GROWTH RATE, BODY COMPOSITION, CELLULAR GROWTH AND ENZYME ACTIVITIES IN LINES OF *TRIBOLIUM CASTANEUM* SELECTED FOR 21-DAY PUPA WEIGHT

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ABSTRACT

Growth rate, body composition, cell number, cell size, and the activity of four dehydrogenase enzymes were studied from 10 to 25 days of age in one control (1C) and three lines (3, 9, 10) of *Tribolium castaneum* that had been subjected to long term selection for large 21-day pupae weight.—Selected lines were two- to three-fold larger in size than the control line throughout development. No major differences in percent of protein were detected among the lines but at any particular age, the selected lines were found to have a higher fat content than the control line. The differences in fat content were closely correlated with development such that all the lines reached very similar levels of percent of fat just prior to pupation. Water content showed an inverse relation to percent of fat.—Selection was observed to have caused major changes in the cellular response to growth. The selected lines had an average of from 17% to 48% larger cells (measured as protein/DNA) and were found to have from 37 to 62% more cells (measured as total DNA) than the control line at all ages from 10 to 19 days of age. In addition, the selected lines had a higher RNA content at all ages studied and higher RNA:DNA ratios at the young ages. In contrast the enzyme activities of ICDH and LDH were 60% lower. The results are interpreted as indicating that a more efficient metabolic machinery had evolved in the rapidly growing selected lines.

A number of experiments involving selection for weight at different stages of development of *Tribolium castaneum* have demonstrated that numerous points along the growth curve are under genetic control (see reviews by BELL 1969; YAMADA 1974). However, none of these studies has attempted to describe the nature of the response to selection for body size in terms of changes in body composition, feed efficiency, metabolic rate or any other biochemical parameter.

Knowledge of the physiological and biochemical alterations associated with changes in body size due to selection may provide a better understanding of the control of genetically determined interrelationships affecting growth and development in animals. Such information would enhance our concepts of quantitative genetics and offer a potentially valuable new dimension within the realm of animal improvement and breeding. For example, CHURCH and ROBERTSON (1966a) found characteristic differences in wet weight and protein, DNA, and RNA content in 10 line of *Drosophila melanogaster* selected for modified body size and developmental time.

Correlated responses in body composition, feed efficiency, and cellular growth as a result of direct selection for body weight and weight gain have been studied by a number of workers using the laboratory mouse. SUTHERLAND, BIONDINI and WARD (1974) have reviewed these studies and concluded that in the mouse there seems to be "little genetic variation for net efficiency of feed utilization or of tissue deposition." However, in most studies a direct increase in gross efficiency (gain/feed) resulting from an increase in appetite and capacity to ingest nutrients has been observed. Also, as reviewed by SUTHERLAND, BIONDINI and WARD (1974) and EISEN (1974), percent fat seems to be the main body component to show genetic differences as a result of selection for growth. EISEN (1974) suggests that some of the differences in results between experiments may be accounted for by differences in physiological age of the lines when selection was performed.

The purpose of the present investigation was to study correlated changes in growth rate, body composition, cellular growth, and the activity of four dehydrogenase enzymes during development in three lines of *Tribolium castaneum* selected for 21-day pupae weight. Preliminary results of this investigation have been reported earlier (MEDRANO, GALL and ROBINSON 1973; GALL and MEDRANO 1974).

MATERIALS AND METHODS

Genetic Stocks

The study utilized four *Tribolium castaneum* lines (lines 3, 9, 10 and 1C) which arose from a single foundation population (GALL 1970) and had undergone mass selection for high 21-day pupa weight. GALL (1971) described the response over the first 30 generations of selection. The present designations of the lines are the same as those used by GALL (1971) except for control line 98 which is referred to here as line 1C. All of the lines were kept in 4 oz. Mason jars in 90% unbleached white wheat flour and 10% dried brewers yeast at 33° and 70% relative humidity.

