Patterns of Proteins Synthesized in the R15 Neuron of *Aplysia*

Temporal Studies and Evidence for Processing

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ABSTRACT The time-course of changes in the pattern of newly synthesized proteins in the R15 neuron of the parietovisceral ganglion of *Aplysia californica* has been studied at 14°C. 5% polyacrylamide gels containing sodium dodecyl sulfate (SDS) have been used to separate newly synthesized (leucine-labeled) proteins from the neuron. We have demonstrated that the pattern of newly synthesized proteins from the R15 neuron does not change significantly if 5-h pulses of labeled leucine are given during the first 72 h of in vitro incubation of the excised ganglion. However, the level of leucine incorporation begins to decline somewhere between 17 and 43 h after the ganglion is isolated; at 43 and 69 h the levels of incorporation fell to 29 and 10% of the initial level, respectively.

A number of conclusions have been drawn from the use of a sequential, double-label type of experiment in the same cell. There is processing of SDS-soluble, 12,000-dalton (12k) material to 6,000–9,000-dalton (6–9k) material. These materials are the two major peaks on gels after long labeling periods and together account for about 35% of all newly synthesized proteins. After synthesis of 12k material, there is a gradual disappearance of 12k (half-life about 8 h) and simultaneous appearance of 6–9k material on the gels, as the postsynthesis "chase" period of ganglia incubation is increased. The processing of 12k to 6–9k material occurs even in the presence of anisomycin, a protein synthesis inhibitor, during the chase period. While the rate of 12k to 6–9k conversion can vary from cell to cell, it appears to remain consistent within, and is characteristic of, any individual R15. We detect no circadian rhythm in either the rate of 12k synthesis or the rate of 12k to 6–9k processing with 5-h label periods. These results are discussed in relation to the roles of 12k and 6–9k material in the R15 neuron.

INTRODUCTION

The size and known morphological and physiological properties of the R15 neuron from *Aplysia californica* make it an ideal subject for the study of protein patterns in relationship to specific cell functions. The R15 neuron contains within its soma approximately 1,200-Å dense-cored vesicles and is presumably neurosecretory (Frazier et al., 1967; Bernstein, 1967). There is evidence that the R15 neurosecretory product is involved in ionic or water balance: an aqueous

extract from R15, when injected into *Aplysia*, causes a significant weight gain (Kupfermann and Weiss, 1976). R15 also possesses an endogenous, bursting pacemaker whose spike output shows a circadian rhythm in frequency (Strumwasser, 1965, 1971; Lickey, 1969; Audesirk and Strumwasser, 1975). Earlier studies have analyzed the newly-synthesized ([^{3}H]leucine labeled) proteins from R15 on sodium dodecyl sulfate (SDS) polyacrylamide gels (Wilson, 1971; Gainer, 1971; Loh and Peterson, 1974).

In this paper we present the results of a more detailed analysis of some temporal aspects of protein metabolism in the R15 neuron. We have looked for changes in the pattern of proteins synthesized at various times up to 3 days after removal of parietovisceral (abdominal) ganglia (containing R15) from *Aplysia*. We have searched for circadian rhythms in certain aspects of protein metabolism in the neuron. "Pulse-chase" experiments, with 5 h of labeling with radioactive leucine followed by 5, 10, or 15 h of chase (with another radioactive "tag" present during the last 5 h of incubation) have been performed in order to follow the metabolism of newly synthesized proteins. These pulse-chase experiments have indicated that, after synthesis of 12,000-dalton (12k) material in R15, processing of the 12k material to 6,000–9,000-dalton (6–9k) material occurs. Previous studies from this laboratory (Arch, 1972) have shown that, in a set of neurosecretory neurons (the bag cells in the same ganglion) producing the 6,000-dalton polypeptide egg-laying hormone, a precursor-product relationship occurs in which a 25,000-dalton polypeptide is processed into 12,000-dalton and ultimately 6,000-dalton products.

