CONTENTS OF NUCLEIC AND AMINO ACIDS AND RATE OF PROTEIN SYNTHESIS IN DEVELOPING FLIGHT MUSCLES OF *LOCUSTA MIGRATORIA*

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Abstract—1. Changes in the contents of DNA, RNA, free amino acids (FAA) and protein as well as in the rate of protein synthesis *in vivo* were measured in the developing flight muscles of *Locustamigratoria*.

2. The DNA concentration rises temporarily at the end of the last larval instar, possibly in relation to the process of tracheolization.

3. RNA content rises considerably until day 6 after the terminal ecdysis and thereupon decreases steadily.

4. Among the amino acids taurine changes the most drastically, raising its contribution to the FAA pool from 6.5 to finally 39 mol%.

5. Rates of protein synthesis are significantly higher in adults than in larvae. The synthesis remains relatively high even after completion of muscle development.

INTRODUCTION

The precursors of the flight muscles of hemimetabolous insects are formed early in development, probably during embryogenesis. For example, in *Schistocerca gregaria* all the future adult pterothoracic muscles are already present in the hatchling; only the dorsal longitudinal muscles are well-developed and probably used in hatching (Bernays, 1972). After hatching these muscles become nonfunctional, persisting as rudiments until they develop into adult indirect flight muscles (Thomas, 1954). Also in the locust, *Chortoicetes terminifera*, the later adult flight muscles were shown to be present as undeveloped muscles in the first instar larvae (Tiegs, 1955).

In *Locusta migratoria* the weight of the flight muscles increases more than 2-fold during the fourth larval instar and about 16-fold in the fifth instar (Hill & Goldsworthy, 1968). The real development of the dorsal longitudinal muscles from the precursor stage starts in the fifth instar and is characterized by substantial increases in the amount of contractile proteins, in the size of mitochondria, and in the activity of enzymes important in flight metabolism (Brosemer *et al.*, 1963; Beenakkers *et al.*, 1975). The most drastic changes take place during the first 3–4 days after the adult ecdysis. During this "phase of final differentiation" (Brosemer *et al.*, 1963), all cell components develop their typical adult qualities. This applies also to the enzyme pattern representing the main catabolic pathways (Beenakkers *et al.*, 1975). After this differentiation phase only quantitative changes take place in muscular components. Development is achieved about 8 days after the final ecdysis.

It was shown by Poels & Beenakkers (1969) that after implantation of active corpora allata (CA) in young fifth instar larvae locust flight muscle development was strongly inhibited. Since then, our research group has investigated the influence of juvenile hormone (JH) on flight muscle development, studying the effects of allatectomy as well as implantation of CA in both larvae and adults on muscle weight, protein content and specific activities of enzymes from the main catabolic pathways (Beenakkers, 1973; Beenakkers & Van den Broek, 1976). Moreover, electron microscopic investigations of flight muscle development under these experimental variations have been made recently together with haemolymph JH-titer determinations (Van den Hondel-Franken *et al.*, 1979).

Within the scope of these investigations on flight muscle development and its regulation, the present study deals with the changes in the basal constituents of locust flight muscle during larval and adult development. The contents of nucleic acids and proteins were determined as well as the composition of the free amino acid pool. In addition we have estimated the rates of muscle protein synthesis *in vivo* throughout development. This first study on the above-mentioned parameters refers to the normal development of locust flight muscles.

MATERIALS AND METHODS

Animals

Migratory locusts, *Locusta migratoria migratorioides*, were reared in the laboratory under crowded conditions at 30°C, 40% r.h., and with a 12 hr photoperiod. They were fed with reed or with equal parts of wheat and rye leaves, supplemented with rolled oats. In the experiments female locusts of specific ages during fifth instar and adult development were used.