Selection was suspended according to the procedures described by GALL (1971) in lines 3 and 10 at generations 31 and 32, respectively, at which time their average 21-day pupa weights were 4.43 and 4.96 mg. Selection was suspended in line 9 at generation 53 where it had attained a mean weight of 5.84 mg; selection intensities of 10 out of 50 males and 30 out of 75 females had been continued to that point. The 21-day pupa weight realized heritability for line 9 was 0.06 from generation 31 to 50 as compared to 0.28 from generations 0 to 30. After suspension of selection all lines were maintained by mass mating randomly selected 21-day pupae obtained by weighting *in mass* two samples of 10 males and four samples of 10 females. The control line, 1C, had been maintained in the same manner since generation 23.

The present study was started at generation 51 for lines 3, 10, and 1C and generation 52 for line 9 at which time their average 21-day pupa weights were 3.86, 4.31, 2.12, and 5.79 mg, respectively. The lines were expanded by setting *in mass* all of the available adults (20 to 40 per line) for seven consecutive 24-hour egg collections. Twenty-nine days after the last egg collection all adults produced in each line were pooled. For each line, 10 random samples of 60 adults each were then set for 8-hour egg collections at sequential time intervals to provide progeny for analysis at 10, 12, 14, 16, 19, 21, and 25 days of age. At a given assay age, all organisms found in the 10 bottles of each line were pooled, random samples weighted, and randomly assigned to sets of four replicates to be used in each of the biochemical assays that followed. A growth curve was calculated for each line from the average weight of 40 samples for ages 10 through 16 and at 25 days, and 16 samples for ages 19 and 21 days.

Analytical Procedures

Body composition was evaluated from 10 to 25 days of age. To determine water content, tissue samples of approximately 25 mg were dried at 105° for 24 hours in individual 6 cm aluminum pans. Lipids were then extracted by homogenizing the dried samples in 1 ml of a 2:1 chloroform:methanol mixture with a Potter-Elvehjem teflon type homogenizer (FOLCH, LEES and STANLEY 1957). The homogenate was washed with 0.24 volumes of 0.5 N NaCl and centrifuged for 10 minutes in a clinical centrifuge. The upper layer was discarded and the supernatant filtered with a 4.25 cm Whatman 1 filter paper into a pre-weighed 6 cm aluminum pan. The centrifuge tube and filter paper were washed five times with 1 ml aliquots of 2:1 chloroform:methanol. The combined supernatant and washings were taken to dryness on a hot plate and weighed.

DNA, RNA, and protein were determined on a separate series of samples weighing 25 to 30 mg by the method of WANNEMACHER, BANKS and WUNNER (1965) modified by homogenizing the tissue in 1:200 (w/v) solution of cold 0.12 N NaCl and precipitating with 0.25 volumes of 1.2 N percholoric acid. The protein values obtained reflect the relative rather than the absolute amount of protein since some chitinous material was lost during extraction of the homogenates. Because of the presence of an interfering polyhydroxy compound (DEVI *et al.* 1963), DNA and RNA concentrations were not determined for adults.

Activities of four dehydrogenase enzymes, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (G6PD), 6-phospho-gluconate dehydrogenase (EC 1.1.1.44) (6PGD), lastic acid dehydrogenase (EC 1.1.1.28) (LHD), and NADH-linked isocitrate dehydrogenase (EC 1.1.1.41) (ICDH) were determined by measuring spectrophotometrically rate of NADPH formation (oxidation of NADH in case of LDH) through modifications of the methods of BALDWIN and MILLIGAN (1966) as outlined by GALL (1970).

RESULTS

Growth and development: Figure 1 illustrated the effect of selection on the size and growth response of the selected and control lines from 10 to 25 days of