The knowledge gained in these temporal studies affects the interpretation of earlier results on protein synthesis in R15 and serves as a foundation for further studies on the interactions between protein metabolism and physiological function in the R15 neuron. Processing of newly synthesized proteins in the soma of R15 was first described in an abstract (Strumwasser et al., 1973). The insensitivity of processing to an inhibition of protein synthesis was described in another abstract (Wilson and Strumwasser, 1975).

MATERIALS AND METHODS

Animals

The gastropod mollusc, *Aplysia californica*, was obtained from Pacific Biomarine Supply Co., Venice, Calif. Animals weighed from 150 to 400 g, and were maintained at 13–15°C under 12:12 light-dark (LD) conditions.

Ganglia Excision and Incubation

Parietovisceral (abdominal) ganglia were dissected and rinsed in filtered sea-water (FSW) for 2 h, unless other preincubation conditions are indicated. All ganglion manipulations were at 13–15°C but temperature regulation was good to ±0.5°C during any one experiment. Incubation medium (IM), unless otherwise indicated, contained (in mM) 450 NaCl, 10 KCl, 21 $MgCl_2$, 28 $MgSO_4$, 13 $CaCl_2$, 10 Tris-Cl (pH 7.7), 10 g/liter glucose, vitamins for minimum essential medium, and essential amino acids for minimum essential medium at $^1/_{40}$ the recommended concentration (Eagle, 1959). Incubation medium was filtered (Millipore, 0.22 μm), and stored frozen until use. Radioactive leucine was added, at the

time of the experiment, in a final volume of 0.9 ml into 5 ml of incubation medium and consisted of 5.2 μg of either [^{3}H]leucine (250 μCi; Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y., about 55 Ci/mmol) or [^{14}C]leucine (12.5 μCi; Schwarz/Mann, about 310 mCi/mmol). When anisomycin (Pfizer Inc., New York) was used, the concentration in IM was 5.4 μg/ml.

Double-Labeling

In those experiments employing both [^{3}H] and [^{14}C]leucine incubations of individual ganglia, ganglia were rinsed twice in incubation medium immediately after the first labeling period in order to reduce carry-over of label to the second labeling period. There were no significant pattern differences between [^{14}C] and [^{3}H]leucine-labeled R15 neurons. Nevertheless, each set of double-label experiments had about even frequencies of ^{3}H and ^{14}C being used as the first label. Typically, each labeling period was for 5 h.

R15 Neuron Dissection and Protein Extraction

R15 neurons were dissected from the ganglion as described in Wilson, 1971. Cells were then homogenized in small glass grinders using three rinses of 8 μl each of 10% glycerol, 0.0015% bromphenol blue, 0.2% sodium dodecyl sulfate, 2% 2-mercaptoethanol, and 0.01 M sodium phosphate, pH 7.1. Samples were frozen until used. After thawing, samples were heated to 65°C for 25 min, then centrifuged at 20,000 *g* for 15 min.

Gel Electrophoresis and Counting

Gel electrophoresis was on miniature, 5% polyacrylamide gels, at pH 7.1, in the presence of sodium dodecyl sulfate, and gave a molecular-weight distribution of polypeptides from the single neurons. Precision-bore glass tubing (Drummond Scientific Co., Broomall, Pa.; about 1.4 mm ID) was used for the gels. Calibration of the gel system by molecular weight was performed by comparing the migration of bovine serum albumin, chymotrypsinogen, ovalbumin, myoglobin, cytochrome C, and insulin with bromphenol blue in Coomassie-blue stained gels (Wilson, 1971). See Wilson, 1971 for details on procedure and for demonstration that counts in the gels are predominantly incorporated into newly synthesized proteins. Gel slicing and counting were as described in Ward et al., 1970. Counting efficiency was 41% for ^{3}H and 58% for ^{14}C. Backgrounds were 17 and 8 cpm, respectively. Overlap corrections for double-label counting were performed whenever such corrections were significant.

