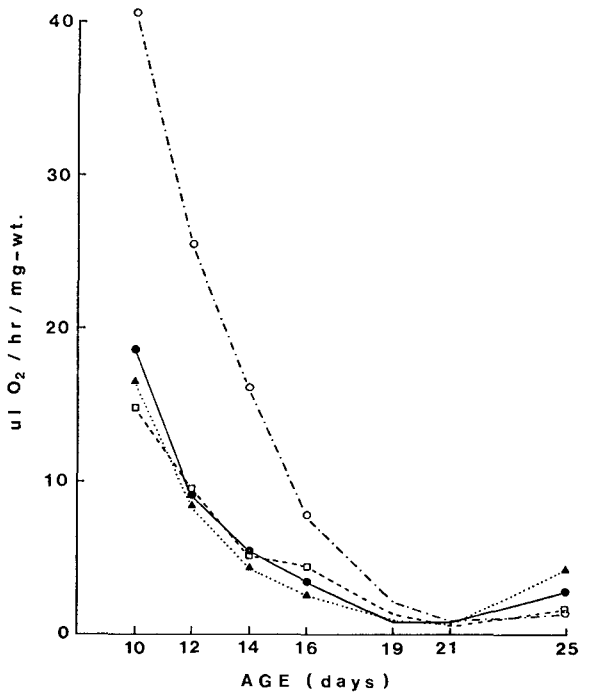

FIGURE 2.—Metabolic rate per unit weight measured as μl of oxygen consumed/hr/mg body weight in Tribolium selected lines 9 (●——●), 10 (□---□), and 3 (▲···▲), and control line 1C (○-·-○).

TABLE 3

Respiratory quotients (R.Q. = CO_2/O_2) of Tribolium at different ages

Line	Age (days)						
	10	12	14	16	19	21	25
9	0.98	0.98	0.98	1.18	0.68	1.67	1.03
10	1.00	1.00	1.12	1.03	0.78	1.61	0.84
3	0.97	0.98	1.13	0.94	0.76	1.82	1.00
1C	0.99	0.98	1.08	0.94	0.80	1.50	0.86

Observations at day 19 contrast with R.Q. values for 21-day pupae which were all considerably above 1.0. R.Q. values above 1.0 were observed by MELAMPY and WILLIS (1939) during the larval stage in honeybees and by BLEIBTREU in 1901, as quoted by KLEIBER (1961), in geese consuming large amounts of grain. Both authors attributed the effect to increased CO_2 production caused by the synthesis of oxygen-poor lipids from carbohydrate which contained relatively more oxygen. Although the lipid content of 21-day pupae (MEDRANO and GALL 1976) in the four lines was high (18.7% fat on the average), it does not seem plausible to apply the above interpretation to the observed R.Q. values particularly when the organisms were not increasing in body weight or consuming any food. More likely, it seems that the observed values may be explained as resulting from a phenomenon of cyclic, gigantic releases of CO_2 that has been observed in several species of insects at rest (PUNT, PARSER and KUCHLEIN 1957) and in pupae of Lepidoptera (SCHNEIDERMAN and WILLIAMS 1953; BUCK and KEISTER 1955). As indicated by BUCK and KEISTER (1958) the phenomenon seems to be widespread in all insects under conditions where oxygen supply is high relative to demand. SCHNEIDERMAN and WILLIAMS (1953) observed R.Q. values varying from 0.1 to 3.0 during successive hours in diapausing pupae of Cecropia silkworm. They found that a continuous oxygen uptake was maintained, but CO_2 was released discontinuously in "bursts" every few hours. BUCK and KEISTER (1955) have indicated that a mechanical stimulus, such as handling of the pupae in weighting and insertion in the respirometer flasks may trigger the CO_2 release, and that in some cases these "bursts" can be delayed for up to 1 hour after handling. An increase in temperature has also been demonstrated (SCHNEIDERMAN and WILLIAMS 1955) to have the effect of increasing burst frequency as well as the interburst rate of release. There is no direct evidence from the literature that this phenomenon occurs in Tribolium pupae, nevertheless, in the present study the experimental conditions were appropriate for the expression of such a behaviour. The pupae were removed from an incubator kept at 33° and weighed. They were then kept at room temperature (approximately 19°) from 3 to 4 hours prior to being placed in the respirometer flask where they were allowed to equilibrate for 1 hour at 33° before CO_2 and O_2 were measured. As indicated above, the handling processes and the temperature gradients the organisms were subjected to may have triggered a synchronized response enhancing the CO_2 released in comparison to oxygen intake during the hour that CO_2 was measured.