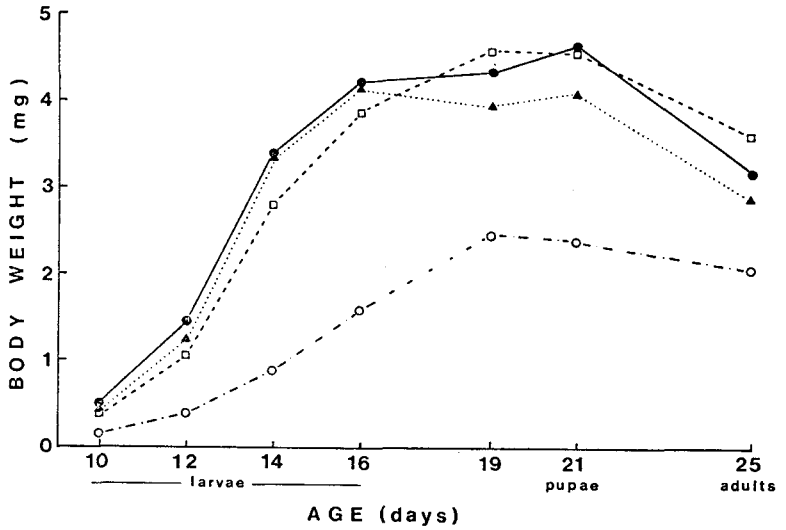

FIGURE 1.—Growth curves (mean body weight) of three selected lines of *Tribolium*; lines 9 (●——●), 10 (□---□), and 3 (▲·····▲), and control line 1C (○-·-○). The values at age 19 represent: for line 9, the mean of 12 samples of a mixture of larvae and pupae in 1:1 proportions and four samples of pupae; for line 10, 12 samples of larvae and pupae in 5:1 proportions and four samples of larvae; for line 3, 16 samples of pupae; for line 1C, 16 samples of larvae.

age. The growth response is drawn as a continuous line to facilitate reading of the graph although Tribolium development, as described by ENGLERT and BELL (1963), consists basically of three discrete segments. These three segments can best be described as: (1) larval growth from hatching to approximately 16 to 18 days (counting day 0 as the day of egg collection) and is a continuous exponential growth period (BERTALANFFY 1960); (2) the prepupal or so-called "white larvae" stage from 18 to 20 days at which time maximum size has been achieved and the pupal stage of from approximately 19 to 23 days of age during which a slight weight loss occurs; and (3) the adult stage from 23 to 25 days onward which is generally a period of constant weight.

Significant ($P < 0.01$) differences in size between control and selected lines and among selected lines were detected at all ages (Table 2). The selected lines were approximately 300% heavier than the unselected control from 10 to 16 days of age and approximately 186% heavier than control as pupae at 21 days. A marked increase in growth rate in the selected lines was observed from 10 to 16

TABLE 1

Body composition of selected and control lines of Tribolium at different ages ($n = 4$)

	Line							
	9		10		3		1C	
Days	μg	%	μg	%	μg	%	μg	%
Protein								
10	56	11.6	42	11.3	38	9.6	19	11.6
12	181	12.8	130	13.7	143	11.8	43	12.4
14	592	18.0	505	18.5	594*	17.9	123	14.0
16	712	17.5	676*	17.1	655	16.7	254	15.8
19	941	21.8	973	21.5	910	23.9	496	20.2
21	1105	23.4	910	20.1	853	20.9	572	23.9
25	492	15.2	540	15.6	391	13.9	345	16.5
Fat								
10	35	7.4	26	6.8	31	7.3	9	5.8
12	163	12.4	120	12.1	133	12.0	31	9.0
14	530	15.7	411	14.7	646	19.5	90	9.9
16	831	19.6	626	16.5	748	19.6	203	12.9
19	854	19.5	940	20.3	736	18.9	438	18.2
21	873	19.7	853	18.8	797	19.2	415	17.6
25	393	12.9	449	12.6	417	13.7	223	10.9
Water								
10	325	68.1	256	67.0	288	67.2	101	65.7
12	887	67.2	663	66.8	734	66.3	221	64.7
14	2097	62.3	1750	62.9	1925	58.4	590	64.7
16	2345	55.4	2173	57.3	2112	55.4	977	62.0
19	2372	54.1	2539	54.8	2207	56.6	1334	55.4
21	2460	55.4	2605	57.4	2340	56.2	1325	56.2
25	1684	55.3	1937	54.4	1650	54.4	1188	58.0

* $n = 3$.