RESULTS

Varying Preincubation Times

As a preliminary to the detailed analysis of protein synthesis in the R15 neuron, we have examined whether the patterns of newly synthesized proteins on gels reflect a stable, continuing condition, or a momentary one produced by certain aspects of ganglia excision from *Aplysia* before labeling. First we found that 2-h incubations in [^{3}H]leucine immediately after ganglia excision gave protein synthesis patterns similar to those obtained after 4–5 h of incubation. Then a series of 5-h labelings were performed at various times after ganglion dissection from the *Aplysia*, and R15 neurons were dissected for analysis immediately after labeling. Fig. 1 indicates that there was little or no change in gel pattern when the 5-h labeling was performed 3 days after dissection (compare with Fig. 2). Table I summarizes the percent of total gel counts in the 12k and 6–9k regions of

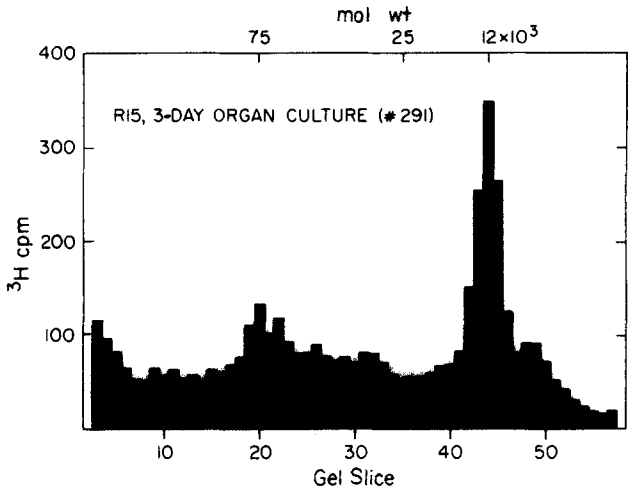

FIGURE 1. Protein synthesis pattern of a single R15 neuron after 3 days of ganglion incubation. An abdominal ganglion was dissected from *Aplysia* and incubated in about 8 ml. IM, with 100 U each per milliliter of penicillin and streptomycin (Microbiological Associates, Bethesda, Md.), for 69 h. IM was changed at daily intervals. The ganglion was then incubated for 5 h in IM with [^{3}H]leucine. The R15 neuron soma was isolated and its proteins extracted for analysis on an SDS polyacrylamide gel. Bromophenol blue acted as an internal marker for the determination of molecular weight distribution.

R15 gels obtained with intervals between dissection and the start of a 5-h labeling period of 7, 12, 17, 43, and 69 h. These results indicate that the 12k region of the R15 gels is remarkably stable, containing about 24% of the total gel counts whether incubation in label is started 7 or 69 h after dissection. The 6–9k regions of R15 gels account for about 11% of the total counts in the 7–43-h cases but appears to be somewhat reduced ($P = 0.025$ by t test) to 7.4% at 69 h. The most striking effect of increasing delay between dissection and the start of incubation is on the total incorporation of leucine into proteins of R15. The level at 69 h is about 10% of that at 7 h and about 35% of that at 43 h. Thus, there is at least a 72-h period during which the pattern of proteins synthesized appears to be relatively stable as well as at least a 17-h period in the stability of the level of total incorporation of leucine into proteins. All protein synthesis experiments described in the following sections of this paper were performed within the first 24 h after ganglion dissection.

Pulse-Chase Analysis

We have taken advantage of double-labeling to analyze the early metabolism of newly synthesized proteins in R15. After brief (2 h) preincubations, ganglia were bathed in medium containing radioactive (^{3}H or ^{14}C) leucine. After rinsing in nonradioactive medium, ganglia were further incubated for 5, 10, or 15 h, with radioactive (^{14}C or ^{3}H) leucine present for the final 5 h of incubation. Thus, each R15 neuron was double-labeled with [^{3}H] and [^{14}C]leucine, with the second label