It should be pointed out that although the CO_2 "bursts" occur, SCHNEIDERMAN and WILLIAMS (1953) have shown that if respiratory exchange is measured for a long period of time the average R.Q. still is around 0.7, as expected for fatty acid oxidation during the pupal stage (see review by AGRELL and LUNDQUIST 1973). This would indicate that the CO_2 "bursts" do not cause any peculiar changes in the intermediary metabolic functions during the pupal stage, but as suggested by BUCK and KEISTER (1955) they may be a consequence of a response of the spiracles to minimize transpiratory water loss.

At 25 days, adults in the early developing lines 3 and 9 appeared to have initiated feeding earlier and relied on carbohydrate oxidation for energy (R.Q. values of 1), whereas the later developing lines 10 and 1C at 25 days, perhaps still a little immature, relied on stored protein and fat as main sources of energy.

Glucose utilization: When an organism is given a radioactive labelled compound such as [U-C^{14}] glucose in the feed, the compound must be digested, incorporated in the system, equilibrated with the organism's metabolic pool of glucose, and then metabolized before the label is recovered as CO_2. By regressing the amount of labelled CO_2 production on incubation time it is possible to obtain comparative estimates of rate of glucose oxidation for the lines. The slope of the regression line will be a composite estimate incorporating effects at all the steps participating in the process of glucose utilization. However, it is also a good indicator of the rate constant of glucose oxidation in the system, i.e., the rate at which it is metabolized and passed through the system.

The apparent constant for glucose oxidation for each line during the active growth stage is presented in Table 4 as the regression of CO_2 produced, in cpm/mg, on incubation time from hours 2 to 6. The mean cumulative CO_2 production in consecutive incubation hours is also presented for comparison. The

TABLE 4

Mean CO_2 production at cumulated hourly intervals ($n = 4$), after allowing Tribolium larvae to feed on U-C^{14} glucose labelled flour, and the rate constant of glucose oxidation as the regression of CO_2 production on incubation hours 2 to 6 ($df = 19$)

Age	Line	Incubation hours						Regression	
		1	2	3	4	5	6	b ± S.E.	r
Days		cpm/mg-tissue						cpm/mg/hr	
10	9	3	9	80	204	404	504	131 ± 12	0.84
	10	3	11	62	114	233	328	81 ± 8	0.91
	3	11	53	147	282	466	437	109 ± 18	0.82
	1C	20	53	277	713	1041	1514	369 ± 23	0.96
12	9	1	17	67	141	371	303	87 ± 23	0.73
	10	1	6	38	146	245	279	75 ± 8	0.83
	3	3	27	163	191	291	336	75 ± 9	0.81
	1C	6	83	230	425	509	873	186 ± 24	0.91
14	9	1	4	50	183	206	373	89 ± 12	0.89
	10	1	5	41	139	173	235	59 ± 5	0.77
	3	0	14	78	80	193	238	56 ± 9	0.89
	1C	3	31	156	443	727	942	239 ± 16	0.98

TABLE 5

Average percent and mean squares showing the linear contrast of control (ctrl.) versus *selected (sel.) lines and a linear contrast among the three selected lines for total ingested C^{14} expired as CO_2 for ages 10, 12 and 14 days (values were calculated from incubation hours 4, 5 and 6)*

Line	Age (days) 10	12	14
	%	%	%
9	60	44	35
10	50	50	36
3	56	42	34
1C	67	55	43

Source	df	Mean squares 10	12	14
Ctrl. *vs.* sel.	1	1437.70**	1344.47**	525.14**
Among selected	2	89.63**	9.03	26.37
Error	44	16.04	19.25	14.52

** $P < 0.01$.

selected lines had consistently and significantly ($P < 0.001$) two- to three-fold lower rates of glucose oxidation than control at the three ages studied. No significant differences among the selected lines were detected except at day 14 where line 9 had a higher rate of oxidation than the other two lines. In addition, absolute amount of CO_2 produced during the 6-hour period was significantly lower for the selected lines than for the control.

These results indicate that the control line moved substrates through its metabolic system at a rapid rate but without achieving the growth observed for the selected lines. This suggests that the control line indulges in wasteful energy production, i.e., it produces little utilizable energy for growth but simply releases heat from substrates. The latter conclusion was supported by differences in the percent of total ingested carbon expired as CO_2 (Table 5). The selected lines expired significantly ($P < 0.01$) less CO_2 than controls from 10 to 14 days retaining a higher proportion of ingested carbon for synthesis of body substance. Line 10 retained more carbon than the other two selected lines at day 10 but tended to retain less at day 12. Rate of glucose utilization and proportion of ingested carbon expired as CO_2 were also measured at 16 and 25 days. No differences among lines were detected in CO_2 expired after 14 days of age.