Preparation of the flight muscles

Locusts were anaesthetized with carbon dioxide applied for 1 min. Dorsal longitudinal flight muscles were dissected out and immersed immediately in liquid nitrogen. Muscles from five animals were pooled and the wet weight determined. The muscle tissue was homogenized in 9 ml ice cold aqua bidest using an ultra-Turrax disintegrator (Janke

and Kunkel). To 7 ml of the homogenate 0.5 ml of 7.5 N perchloric acid (PCA) was added. After 30 min on ice the precipitated material was pelleted by centrifugation (15,000 g for 10 min). The pellet was washed twice with 2.5 ml of 0.5 N PCA. The three supernatants were pooled: *S1-fraction*, containing the free amino acids. The washed pellet was resuspended in 4.5 ml of 0.35 N NaOH and RNA was hydrolyzed overnight at 32°C. DNA and proteins were precipitated by the addition of PCA to a final concentration of 0.5 N. After centrifugation the pellet was washed twice with 0.5 N PCA. The three supernatants were pooled: *S2-fraction*, containing the nucleotides of hydrolysed RNA. The washed pellet was resuspended in 3 ml of 0.5 N PCA and DNA was hydrolysed at 80°C for 30 min. After cooling and centrifugation the pellet was hydrolysed twice more for 20 min in, respectively, 2 and 1.5 ml of 0.5 N PCA. The three supernatants were pooled: *S3-fraction*, containing the DNA-nucleotides. The pellet was dissolved in 0.5 N NaOH: *P-fraction*, containing proteins.

Determination of proteins, RNA and DNA

Protein contents of homogenate and P-fraction were measured according to the method of Lowry et al. (1951) as modified by Schacterle and Pollack (1973). Bovine serum albumine was used as a standard.

RNA was estimated in the S2-fraction with orcinol reagent, according to Lin & Schjeide (1968), with yeast RNA as a standard. DNA was determined in the S3-fraction with diphenylamine reagent, according to Burton (1968). Calf thymus DNA was used as a standard.

Analysis of the amino acids

Qualitative and quantitative analyses of free amino acids in the S1-fraction were performed using an automatic analyzer as described by Van Marrewijk & Ravestein (1974).

[^{14}C]leucine incorporation

In vivo synthesis of flight muscle proteins was estimated from the incorporation rate of [^{14}C]leucine injected. Each locust received 0.1 μCi [U-^{14}C]leucine (298 mCi/mmol) in

a vol of 1 μl, which was injected abdominally with a 50 μl micro-syringe operated by means of a repeating dispenser (Hamilton). Locusts were kept at 30°C and sacrificed at specific times after receiving the injection. Flight muscles were dissected out and prepared as described above. The P-fraction was used for measuring [^{14}C]leucine incorporation.

Determination of radioactivity

Radioactivity of free leucine in the S1-fraction was measured by passing the column eluent of the automatic analyzer through a liquid scintillation counter fitted with a flowcell, as described by Van Marrewijk & Zandee (1975).

For determination of radioactivity incorporated into the proteins 1 ml of the P-fraction was neutralized with concentrated HCl; 10 ml of toluene–Triton X-100 (2:1, v/v) was added, the toluene containing 0.4% Omnifluor (NEN Chemicals). Radioactivities were measured with a Nuclear Chicago liquid scintillation counter, model Mk-II, equipped with an automatic external standardization.

RESULTS

Concentration of flight muscle nucleic acids and proteins during development

Figure 1 summarizes the fresh weights and the concentrations of DNA, RNA and protein in locust flight muscles during development. The muscle weight increased until about the twelfth day after the ecdysis and then remained almost constant.

DNA content reached a peak at the end of the last larval instar and decreased during the first days of adult life to return finally to the starting level measured at the onset of muscle development. The DNA concentration on day 8 of the larval instar was significantly higher (5% level) than all other values, except those on day 1 and 2 after the fifth ecdysis.
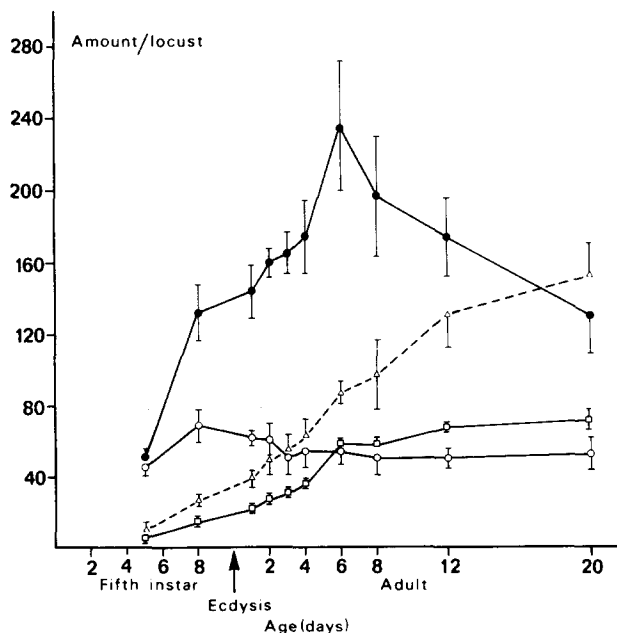