TABLE I

EFFECTS OF INCREASING DELAY BETWEEN DISSECTION OF ABDOMINAL GANGLION AND INCUBATION IN RADIOACTIVE MEDIUM

Time after ganglion dissection[a]	[^{3}H] or [^{14}C]leucine, incorporation[b]	Total cpm in gel[c]		
		12k	6–9k	*N*
h	*pg*	%	%	δ
7	140±90	24.0±4.8	11.2±3.3	35
12	150±100	24.2±6.7	10.9±3.7	9
17	130±100	21.3±2.9	9.9±2.2	10
43	40±23	24.6±1.5	10.7±3.8	3
69	14±7	24.6±1.2	7.4±2.2	4

[a] All incubations after dissection were in IM (8–10 ml) after rinsing ganglia in FSW. Penicillin and streptomycin (100 U each per milliliter; Microbiological Associates) was present in the 69-h ganglia with changes of medium made daily. Labeling was for 5 h in all cases.

[b] Concentration of leucine present in 7-, 12-, and 17-h ganglia was 0.88 μg/ml; in 43–69-h ganglia, leucine was 0.13 μg/ml.

[c] cpm from four gel slices (1 mm/slice) or three gel slices (1.3 mm/slice) were summed for each region; bromophenol blue was used as a marker "tracking" dye for standardization. Total cpm in gel excludes top four or five slices where unincorporated leucine is present (Wilson, 1971). Numbers shown are means ± sample standard deviations.

serving as an internal control, always 5 h in length and with no chase period. The label incorporated during the first incubation period was then used to observe the metabolic events, which occur shortly after protein synthesis.

Table II indicates that there is a slow decrease (over about 10 h) of 12,000-dalton (12k) material (from about 24 to 10–11%) and simultaneous increase of 6,000–9,000-dalton (6–9k) materials (from about 11 to 18–23%), with good conservation of the sum of these materials in the two regions. In other words the half-life of the 12k material was about 5–10 h at 14°C and its disappearance was correlated with the appearance of increasing amounts of the 6–9k material. Only in 5-10-5 experiments (5 h of labeling followed by 15 h of chase, with a second label present during the last 5 h) was there a significant reduction in the early-labeled, 6–9k material still present in the R15 soma. The mean ratios of 6–9k/12k material range from 0.48 to 0.50 whether the second label is given early (5-5) or late (5-10-5); for the first label which allows processing to occur, this ratio increases from 1.60 (5-5) to 2.15 (5-5-5) and is stable thereafter. Typical patterns are shown in Fig. 2 (5-5; 5-10-5).

There was wide variation as to the percent of 6–9k/12k material from cell to cell (e.g. 50–300% in the first label of a 5-5 experiment, $n = 31$). However, it is quite interesting that there is a high correlation (sample correlation coefficient = 0.80) between the percent ratio of 6–9k to 12k material in the first and second label within any individual R15 (Fig. 3). This implies that different R15 cells may be processing 12k material at different rates but that the rate is consistent and characteristic within each cell.

During chase periods, the presence of anisomycin, an inhibitor of protein synthesis in *Aplysia* (Schwartz et al., 1971), does not interfere with the shift of label from 12k to 6–9k (Table III and Fig. 4). This indicates that further protein

TABLE II

PULSE-CHASE SERIES IN THE R15 NEURON

Procedure[a]	N	Label	Total cpm[b] 12k	Total cpm[b] 6–9k	Sum (12k+6–9k)	Ratio (6–9k/12k)
			%	%		
5†-5*	35	†	13.9±3.8	20.5±4.4	34.4±5.4	1.60±0.49
		*	24.0±4.8	11.2±3.3	35.0±5.7	0.49±0.19
5†-5-5*	9	†	11.1±2.5	23.1±6.3	34.1±7.1	2.15±0.70
		*	24.2±6.7	10.9±3.7	35.2±7.8	0.48±0.24
5†-10-5*	10	†	9.7±2.4	17.9±4.6	27.6±5.8	1.93±0.70
		*	21.3±2.9	9.9±2.2	30.2±3.9	0.50±0.20

[a] Numbers listed are hours. Periods with label are indicated by † and *. Thus, 5†-5-5* indicates 5 h of labeling followed by 10 h of chase, with a second label present during the last 5 h of chase.
[b] Percent of gel cpm in three slices (3.9 mm) of gel in the region indicated.

synthesis is not involved in the increased proportion of radioactive material appearing in the 6–9k region of the gels during the chase period.