DISCUSSION

Metabolic body size: All living organisms are transformers of energy. The participation of all the cells of the organism in this process results in a direct association between the intensity of energy transformation and changes in body weight (KAYSER and HEUSNER 1964).

KLEIBER (1961) from his earlier work, concluded that the limits for the relation between body size and metabolic rate in animals lies between the positive correlation of metabolic rate/animal and body weight and the negative correlation of metabolic rate/unit weight and body weight. Within these limits, through comparative studies, KLEIBER showed that in homeotherms metabolic rate is proportional to the 3/4 power of body weight. This unit of metabolic body size has been very useful in homeotherms in the evaluation of food intake, feed efficiency, level of metabolism, etc. independent of body size.

In insects, although extensive work has been done in the area of respiratory metabolism, there is no absolute consensus on the appropriate exponent to relate body weight and rate of metabolism. BERTALANFFY (1957) distinguished three basic metabolic types in animals. One of his classifications was metabolic rate proportional to body weight. He considered this type to be characteristic of organisms with an exponential growth curve such as growing insect larvae and hemimetabolous insects. He also indicated that this relationship was appropriate in comparing pupae of different but related species. EDWARDS (1953) indicated the exponent to be 1.0 for larvae of holometabolous insects which include coleoptera. LUDWIG in 1937, as quoted from KAYSER and HEUSNER (1964), also proposed an exponent of 1.0 for invertebrates with tracheal respiration. ZEUTHEN (1953) indicated an exponent of 0.95 for invertebrates of less than 40 mg. This size range would include most insects although his work was done with larvae of marine organisms. TESSIER (1931), working with *Tenebrio molitor*, found an exponent of 0.7 for basal metabolism, but when active larval growth was considered the exponent increased to 0.95. KAYSER and HEUSNER (1964) presented some unpublished results of STUSSI and HEUSNER. For hymenoptera they reported an exponent of 0.92 with a 95% confidence interval of $0.82 \leq b \leq 1.02$, and suggested a value of 0.77 ($0.45 \leq b \leq 1.09$) for coleoptera. The developmental stage of the organisms was not stated.

In addition, KAYSER and HEUSNER (1964, quoted in free translation) have indicated that there are two aspects to the relation of weight to metabolic intensity. One is the case in which the increase of weight is due to the increase in cell volume. Since O_2 penetrates the cell through the surface, and since surface area grows proportionally to the square of the linear dimensions between large and small animals, the use of energy would be proportional to weight to the 2/3 power. The other aspect is the case in which the increase in weight is due to a cellular multiplication with the cellular volume remaining constant. Then in comparing animals of different size the energy utilization would be proportional to the power of 1.0. ELLENBY (1953) investigated the relationship between oxygen consumption and cell size by comparing diploid and triploid prepupae of *Drosophila melanogaster*. The two groups of animals had the same body weight, but the cells of the triploids were 1.5 times the size of those of the diploid. The rates of O_2 consumption per mg tissue per hour were the same for both groups, indicating the cell size had no influence on general level of metabolism.

Generalizing from the conclusions of ELLENBY (1953) and KAYSER and HEUSNER (1964), the assumption of an exponent of 1.0 appears to be justified.

Insect growth is observed to result from increases in both cell number and cell size, but since the latter does not influence metabolic rate, the relationship between size and metabolic rate should be directly related to body mass.

The rate of oxygen utilization in the present investigation, having been measured on groups of individuals, but did not permit accurate estimation of an appropriate exponent. The magnitude of the differences in size between selected and control lines demanded that a correction for weight be made to allow any valid comparisons. Consequently, an exponent of 1.0 was assumed for Tribolium in lieu of any other information on this species. The data presented in Figures 1 and 2, expressing respiration rate per animal and per unit body size, represent the outer limits of the relationship between body weight and rate of metabolism as suggested by KLEIBER (1961).

General considerations: Genetically controlled mechanisms that have a regulatory influence on the metabolic and biosynthetic capabilities of Tribolium were subject to change through the process of artificial selection for 21-day pupae weight. The remarkable differentiation in growth rates and size between the selected lines and their base population was a key feature in allowing an assessment of possible differences in biosynthetic activity.

In a preceding paper (MEDRANO and GALL 1976) it was indicated that genetic factors influencing the control of developmental mechanisms markedly increased the number and size of cells in selected individuals. Significant changes in fat and water deposition with age were also noted. At 21 days the final fat content was similar among the lines, although the selected lines contained significantly more (1.6%) fat than the control.