Fig. 1. Changes in muscle fresh weight (□——□, mg) and in the concentrations of DNA (○——○, μg), RNA (●——●, μg) and protein (△---△, mg × 10^{-1}) in the dorsal longitudinal flight muscles of female *Locusta migratoria* during development. Each point is the mean ± standard deviation of 3–5 groups of 5 animals each.

The sharp rise in the concentration of RNA during larval growth was followed by a more gradual increase during the first days of adult life. Beyond a peak on day 6 RNA decreased regularly, returning on day 20 to a level as low as the one found at the end of the last larval instar.

Flight muscle protein showed a gradual increase until day 12 of adult life, followed by a further smaller rise up to day 20. At this time, protein amounted to about 21% of muscle fresh weight.

Free amino acids in the developing flight muscles

The composition of the free amino acid (FAA) pool in the flight muscles throughout development is reported in Table 1. The most pronounced changes are observed for taurine, which concentration on day 20 of adult life is eight times higher than on day 5 of the larval instar. The contribution of taurine to the total FAA pool increased over this period from 6.5 to 30 mol%.

Other amino acids with a marked increase during muscle development are proline and arginine, whose concentrations more than doubled. Glycine maintained a high, rather constant, level throughout the whole developmental period. On day 20 of adult life the four most abundant amino acids (taurine, proline, arginine and glycine) contributed 74 mol% of the total FAA pool. In 5-day-old larvae the contribution of these amino acids amounted to only 34 mol%.

The concentration of glutamic acid, the most predominant amino acid at the onset of flight muscle development (17.6 mol%), decreased sharply around the final ecdysis. It remained low during the whole period of adult life, reaching its lowest level on day 20

with only 10% of the concentration in 5-day-old larvae. Alanine, with more than 10 mol% one of the most predominant amino acids in the larval muscles, reached its highest concentration on the first day after the final ecydsis. During the next 3 days the alanine concentration was reduced to half its maximal value, and this lower level persisted up to day 20. The concentration of the basic histidine was twice as high in the larval flight muscle as in the adult muscle.

The total concentration of FAA increased about 40% during the investigated period of flight muscle development. As is shown in Fig. 2, this increase is completely attributable to taurine. When taurine is excluded, a small gradual decrease of the FAA concentration is observed, reaching its lowest level 12 days after the final ecdysis.

Synthesis of flight muscle proteins in vivo

The rate of protein synthesis during flight muscle development was estimated in vivo from the rate of incorporation of [^{14}C]leucine injected. After incubation the specific activity of muscle protein (dpm/mg protein) was determined. Figure 3 gives an example of [^{14}C]leucine incorporation at different incubation times in 3-day-old adults.

When only the radioactivity incorporated is considered, a very irregular curve is obtained. Therefore the specific radioactivity of free leucine (dpm/nmol leucine) was also determined. From the specific activities of muscle protein and of free leucine, protein synthesis (nmol leucine incorporated/mg protein) was calculated. As can be taken from Fig. 3, a linear relationship is found for at least 60 min between incubation time and nmol leucine incorporated. The