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 body weight and body composition of Tribolium at different ages*

Source	df	Mean squares						
		10	12	14	16	19	21	25
Body wt.					(mg^2)			
Ctrl. *vs.* sel.	1	2.18**	21.52**	155.65**	181.97**	41.96**	49.83**	41.77**
Among sel.	2	0.12**	1.16**	4.23**	1.31**	1.99**	1.40**	4.14**
Error	156†	0.001	0.007	0.041	0.053	0.047	0.041	0.015
Protein wt.					($mg^2 \times 1000$)			
Ctrl *vs.* sel.	1	2.1*	35.5**	562.5**	535.9**	595.0**	442.4**	50.2**
Among sel.	2	0.4	2.8**	9.8	6.6	4.0	69.9	23.2*
Error	12‡	0.4	0.1	8.0	2.9	13.1	19.2	5.0
Fat wt.					($mg^2 \times 1000$)			
Ctrl. *vs.* sel.	1	1.5**	34.8**	577.3**	849.1**	492.9**	545.3**	116.0**
Among sel.	2	0.1**	1.9**	54.8**	42.6**	42.0**	6.3	3.2
Error	12	0.01	0.1	3.5	4.0	1.7	2.9	1.8
Water wt.					($mg^2 \times 1000$)			
Ctrl. *vs.* sel.	1	107.0**	875.9**	5338.7**	4560.9**	3236.5**	3921.6**	971.3**
Among sel.	2	5.1*	52.4**	120.4**	58.4*	110.2**	70.4*	98.4**
Error	12	1.2	2.0	12.3	13.7	14.2	4.1	2.4

* $P < 0.05$.
** $P < 0.01$.
† Error degrees of freedom equal 155 for days 14 and 16, and 60 for days 19 and 21, respectively.
‡ Error degrees of freedom equal 11 for days 14 and 16, respectively.

days of age. For lines 3 and 9, the rapid growth was followed by a reduction in growth rate through pupation whereas line 10, being smaller at day 16, showed slow continuous growth through day 19. This is in contrast to line 1C which showed slow continuous growth through day 19.

Starting at day 16, differences in developmental rate among lines were observed; lines 3 and 9 yielded 9% and 4% pupae, respectively, but no pupae were observed in lines 10 and 1C at this time. At day 19, 76%, 52%, 23%, and 5% pupae were observed in lines 3, 9, 10, and 1C, respectively. The earlier development of lines 3 and 9 was reflected in their decreased growth response after 16 days in comparison with line 10.

Body composition: The means and linear comparisons for protein, fat and water content are shown in Tables 1 and 2. All of the lines show a similar pattern of development of the body components, with each component reaching a maximum at 19 or 21 days of age when cessation of growth occurred and the organism entered the pupal stage.

Analysis of protein, fat, and water content revealed the selected lines to be significantly ($P < 0.01$) higher than the control line at all ages. This difference was a direct reflection of the divergence in body size between the two groups. Among selected lines, significant ($P < 0.05$) differences were detected in protein

content at 12 and 25 days of age, mainly due to higher values of lines 9 and 10. More striking differences were observed among selected lines in fat and water content, reflecting inequalities between the early developing lines 3 and 9 and the later developing line 10. Significant ($P < 0.01$) differences in fat content were observed among the three lines from 10 to 19 days of age with lines 3 and 9 having more fat up to day 16. Similarly, differences in water content were detected during this period and extended to 21 and 25 days of age when the developmental status of the organism may also have accounted for differences in their state of hydration.

Since large differences in body weight existed between the control and selected lines and there was a high positive relationship between body weight and weight of tissue components, more objective comparisons can be made in terms of percent body composition (Table 1). Table 3 presents the linear comparisons for protein, fat and water percentages for all lines. Line differences in protein percent were not evident except at day 14 where the selected lines were significantly ($P < 0.01$) higher than the control line. This difference may be attributed to the slower growth and developmental rate of line 1C in comparison to the rapid growth the selected lines underwent from day 12 to 14. Selection for 21-day pupae weight caused a significant ($P < 0.01$) divergence between control and selected lines in percent fat over all ages. Among selected lines, however, differences were observed only at 14 and 16 days with line 3 being highest at day 14 and lines 3 and 9 being higher than line 10 in percent fat at day 16. The latter observation is undoubtedly related to developmental rate since all selected lines achieved the