Circadian Studies on Protein Synthesis in R15

We have concentrated on attempts to detect changes in the rate of 12k synthesis or in the rate of processing from 12k to 6–9k as a function of time of day. Animals were kept under a 12:12 h light-dark schedule for at least 6 days before use. A series of 5-5 experiments were performed. During the first 5 h of incubation, either [^{3}H] or [^{14}C]leucine was present in the incubation medium. During the second 5 h, the opposite label was present. Individual neurons were dissected for analysis within a short time after the second labeling period. Experiments were begun at various phases relative to the light-dark cycle of the *Aplysia*. The second 5-h labeling periods began at 9, 19, and 23 h after "dawn" (lights-on) for the animals. As indicated in Table IV, there was no significant difference ($P > 0.025$) in the amount of 12k synthesis or 12k to 6–9k processing.

DISCUSSION

Increasing the delay between dissection of the abdominal ganglion from *Aplysia* and 5-h incubation in labeled medium has little or no effect on the patterns of newly synthesized proteins from the R15 neuron, at least up to 3 days of delay. Thus, we tend to view these protein synthesis patterns as "normal" rather than "induced" by ganglion excision from the *Aplysia*. Further support for this point comes from earlier data comparing the in vivo and in vitro patterns of protein synthesis in the R2 neuron of *Aplysia* (Wilson, 1971). The rate of incorporation of leucine into *Aplysia* neuronal protein allows a very approximate general half-life of more than 100 days to be calculated. When compared with reported half-lives of proteins in mammals (1–2 wk; Lajtha and Marks, 1971) the very different temperatures of the organisms must be kept in mind. A Q_{10} of about 3 would account for the difference. The cause of the reduction in total incorporation, when 5-h pulses of label are given after the first day of ganglion incubation, is