Table 1. Free-amino acids and related compounds (μmols/g fresh weight) in the developing flight muscles of Locusta migratoria*

	last larval ecdysis		Developmental day after larval/adult ecdysis				
	5	8	1	4	8	12	20
Taurine	6.59	12.80	17.05	33.29	41.10	46.68	54.43
Phosphoethanolamine	2.00	1.04	1.88	1.15	0.49	0.43	0.49
Aspartic acid	1.74	0.17	0.34	2.48	0.50	0.30	0.18
Threonine	2.11	2.00	1.39	0.72	1.21	1.07	0.74
Serine	4.00	2.21	2.25	3.45	1.69	2.77	2.23
Asparagine	1.85	1.53	1.26	4.04	3.12	2.82	3.92
Glutamic acid	17.69	12.62	3.32	4.91	3.63	3.11	1.75
Glutamine	6.95	6.04	11.27	8.28	9.38	9.82	10.50
Sarcosine	0.92	0.23	—	0.36	—	0.19	0.24
Proline	7.60	8.90	7.01	10.94	14.60	14.06	18.10
Glycine	13.59	13.09	14.04	16.87	14.83	14.78	15.24
Alanine	10.97	11.34	14.38	6.49	7.45	6.32	6.91
Valine	4.56	6.81	5.69	3.86	4.34	3.11	3.51
Methionine	0.47	0.47	0.34	0.28	0.39	0.52	0.30
Isoleucine	0.77	1.91	1.48	1.22	0.75	0.72	0.63
Leucine	0.68	1.39	1.82	0.63	0.85	0.57	0.27
Tyrosine	2.29	2.88	3.17	0.83	0.98	0.48	0.43
Phenylalanine	0.39	0.65	0.68	0.19	0.15	0.42	0.18
β-alanine	0.42	0.31	0.65	0.63	0.64	0.48	0.58
β-aminoisobutyric acid	0.51	0.38	0.18	0.43	0.41	0.25	0.33
γ-aminobutyric acid	0.12	0.15	—	—	—	—	—
Lysine	1.25	2.45	1.41	1.18	0.47	0.45	0.31
Histidine	6.50	7.05	2.70	3.14	3.70	3.15	2.52
Arginine	6.69	11.31	11.98	16.89	14.48	14.37	15.58
Total (excluding taurine)	94.07	94.93	87.24	88.97	84.06	80.19	84.94

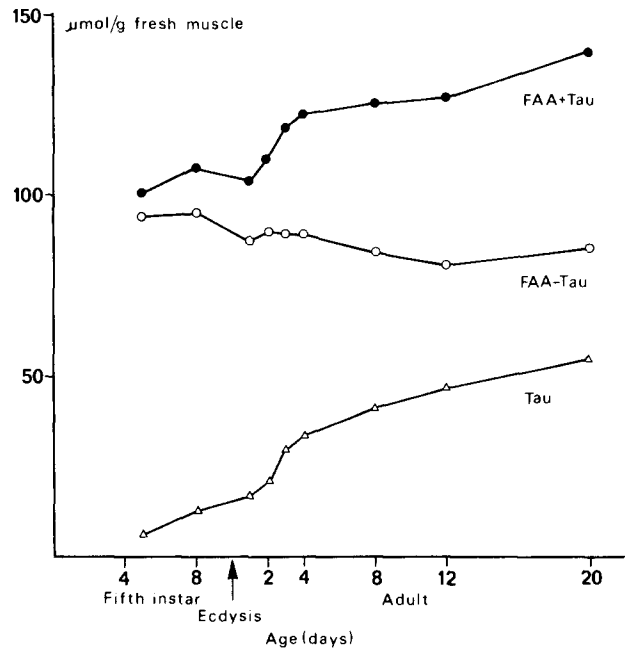* Mean values of 3–5 groups of 5 animals each.

Fig. 2. Contents of taurine and of total FAA including and excluding taurine in the dorsal longitudinal flight muscles of female locusts during development.

increased rate of protein synthesis calculated at incubation times exceeding one hour can easily be explained by the decreasing specific activity of free leucine during prolonged incubation periods. For this reason, leucine specific activities measured at the end of the incubation period will no longer be representative for the whole incubation period and the rates of protein synthesis calculated will be too high. Patterns similar to that of flight muscles of 3-day-old adults were obtained with muscles of 5-day-old larvae and 8-day-old adults. From these results, an incubation time of 45 min was chosen for studies on the rate of

protein synthesis in flight muscles throughout the developmental period.