TABLE 3

Mean squares showing the linear contrast of control (ctrl.) versus *selected (sel.) lines and a linear contrast among the three selected lines, on the body composition, as percent of total body weight, of Tribolium at different ages*

		Mean squares (days)						
Source	df	10	12	14	16	19	21	25
Protein %								
Ctrl. *vs.* sel.	1	1.81	0.28	49.37**	4.79	14.42	17.58	8.27
Among sel.	2	4.79	3.51	0.38	0.63	6.90	11.33	3.06
Error	12†	1.80	1.51	6.65	5.95	4.01	10.82	5.32
Fat %								
Ctrl. *vs.* sel.	1	6.08**	29.34**	136.74**	97.15**	5.61**	7.60*	14.05**
Among sel.	2	0.36	0.15	26.00**	13.00**	1.87	0.81	1.30
Error	12	0.77	0.57	1.06	0.71	0.58	1.05	1.36
Water %								
Ctrl. *vs.* sel.	1	9.47*	13.07**	37.79**	107.83**	0.23	0.05	32.03**
Among sel.	2	1.29	0.82	24.08**	5.03*	6.46**	4.23*	0.96
Error	12	1.19	0.37	0.92	0.91	0.56	0.65	0.94

* $P < 0.05$.
** $P < 0.01$.
† Error degrees of freedom equal 11 for days 14 and 16, respectively.

same level of fatness by day 19. Fat content at a particular age is directly related to developmental time in Tribolium and is a response to the organism's need to increase its energy stores for maintenance during the transitional pupal stage.

Percent water had an inverse relationship to fat and declined from a high of 67% for larvae at day 10 to about 55% during the prepupal and adult stages. The selected lines had significantly lower percent water than the control from 10 to 16 days and at 25 days of age. This divergence was probably a reflection of the differences in fat content observed between the two groups at those ages. The differences among the selected lines at days 14 and 16 were related to differences in fat content; however, the lines also differed in percent water at 19 and 21 days. This result suggested that 21-day pupae in lines 3 and 9 contain more water than either line 10 or the control although the two linear contrasts are not orthogonal.

Simple phenotypic correlations were calculated between the percent body composition components for days 10 through 16 utilizing data from all four lines since the point estimates for the lines were consistent. The results revealed a strong negative relationship between water and fat (−0.86) and between water and protein (−0.67) and a strong positive relationship between fat and protein (0.73). All correlations were significant at $P < 0.01$. The observations were not in agreement with results obtained from mice by LANG and LEGATES (1969). They observed a negative relationship between percent fat and protein and between water and ash and a positive relationship between water and protein in mice from 3 to 8 weeks of age.

Nucleic acids: The nucleic acid content of an organ or an organism and its relationship to body weight or protein content may be utilized as a basic technique in the characterization of animal growth (ROBINSON 1971). It has been demonstrated that the amount of DNA per nucleus of somatic cells is approximately constant for a given species (BIOVIN, VENDRELY and VENDRELY 1949; MIRSKY and RIS 1949; VENDRELY 1955) and that this measurement can be utilized as an index of cell number. DNA content can also be used as a standard reference to demonstrate changes in other cellular constituents (DAVIDSON and LESLIE 1950; THOMAS *et al.* 1953). Therefore, DNA per animal measured serially during development can be utilized as an index of cellular hyperplasia. Similarly, changes in the ratio of protein to DNA are indicative of changes in cell size and can be used to estimate cellular hypertrophy, and the ratio of RNA to DNA serves as an index of cellular capacity for synthesizing protein.