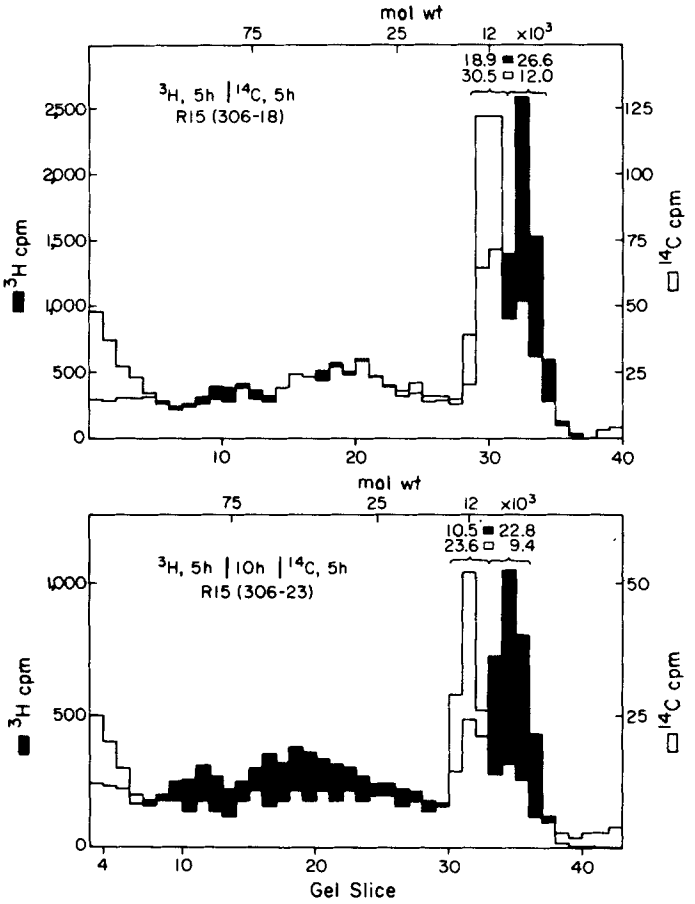

FIGURE 2. Pulse-chase patterns of newly synthesized proteins in R15. Each figure shows a single R15 neuron from a ganglion which was incubated first with [³H]leucine, then with [¹⁴C]leucine. All labeling periods were 5 h. (*a*) The first 5-h labeling period in [³H]leucine was immediately followed by a second 5-h labeling period with [¹⁴C]leucine present. (*b*) The first 5-h labeling period was followed by 10 h of incubation in IM with no added label, and then a 5-h labeling with [¹⁴C]leucine. Also shown are the percent 12k and 6–9k counts and the gel regions used for the calculations for each of the labels.

unknown. Schwartz et al. (1971) reported a reduction in incorporation rate which occurs even earlier after ganglion dissection, under different culture conditions.

The failure to find a circadian rhythm in 12k synthesis or 12k to 6–9k processing does not necessarily contradict the earlier report of Loh and Peterson (1973) of an enhanced synthesis of 12k material at dawn, because these authors used shorter time periods of labeling (1 h) and more frequent sampling times during the day (every 2 h). However, the recent report by Audesirk and Strumwasser (1975) on the effect of ganglion dissection time on the phase of the circadian rhythm in spike output from R15, suggests that a proper study of

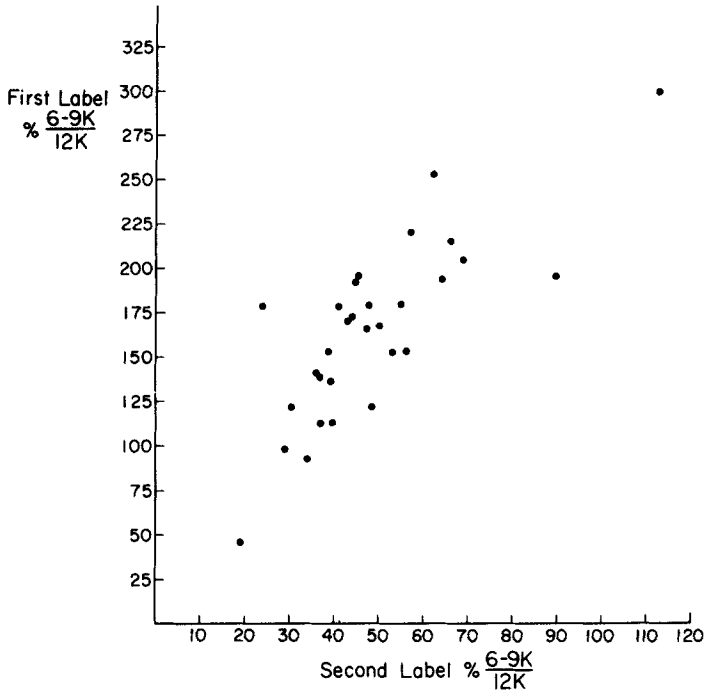

FIGURE 3. Correlation of ratio of 6–9k to 12k labeling between first and second labeling periods. Each ganglion was labeled for 5 h with [^{3}H] or [^{14}C]leucine, followed by 5 h of labeling with [^{14}C] or [^{3}H]leucine. R15 neuron proteins were extracted and separated on polyacrylamide gels, and the percent ratio of counts, 6–9k/12k was determined for each label.

circadian rhythms in protein synthesis may also have to take account of the effects of time-of-dissection. It is possible, in fact, that an actual circadian rhythm in the synthesis of some protein species in R15 could be masked by the disrupting effects of dissections at various times of day.

Gainer and Barker (1974) had reported that synaptic inhibition, by branchial nerve stimulation for 4 h, decreased the level of 12k synthesis (3-h label) by about 21% in experiments performed at 21–23°C. Our circadian studies indicated a constancy of synthesis and processing rates of the 12k material (over a 24-h period) in R15 and hence imply that there is no significant spontaneous synaptic regulation, within the isolated ganglion, of 12k synthesis or processing.