Leucine contents of muscle proteins were estimated in larval and adult flight muscles in 8-day-old animals from both stages. The values obtained in acid hydrolysates of the larval and the adult muscle proteins were, respectively, 9.9 and 10.7 mg leucine/100 mg protein. The mean of these values (10.3 mg) was used for the conversion of "nmol leucine incorporated/mg protein" into "μg protein synthesized". The thus estimated rates of protein synthesis during flight muscle development are presented in Fig. 4. During the
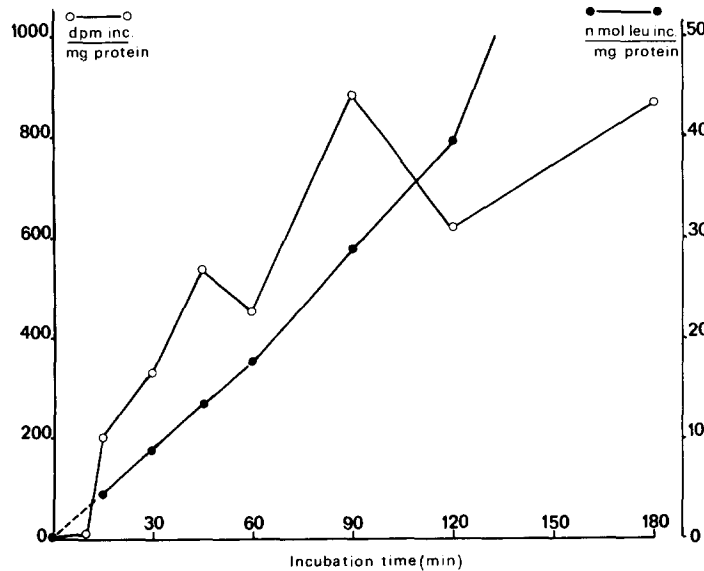

Fig. 3. Incorporation of leucine into flight muscle proteins of 3-day-old adult locusts at different times after injection of [^{14}C]leucine.
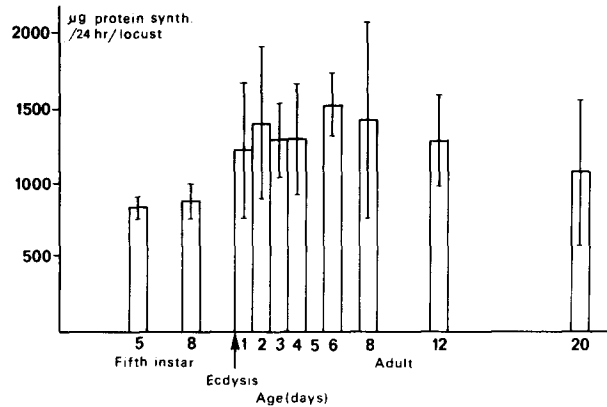
Fig. 4. Rates of protein synthesis *in vivo* in the developing flight muscles of *Locusta migratoria*. Each point is the mean ± standard deviation of 3–5 groups of 5 animals each.

second half of the larval instar period a nearly con-
stant level was measured with a daily synthesis of
about 0.85 mg muscle protein per female. Throughout
adult life protein synthesis was significantly higher.
The rate of synthesis appeared to be increased directly
after the final ecdysis. During the first 12 days of adult
life differences were rather small. The highest rate was
measured on day 6 with about 1.52 mg protein per
24 hr. The lowest rate of protein synthesis was
measured on day 20, viz. 1.08 mg per 24 hr. Due to
the large standard deviation, no statistical significance
could be demonstrated for differences between indivi-
dual values in the adult period.