The DNA and RNA content, the ratios of protein to DNA, and RNA to DNA are presented graphically in Figure 2, and the results of line comparisons are presented in Table 4. As was found with *Drosophila melanogaster* (CHURCH and ROBERTSON 1966b), changes in DNA content during Tribolium development closely followed the curve for protein content. Selection for 21-day pupa weight caused a significant increase ($P < 0.01$) in DNA content per animal at all ages in the three selected lines (Figure 2a). Differences among the selected lines were evident from 10 to 14 days of age when the highest growth rate was realized;

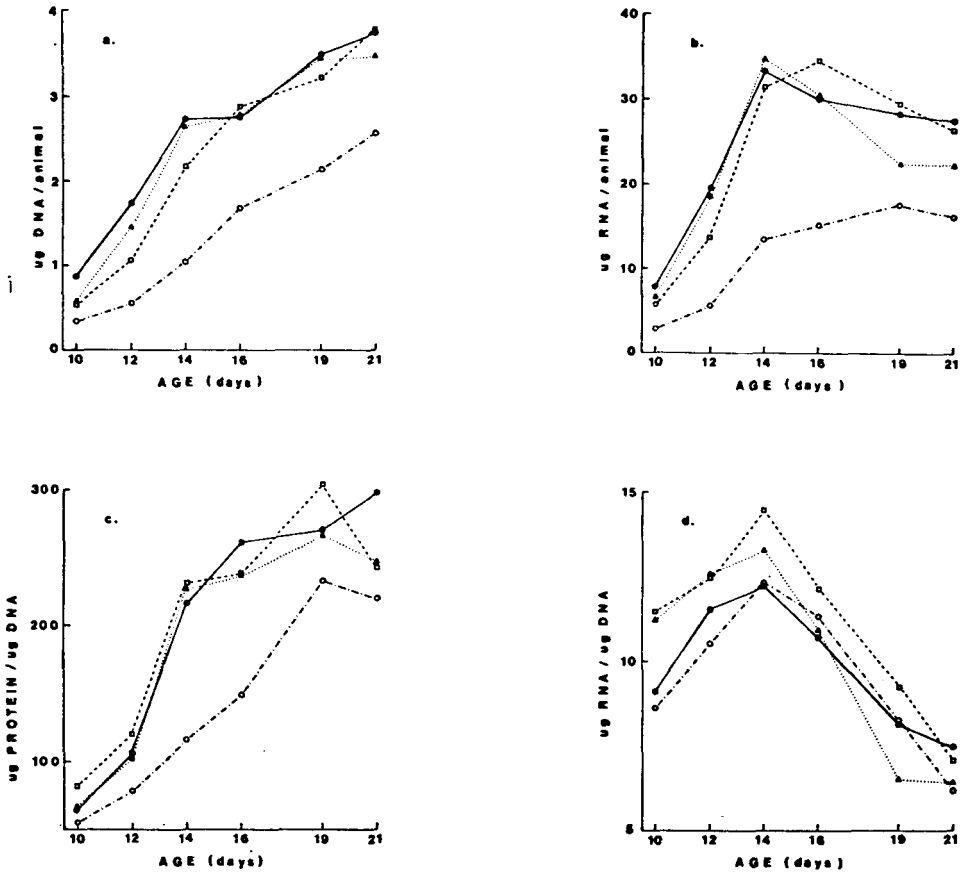

FIGURE 2.—Cellular characteristics during growth of four lines of Tribolium. (a) DNA; (b) RNA; (c) Protein/DNA; (d) RNA/DNA. Selected lines 9 (●—●), 10 (□- - -□), 3 (▲---▲), and control 1C (○-.-○).

the faster growing and developing lines 3 and 9 underwent cellular hyperplasia at a higher rate than line 10.

RNA content per animal was proportional to body size (Figure 2b). It was many times greater than DNA content and increased at a much more rapid rate through larval development. The selected lines exceeded the control line in RNA content at all ages. No differences were detected among selected lines at 14 and 16 days of age which was probably due to the large error variance at those ages, whereas at the other ages differences were directly related to body size.