The persistence of normal synthesis patterns from R15 for at least 3 days after ganglion dissection from *Aplysia*, and the failure to detect significant circadian effects on the synthesis patterns, also are important control experiments for the pulse-chase study of R15 protein metabolism. Thus, the disappearance of 12k material and simultaneous appearance of 6–9k material, observed as chase periods are extended, cannot be accounted for by changes in synthesis occurring with time-after-dissection or time-of-day. In addition, the presence of anisomycin, a protein synthesis inhibitor, during the chase period, did not affect the disappearance of 12k or appearance of 6–9k material, indicating that protein

TABLE III
EFFECT OF PROTEIN SYNTHESIS INHIBITION DURING CHASE

Procedure[a]	N	cpm[b] 12k	cpm[b] 6–9k
		%	%
5*-5 Anisomycin	3	10.5±1.3	20.8±1.1

[a] Numbers listed are hours. Period with [^{3}H]leucine present is indicated by *. 18 μM anisomycin (a gift from Pfizer) was present during the 5-h chase period. Independent experiments indicate that this level of anisomycin is sufficient to block >95% of protein synthesis, both in terms of TCA precipitable counts and count appearing on the gels, in verification of the results of Schwartz et al. (1971).

[b] Percent of total cpm in gel in the regions indicated (four slices; 1 mm/slice) ± sample standard deviation.

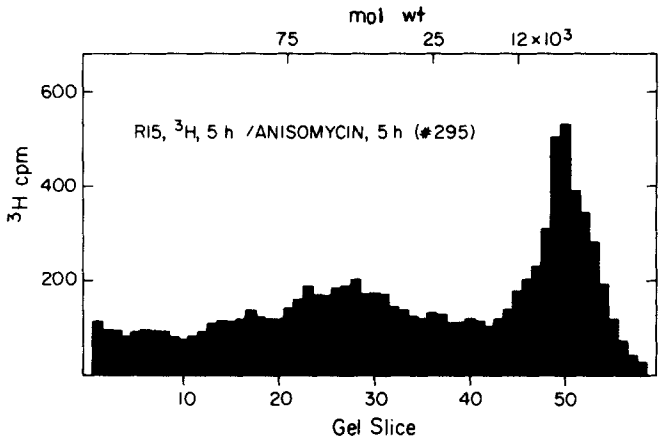

FIGURE 4. Effect of protein synthesis inhibition during chase on the protein-labeling pattern from R15. An abdominal ganglion was incubated for 5 h in IM containing [^{3}H]leucine. The ganglion was further incubated for 5 h in IM containing anisomycin (18 μM) before neuron dissection and homogenization.

synthesis is not required for this change. Thus, it is not the case that the 6–9k protein merely has strange, delayed synthesis kinetics. Instead, the most likely explanation of the results is that processing of 12k to 6–9k material occurs, with a delay at 14°C of 5–10 h after 12k synthesis. After our preliminary report (Strumwasser et al., 1973), this conclusion has also been reached by Loh and Gainer (1975) although another, apparently earlier, report in the same year (Gainer and Barker, 1975) did not consider the possibility of processing of newly synthesized proteins in the same neuron.

The good conservation of the sum of material in 12k and 6–9k regions of the gels during processing strongly suggests that one does not convert half of the 12k material into peptides during the processing, but that there is a splitting of all the 12k material into only a few pieces.

The existence of 12k to 6–9k processing in R15 affects the results of other

TABLE IV

CIRCADIAN ASPECTS OF PROTEIN SYNTHESIS PATTERNS FROM R15

Procedure[a]	Time of beginning second label (hours after dawn)	Label	cpm[b] 12k	cpm[b] 6–9k	Sum (12k+6–9k)	N
			%	%		
5†-5*	9	†	14.5±4.3	21.0±4.7	35.5±5.1	18
		*	24.6±4.6	11.3±3.2	35.8±4.7	18
5†-5*	19	†	14.2±3.8	19.0±4.2	33.2±6.3	11
		*	23.1±8.8	10.0±2.9	34.9±6.3	11
5†-5*	23	†	11.7±1.3	21.6±3.9	33.3±5.1	6
		*	20.4±5.0	12.9±3.7	32.8±7.8	6

[a] Numbers listed are hours.