The amounts of protein synthesized and degraded
were estimated for successive periods throughout
flight muscle development (Table 2). At the end of the
larval period protein concentration (d) amounted to
only 74% of the theoretical maximum (c), which sug-
gests a breakdown of 26% of muscle protein during
this period. During the first 12 days of adult life the
measured protein concentrations (d) amounted to
about 85 to even 95% of the theoretical maximum

values (c). This close correspondence suggests a rela-
tively small protein degradation (e) during the periods
concerned. In the last period (Ad-12d → Ad-20d) the
actual protein concentration amounted to only 68%
of the theoretical maximum. This suggests a simulta-
neous degradation of about one third of total muscle
protein during this period.

DISCUSSION

Growth of the dorsal longitudinal flight muscles of
locusts reared under the conditions described, was
virtually completed at day 12 after the final ecdysis.
The small further increase in muscle fresh weight up
to day 20 of the adult period was mainly due to a
small rise of the protein concentration (Fig. 1).

The rise of the flight muscle DNA concentration in
the second half of the fifth instar period and the sub-
sequent drop during the first days after the imaginal
moult has also been observed in *Schistocerca* (Richard
et al., 1971). In *Locusta* the elevated DNA level coin-
cides with the invagination of tracheoblasts into the

Table 2. Protein synthesis and degradation in flight muscles of *Locusta migratoria* during successive periods of development*

Period	*a* Starting concentration	*b* Calculated synthesis	*c = a + b* Theoretical maximum	*d* Actual concentration	*e = c − d* Degradation	*f* *d/c* × 100
Fifth instar						
5d → 8d	1000	2590	3590	2658	932	74
Adult						
1d → 2d	3898	1233	5131	4872	259	95
2d → 3d	4872	1407	6279	5460	819	87
3d → 4d	5460	1295	6755	6264	491	93
4d → 6d	6264	2829	9093	8775	318	96
6d → 8d	8775	2961	11,763	9768	1968	83
8d → 12d	9768	5428	15,196	13,134	2062	86
12d → 20d	13,134	9456	22,590	15,262	7328	68

* Values represent µg protein in flight muscles of one female.

a: Concentration on first day of period (values from Fig. 1). *b*: Calculations are made with the rates
presented in Fig. 4. For periods of more than one day the mean of the rates on the first and the last day
of the relating period are used. *c*: Theoretical final concentration in the absence of protein degradation.
d: Protein actually present on last day of period (values from Fig. 1). *e*: Difference between theoretical
and actual values attributed to degradation. *f*: Actual protein concentration as a percentage of theoreti-
cal maximum.

flight muscles, which takes place during the "moulting interval" (Brosemer et al., 1963). Once tracheolization is completed, the DNA concentration returns to the level present on the 5th day of the larval instar period. This might suggest a causal relationship between the elevated DNA concentration and tracheolization.

The high concentration of RNA in 6-day-old adults may indicate a high protein synthesizing capacity. Indeed, we measured the highest rate of synthesis on day 6, though the difference from the other days was not significant (Fig. 4).

The individual contribution of some specific amino acids to the FAA pool changes drastically during development. The sharp rise in proline concentration between the first and fourth day after the imaginal ecdysis coincides with the beginning of flight capacity; a role of proline in locust flight can be taken from experimental data obtained by Worm and Beenakkers (in preparation), who observed a decrease of proline in locust flight muscle during flight.

The increase of the arginine concentration during development is probably attributable to the role of arginine phosphate as the phosphagen of insects, which has the same function as creatine phosphate in vertebrate muscle (Gilmour, 1965), and thus may participate in flight metabolism.

The sharp decrease of the glutamate concentration after the final ecdysis possibly results from the more aerobic character of the adult muscle after completion of the tracheolization. The aerobic muscle will have an enhanced capacity to oxidize 2-oxoglutarate derived from glutamate.

Taurine shows the most spectacular changes of all amino acids studied. The function of this by far most abundant amino acid in the developed flight muscle is not clear. It has been suggested by Gilmour (1961) that in insect muscles taurine could function as a phosphagen, just like arginine. So far, such a role has only been reported for some annelids (Jacobsen & Smith, 1968). In mammalian hearts, where it represents nearly 50% of the FAA pool, taurine is suggested to play a role in the control of K^+ and Ca^{2+} movements, thus affecting cardiac function (Remtulla et al., 1978). A function of taurine as a neurotransmitter has also been suggested (Kaczmarek & Davidson, 1971).