As evidenced by the protein to DNA ratio (Figure 2c), the rate of cellular hypertrophy was significantly ($P < 0.01$) higher in the three selected lines during larval growth, a pattern similar to that observed for the increase in cell number of selected lines. There was no apparent differences among selected lines beyond 12 days of age although line 10 had larger cells at day 10 and 12. There were significant differences in RNA:DNA ratios among the selected lines (Figure

TABLE 4

Mean squares showing the linear contrast of control (ctrl.) versus *selected (sel.) lines and a linear contrast among the three selected lines, on DNA and RNA per animal, and the ratios of protein to DNA, and RNA to DNA in Tribolium at different ages*

Source	df	Mean squares					
		10	12	14	16	19	21
DNA/animal				(μg)			
Ctrl. *vs.* sel.	1	0.298**	2.329**	5.904**	3.680**	4.657**	3.379**
Among sel.	2	0.144**	0.394**	0.320**	0.010	0.095	0.096
Error	12†	0.007	0.008	0.030	0.093	0.074	0.053
RNA/animal				(μg)			
Ctrl. *vs. sel.*	1	43.05**	407.34**	1135.84**	446.48**	253.70**	257.73**
Among sel.	2	4.69**	40.84**	10.08	21.14	57.38**	31.20**
Error	12	0.21	0.71	5.60	20.01	0.61	1.41
Protein/DNA				(mg/μg $\times$ 100)			
Ctrl. *vs.* sel.	1	0.07**	0.26**	3.47**	2.76**	0.66*	0.55
Among sel.	2	0.04**	0.05*	0.02	0.07	0.18	0.36
Error	12	0.003	0.01	0.08	0.13	0.11	0.15
RNA/RNA				(μg/μg)			
Ctrl. *vs.* sel.	1	11.248**	7.802*	2.839	0.045	0.182	1.584**
Among sel.	2	6.678*	1.326	4.869*	1.955	7.542**	1.044*
Error	12	1.026	0.381	1.153	1.359	0.339	0.164

* $P < 0.05$.
** $P < 0.01$.
† Error degrees of freedom equal 11 for days 14 and 16 of DNA and RNA per animal, and ratios of protein and RNA to DNA, respectively.

2d) but of particular interest was the general pattern of the change in the ratio. The synthesizing capacity of the cells increased rapidly during larval growth and peaked at 14 days of age when the lines were approaching a reduction in average growth rate. The ratio then declined sharply to reach a minimum at day 21.

Enzyme activities: The means and linear comparisons for the activity of four dehydrogenase enzymes are given in Tables 5 and 6. As shown by GALL (1970), the activities of 6PGD and LDH (O.D./min/mg) were generally low during the pupal and adult stages. The activity of LDH for the selected lines was in most cases significantly lower than the control line while 6PGD tended to be higher in the selected lines during early growth but lower at later ages. Also, as described by GALL (1970), the activities of ICDH and G6PD in the selected lines were initially low, achieving a maximal value at days 12 and 14, respectively. The selected lines were significantly lower than the control line in ICDH activity at most ages, whereas few differences were detected for G6PD.

TABLE 5

Enzyme activities (O.D./min/mg tissue × .01) for selected and control lines of Tribolium at different ages (n = 4)

Age	Line 9	10	3	1C
G6PD				
10	2.52*	5.46	4.55	5.97
12	5.04	5.65	5.30	4.90
14	5.60	6.55	5.78	4.93
16	3.61	3.09	2.77	3.35
19	1.78	1.66	1.16	1.96
21	1.25	1.18	0.90	1.59
25	3.58	3.05	3.88	2.55
6PGD				
10	5.08	6.67	6.68	5.36
12	5.42	6.55	6.88	5.33
14	4.96	5.92	6.91	4.64
16	4.04	4.26	5.41	5.00
19	2.91	3.35	2.78	4.14
21	2.27	2.23	2.51	2.47
25	0.09*	0.56	0.05*	1.62
ICDH				
10	15.39	19.23	15.49	18.85
12	16.37	16.75	15.77	19.97
14	14.66	13.44	14.08	20.21
16	11.45	12.33	12.04	17.82
19	10.38	10.91	6.40	14.95
21	7.49	7.52	6.42	6.09
25	0.54*	0.57*	0.25*	3.88
LDH				
10	1.08	2.32	2.09	2.63
12	0.49	0.91	0.82	1.23
14	0.11	0.42	0.90	1.33
16	0.51	0.78	0.84†	0.62
19	0.12*	0.49*	0.12	0.65
21	0.07	0.20	0.0	0.19

* n = 3.
† n = 2.