[b] Percent of total cpm in the gel at the regions indicated (three slices, 3.9 mm of gel) ± sample standard deviations.

researchers. One must be cautious in interpreting synthesis rates of proteins from measurements which compare radioactivity to optical density in gel profiles (Loh and Peterson, 1974). The low incorporation relative to staining of the 6–9k material which these authors reported was not caused by low synthesis rates for the 6–9k material, but was undoubtedly due to the delay between 12k synthesis and processing. One must also use caution in comparing earlier reported data on R15 synthesis and turnover patterns due not only to the existence of processing but to the wide range of temperatures (13–23°C) and incubation times (1–24 h) which have been used by the various researchers. The rate of processing, and hence, the relative abundances of 6–9k and 12k materials will depend upon these variables. Thus, the existence of processing of 12k material explain such divergent earlier reports as the R15 patterns showing little 6–9k labeling (Loh and Peterson, 1974) and those showing significant 6–9k labeling (Gainer, 1971).

For other *Aplysia* neurons, the ratio of counts in various regions of the gels is very repeatable from animal to animal (Wilson and Berry, 1972). Therefore, the variable ratios of 6–9k/12k material among R15 cells (Fig. 3) indicate that there is a variable rate of processing in these cells. We have found ways of altering the processing rate when pacemaker waves in R15 are blocked by substitution of acetate or propionate for chloride (Strumwasser, 1973; Strumwasser and Wilson, manuscript in preparation), thus supporting the idea that the rate of processing is under the control of some aspect of neuron functioning. When spike production, but not pacemaker waves, are blocked by TTX neither synthesis nor processing of 12k material is affected.

There has been considerable speculation concerning the roles of the low molecular weight materials in R15 and other *Aplysia* neurons (Strumwasser, 1973; Loh and Peterson, 1974; Gainer and Wollberg, 1974; Wilson, 1974; and Berry, 1975). These speculations have centered on low molecular weight materials participating in neurosecretion or spontaneous activity. There is one other well-documented case of processing of polypeptides in *Aplysia*. Arch (1972) has shown that there is a 25,000-dalton precursor to the 6,000-dalton neurosecretory polypeptide in the bag cells of the abdominal ganglion; this 6,000-dalton

polypeptide induces egg-laying in *Aplysia* (Toevs, 1970). In light of this, and because of its size, it is tempting to conclude that the 6–9k material is involved in neurosecretion in R15. Its role as a neurosecretory protein also would explain its apparent high production rate, since secreted proteins would require replacement. Its short half-life in the soma might reflect transport to axon terminals (Loh and Gainer, 1975), and 12k processing might be involved with packaging into granules. However, a careful comparison of the kinetics of disappearance of 6k material in the bag cells, a well-characterized neurosecretory system in *Aplysia*, and the disappearance of 6–9k material in R15 remains to be performed. It might be expected that these two types of neurosecretory neurons would transport their products with similar rates. Such a comparison has recently been made for R15 and L11 (presumed not to be neurosecretory) by Berry (1976). Colchicine, which blocks axoplasmic flow, caused accumulation of low-molecular-weight materials in L11, but not in R15, after 2 h of labeling and 8 h of chase. All of these points are indirect, however, and something more than circumstantial evidence is needed to determine what the actual function of the 6–9k material is. We have some evidence of a causal relationship between the functioning of the mechanism involved with pacemaker oscillations in R15 and the processing of 12k to 6–9k material (Strumwasser and Wilson, manuscript in preparation).

This paper presents insights into protein synthesis and processing in an individual neuron and also supplies information necessary for studies on changes in protein metabolism brought about by physiological manipulations of the R15 neuron.

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