For our studies on protein synthesis we have taken the total FAA pool of the muscles as the precursor pool. However, there do exist contradictory views about this, some considering the extracellular amino acid pool as the real precursor pool (Airhart et al., 1974; Van Venrooij et al., 1974), whereas others conclude that the total FAA pool provides precursors for protein synthesis (Fern & Garlick, 1974; Seglen & Solheim, 1978). In the locust flight muscle we have estimated the amount of protein synthesized at different times after [^{14}C]leucine injection (Fig. 3). During at least 1 hr a linear course was obtained when protein synthesis was calculated from the leucine specific activity in the whole FAA pool. It therefore seems reasonable to consider this pool as the precursor pool for protein synthesis in locust flight muscles.

Protein is synthesized at a significantly higher rate after the final ecdysis than before (Fig. 4). During the first 3–4 days of adult life, this protein synthetic activity will be pertinent to the process of differentia-

tion, when sarcomeres and mitochondria develop their typical adult characteristics and also the adult enzyme pattern is formed (Brosemer et al., 1963; Beenakkers et al., 1975). In the next developmental period, which lasts up to the end of muscle development, a high protein synthetic activity is needed for doubling of the contractile elements and the enzyme activities, together with an increase of the mitochondrial volume up to 30% of total muscle volume (Brosemer et al., 1963).

In all successive periods studied the estimated amounts of protein synthesized exceeded the increase of the protein concentration, the difference being attributed to protein degradation (Table 2). Synthesis and increase corresponded most closely during the first week after the final ecdysis, suggesting a relatively small degradation. Protein degradation estimated for the differentiation phase may be correlated with the breakdown of some enzymes taking place during this period (Brosemer et al., 1963; Beenakkers et al., 1975).

During the last adult period (Ad-12d → Ad-20d), when muscle development is completed, protein synthesis still continues at a rather high level. The major function of this synthesizing activity seems to be renewal of proteins already present, i.e. their turnover. In 20-day-old adults, still a mean synthesis of about 1.1 mg protein per animal was measured, this synthesis serving exclusively the turnover of proteins. Assuming the permanence of this steady state between synthesis and degradation for a longer period, this would mean a daily renewal of 7% of total muscle proteins, resulting in a half-life of 7 days. This rate of turnover is high compared with that of vertebrate muscles; total protein from rat muscle, for instance, has a half-life of 30 days (Lehninger, 1975). The high turnover rate in locust flight muscle may be correlated with the high metabolic capacity of this tissue.

The data on protein synthesis and degradation during development should be interpreted carefully, as is the case with corresponding results obtained by using separate muscle protein classes, since there will be a marked heterogenity in turnover rate of different proteins. Nevertheless, a general insight in protein metabolism during development focusses attention to those periods in which similar studies on individual proteins will be promising for research on regulatory processes.

REFERENCES

Airhart J., Vidrich A. & Khairallah E. A. (1974) Compartmentation of free amino acids for protein synthesis in rat liver. Biochem. J. 140, 539–548.

Beenakkers A. M. Th. (1973) The influence of corpora allata on flight muscle development in locusts. J. Endocr. 57, 52.

Beenakkers A. M. Th. & Van den Broek A. Th. M. (1976) Juvenile hormone and the development of locust muscles. Gen. comp. Endocr. 29, 295.

Beenakkers A. M. Th., Van den Broek A. Th. M. & De Ronde Th. J. A. (1975) Development of catabolic pathways in insect flight muscles. A comparative study. J. Insect Physiol. 21, 849–859.

Bernays E. A. (1972) The muscles of newly hatched Schistocerca gregaria larvae and their possible functions in hatching, digging, and ecclysial movements (Insecta: Acrididae). J. Zool. 166, 141–158.

BROSEMER R. W., VOGELL W. & BÜCHER TH. (1963) Morphologische und Enzymatische Muster bei der Entwicklung indirecter Flugmuskeln von *Locusta migratoria. Biochem. Z.* **338**, 854–910.