Larvae of the large selected lines consumed more food per unit weight (C.I.) throughout the active growth stages and utilized it more efficiently than the control. TIMON and EISEN (1970) interpreted a similar measurement of food intake in mice as indicating a change in appetite. The high demands for growth in a limited period of time by the selected lines not only resulted in considerable improvement in gross efficiency (E.C.I.) but also in efficiency of utilization of digested food (E.C.D.). The net effect of the improved efficiencies was that the selected lines showed a marked reduction in food intake per unit of body weight. This relationship, particularly during periods of rapid growth, tends to indicate that the large lines decreased the demand for food to supply their maintenance requirements, resulting in more food being utilized for growth functions. The efficiency of conversion of digested food is of particular importance in arriving at this conclusion because it is not directly affected by digestibility. It depends mainly on factors affecting the proportional amounts of digested food utilized for growth and maintenance.

In addition, the conclusion of a decreased maintenance cost associated with increases in weight gain and feed efficiency is supported by the low energy expenditure, reflected in the relative rates of oxygen consumption (Figures 1 and 2) and the lower proportion of ingested carbon expired as CO_2 (Table 4) observed for the large selected lines. One of the most significant differences between the

selected and control groups was the striking divergence in rate of metabolism per unit body size, since it demonstrates the direct effect of selection on metabolic regulation. If selection had not achieved an accumulation of genes associated with effective regulation of metabolism, the large difference in the size of the individuals, and the concomitant differences in cell number, would lead to the expectation that oxygen consumption per individual in the large selected lines would be two- to three-fold greater than in the control line. This was observed by MELLAMPY and WILLIS (1939) in a comparison of respiratory metabolism of the female honeybee (*Apis mellifica*) during larval and pupal development; queens that were approximately twice as big as the workers utilized, according to expectations, two to three times more oxygen than the workers, resulting in similar metabolic rates per unit body size. The present observations on individuals genetically different in body weight clearly indicate that on a per-individual basis the control and selected line large utilized comparable amount of oxygen whereas when expressed on an equivalent basis, per unit weight, the rate of respiratory metabolism was two- to three-fold lower in the selected lines throughout the active growth stages.

Moreover, the rate constant of glucose oxidation was markedly higher for the control line indicating these individuals maintain a much more active turnover of metabolites without sufficient retention to achieve a higher growth rate. The individuals seem to indulge in the wasteful utilization of energy, perhaps as indicated by MILLIGAN (1971), through a pump-leak system of ion transfer, which ". . . would be a case of complete inefficiency of energy utilization, as all of the energy of the nutrients degraded to serve the cost of the transport component would appear as heat". Selection for large size, on the other hand, improved efficiency of energy utilization probably through greatly reducing the energetic costs in the turnover of cell constituents and the maintenance of ionic distributions.

Growth in selected lines of Tribolium is characterized by a rapid synthesis of structural proteins, and by lipogenesis. Selection for fast growth rate appears to have increased the frequency of genes which control some functional mechanisms to increase biosynthetic capacity with minimal energy waste as well as genes which regulate the proliferation and size of cells during development of the organism.

We wish to thank DR. R. L. BALDWIN and DR. Y. T. YANG for their helpful criticism during the preparation of this manuscript, and BARBARA B. MEDRANO for her valuable technical assistance.

LITERATURE CITED

AGRELL, I. P. S. and A. M. LUNDQUIST, 1973 Physiology and biochemical changes during insect development. In: *The Physiology of Insecta*, edited by M. ROCKSTEIN. Vol. I , Academic Press, New York.

BERTALANFFY, L. VON, 1957 Quantitative laws in metabolism and growth. Quart. Rev. Biol. **32**: 217–231.

BHATTACHARYA, A. K. and G. P. WALDBAUER, 1969a Quantitative determination of uric acid in insect feces by lithium carbonate extraction and the enzymatic-spectrophotometric method. Ann. Entom. Soc. Am. **62**: 925–927. ——, 1969b Faecal uric acid as an indicator in the determination of food utilization. J. Insect Physiol. **15**: 1129–1135. ——, 1970 Use of faecal uric acid method in measuring the utilization of food by *Tribolium confusum*. J. Insect Physiol. **16**: 1983–1990.

BUCK, J. and M. KEISTER, 1955 Cyclic CO_2 release in diapausing Agapema pupae. Biol. Bull. **109**: 144–163. ——, 1958 Cyclic CO_2 release in diapausing pupae—II Trachael anatomy, volume and pCO_2; blood volume; interburst CO_2 release rate. J. Insect Physiol. **1**: 327–340.