DISCUSSION

Long term selection for 21-day pupae weight in *Tribolium castaneum* has altered the basic cellular pattern of growth. The large lines have acquired the ability to increase their size two- to three-fold above that of the unselected line by developing a genetically controlled developmental process which markedly increases both cell number and size. The changes in these two basic processes resulted in a marked increase in total RNA, protein, fat, water and weight.

TABLE 6

Mean squares showing the linear contrast of control (ctrl.) versus *selected (sel.) lines and a linear contrast among the three selected lines on the activity of four dehydrogenase enzymes in Tribolium at different ages*

Source	df	Mean squares (days)						
		10	12	14	16	19	21	25
G6PD								
Ctrl. *vs.* sel.	1	7.88*	0.55	3.26	0.12	0.55	0.69**	2.71**
Among sel.	2	7.56**	0.37	1.02	0.72	0.43	0.13	0.71
Error	12†	0.88	1.26	2.48	0.85	0.23	0.08	0.19
6PGD								
Ctrl. *vs.* sel.	1	1.86	2.70*	4.97**	0.56	3.79**	0.05	5.21**
Among sel.	2	3.41*	2.36**	3.79**	2.18**	0.36	0.09	3.57
Error	12‡	0.64	0.32	0.17	0.19	0.13	1.80	0.08
ICDH								
Ctrl. *vs.* sel.	1	13.85	40.43**	113.50**	103.64**	98.13**	3.36	32.56**
Among sel.	2	19.20	0.98	1.49	0.80	24.38**	1.28	0.09
Error	12	5.35	1.20	0.56	1.57	1.35	2.63	0.53
LDH								
Ctrl. *vs.* sel.	1	1.93*	0.71*	2.18**	0.01	0.50**	0.03	—
Among sel.	2	1.74*	0.19	0.65**	0.10	0.14	0.04	—
Error	12§	0.38	0.10	0.04	0.20	0.04	0.01	—

* $P < 0.05$.
** $P < 0.01$.
† Error degrees of freedom equal 11 for day 10.
‡ Error degrees of freedom equal 10 and 9 for day 25 of 6PGD and ICDH, respectively.
§ Error degrees of freedom equal 10 for days 16 and 19.

When the components of tissue were compared as percentages of body weight, fat was found to be consistently higher in the large lines at all ages. However, the increase in fat was very highly correlated with developmental rate or physiological age of the lines. The early developing lines 3 and 9 reached maximum fat content 3 days earlier than the later developing lines, 10 and 1C. It seems from the general pattern of the results that all lines reached similar fat content prior to pupation. Quantification of this statement would require further observations between the ages of 14 and 19 days. However, it is of interest to point out that at the age intervals measured, the greatest increases in fat content were positively correlated with weight gain.

In all the lines percent water decreased with age in proportion to the increase in fat, and no major differences in percent protein were observed among the lines except at 14 days, the end of the highest growth period. At this age the selected lines contained about 4% more protein than the control.

Enzyme activities generally followed the pattern of RNA concentration calculated per unit of body weight, reflecting the effect of protein synthesis in the cells.

RNA concentration decreased sharply in all lines from 10 to 19 days and was highest in the control line throughout this period. In concert with this, the activities of ICDH and LDH in the control line were 60% higher per unit weight than those seen in the selected lines.

The data indicate that selection for large 21-day pupae has not caused major changes in body composition of the organism prior to pupation. The developmental patterns to achieve a large size has been modified by changes in the cellular responses to growth. Rapid DNA synthesis during larval growth is followed by rapid RNA synthesis which is in turn succeeded by a high rate of protein synthesis. These relationships occurred at an accelerated rate in the three selected lines and, consequently, resulted in a more rapid increase in cell size. The general pattern indicated that during the accelerated period of larval growth, the demands for a rapid increase in tissue weight were met by a combination of the processes of cellular hyperplasia and hypertrophy similar to that observed in some mammals before weaning (ROBINSON 1969). Lower enzymatic rates in large lines tend to suggest a more efficient metabolic machinery for growth. This topic is the subject of another publication (MEDRANO and GALL 1976).

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