BURTON K. (1968) Determination of DNA concentration with diphenylamine. In *Methods in Enzymology.* Vol. 12B (Edited by GROSSMAN L. & MOLDAVE K.) pp. 163–166. Academic Press, New York.

FERN E. B. & GARLICK P. J. (1974) The specific radioactivity of the tissue free amino acid pool as a basis for measuring the rate of protein synthesis in the rat *in vivo. Biochem. J.* **142**, 413–419.

GILMOUR D. (1961) *The Biochemistry of Insects.* Academic Press, New York.

GILMOUR D. (1965) *The Metabolism of Insects.* Oliver & Boyd, Edinburgh.

HILL L. & GOLDSWORTHY G. J. (1968) Growth, feeding activity and the utilization of reserves in larvae of *Locusta. J. Insect Physiol.* **14**, 1085–1098.

JACOBSEN J. G. & SMITH L. H. JR (1968) Biochemistry and physiology of taurine and taurine derivates. *Physiol. Rev.* **48**, 424–511.

KACZMAREK L. K. & DAVISON A. N. (1971) The role of taurine. *Biochem. J.* **123**, 45P.

LEHNINGER A. L. (1975) *Biochemistry.* 2nd edn. Worth Publishers, New York.

LIN R. I. S. & SCHJEIDE O. A. (1969) Microestimation of RNA by the cupric ion catalyzed orcinol reaction. *Analyt. Biochem.* **27**, 472–483.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L. & RANDALL R. J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

POELS C. L. M. & BEENAKKERS A. M. TH. (1969) The effects of corpus allatum implantation on the development of flight muscles and fat body in *Locusta migratoria. Ent. exp. appl.* **12**, 312–324.

REMTULLA M. A., KATZ S. & APPLEGARTH D. A. (1978) Effect of taurine on ATP-dependent calcium transport in guinea-pig cardiac muscle. *Life Sci.* **23**, 383–390.

RICHARD C., TANGUAY R. & CHAUDHARY K. D. (1971) Nucleic acid and protein variations in relation to ultrastructural changes in the developing thoracic muscle of the African desert locust, *Schistocerca gregaria* Forsk. *Cytobios* **3**, 145–160.

SCHACTERLE G. R. & POLLACK L. L. (1973) A simplified method for the quantitative assay of small amounts of protein in biologic material. *Analyt. Biochem.* **51**, 654–657.

SEGLEN P. O. & SOLHEIM A. E. (1978) Valine uptake and incorporation into protein in isolated rat hepatocytes. *Eur. J. Biochem.* **85**, 15–25.

THOMAS G. J. (1954) The post-embryonic development of the flight muscles of *Lamarckiana* sp. (Orthoptera) and a brief comparison of these with those of *Saussurea stuhlammiana* (Karsch) and *Tanita dispar* (Miller). *Proc. R. Ent. Soc. Lond.* **A**, 23–30.

TIEGS O. W. (1955) The flight muscles of insects—their anatomy and histology; with some observations on the structure of striated muscle in general. *Phil. Trans. R. Soc. Lond.* **B238**, 221–348.

VAN DEN HONDEL-FRANKEN M. A. M., BEENAKKERS A. M. TH. & HUIBREGTSE-MINDERHOUD L. (1979) Flight muscle development and JH-titer after implantation of active corpora allata in developing locusts. *Gen. comp. Endocr.* In press.

VAN MARREWIJK W. J. A. & RAVESTEIN H. J. L. (1974) Amino acid metabolism of *Astacus leptodactylus* Esch. —I. Composition of the free and protein-bound amino acids in different organs of the crayfish. *Comp. Biochem. Physiol.* **47B**, 531–542.

VAN MARREWIJK W. J. A. & ZANDEE D. I. (1975) Amino acid metabolism of *Astacus leptodactylus* Esch. —III. Studies on the biosynthesis of α- and β-alanine from aspartate. *Comp. Biochem. Physiol.* **51B**, 371–373.

VAN VENROOIJ W. J., MOONEN H. & VAN LOON-KLAASSEN L. (1974) Source of amino acids used for protein synthesis in HeLa cells. *Eur. J. Biochem.* **50**, 297–304.