EDWARDS, G. A., 1953 Respiratory metabolism. In: *Insect Physiology*, edited by K. D. ROEDER. Wiley, New York, 1100 pp.

ELLENBY, C., 1953 Oxygen consumption and cell size. A comparison of the rate of oxygen consumption of diploid and triploid prepupae of *Drosophila melanogaster* Meigen. J. Exp. Biol. **30**: 475–491.

FALCONER, D. S., 1960 Selection of mice for growth on high and low plane of nutrition. Genet. Res. **1**: 91–113.

FOWLER, R. C., 1962 The efficiency of food utilization, digestibility of foodstuffs and energy expenditures of mice selected for large or small body size. Genet. Res. **3**: 51–68.

GALL, G. A. E., 1970 Biochemical genetics of *Tribolium*. I. The foundation population. J. Exp. Zool. **173**: 225–230. ——, 1971 Replicated selection for 21-day pupa weight of *Tribolium castaneum*. Theor. Appl. Genetics **41**: 164–173.

HAWK, P. B., B. L. OSER and W. H. SUMMERSON, 1926 *Practical Physiological Chemistry* (9th ed.). P. Blakiston's Co., Philadelphia.

KAYSER, CH. and A. HEUSNER, 1964 Étude comparative du métabolisme énergetique dans la série animale. J. Physiol. Paris **56**: 489–524.

KLEIBER, M., 1961 *The Fire of Life: An Introduction to Animal Energetics*. John Wiley & Sons, New York, 454 pp.

LANG, B. J. and J. E. LEGATES, 1969 Rate, composition and efficiency of growth in mice selected for large and small body weight. Theor. Appl. Genetics **39**: 306–314.

MEDRANO, J. F. and G. A. E. GALL, 1976 Growth rate, body composition, cellular growth and enzyme activities in lines of *Tribolium castaneum* selected for 21-day pupa weight. Genetics **83**: 379–391.

MELAMPY, R. M. and E. E. WILLIS, 1939 Respiratory metabolism during larval and pupal development of the female honeybee (*Apis mellifica* L.). Phys. Zool. **12**: 302–311.

MEYER, H. H. and G. E. BRADFORD, 1974 Estrus ovulation rate and body composition of selected strains of mice on ad libitum and restricted feed intake. J. Anim. Sci. **38**: 271–278.

MILLIGAN, L. P., 1971 Energetic efficiency and metabolic transformations. Fed. Proc. **30**: 1454–1458.

PUNT, A., W. J. PARSER and J. KUCHLEIN, 1957 Oxygen uptake in insects with cyclic CO_2 release. Biol. Bull. **112**: 108–119.

RAHNEFELD, G. W., R. E. COMSTOCK, W. I. BOYLAN and M. SINGH, 1965 Genetic correlations between growth rate and feed per unit gain in mice. J. Anim. Sci. **24**: 1061–1066.

SCHNEIDERMAN, H. A. and C. M. WILLIAMS, 1953 The physiology of insect diapause. VII. The respiratory metabolism of the Cecropia silkworm during diapause and development. Biol. Bull. **105**: 320–334. ——, 1955 An experimental analysis of the discontinuous respiration of the Cecropia silkworm. Biol. Bull. **109**: 123–143.

SUTHERLAND, T. M., P. E. BIONDINI, L. H. HAVERLAND, D. PETTUS and W. B. OWEN, 1970 Selection for rate of gain, appetite and efficiency of feed utilization in mice. J. Anim. Sci. **31:** 1049–1057.

TESSIER, G., 1931 Recherches morphologiques et physiologiques sur la croissance des Insectes. Trav. Stat. Biol. Roscoff **9:** 29–238.

TIMON, V. M. and E. J. EISEN, 1970 Comparison of *ad libitum* and restricted feeding of mice selected and unselected for post weaning gain. I. Growth, feed consumption and feed efficiency. Genetics **64:** 41–57.

UMBREIT, W. W., R. H. BURRIS and J. F. STAUFFER, 1964. *Manometric Techniques* (4th ed.). Burgess Publishing Company, Minneapolis.

WALDBAUER, G. P., 1968 The consumption and utilization of food by insects. Adv. Insect Physiol. **5:** 229–288.

ZEUTHEN, E., 1953 Oxygen uptake as related to body size in organisms. Quart. Rev. Biol. **28:** 1–12.

Corresponding editor: R. W. ALLARD