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Conservation of Web Proteins in the Spider, *Araneus diadematus*

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ABSTRACT Experiments placing "cold" spiders on radioactive webs and measuring the specific activity of successive webs shows that silk protein is highly conserved during the web building cycle. This conservation of web protein is not affected by feeding the spider flies or cold amino acids. It is considered that the timing of web eating to web construction is critical to this efficient re-cycling.

Although technically spiders are distinguished from other creatures by such features as having eight legs and the fusion of the head and thorax what is strikingly unique is their ability to build snares for obtaining their food. For this process to be successful the energy content of the food obtained must be larger than the energy expended to obtain it. The two major factors in this process are the amount of food captured in the web and the expenditure that the spider has to make synthesising, constructing and operating the web. Since the spider has, apparently, little control over food capture, it is obviously advantageous to economise as much as possible on the latter. In this article the amino acid balance of the spider, as it pertains to web-building, is examined. The work reported is divided into three main parts: The efficiency of the re-cycling of protein through successive web-building, the changes in the amino acid pool of the ampullate gland during the silk synthesising cycle, and the frequency of replacement of the orb-web under natural conditions.

MATERIALS AND METHODS

Adult female *Araneus diadematus* (Cl) spiders were used. The laboratory experiments on web building were carried out in individual cages (20″ × 20″ × 4″) with removable glass windows. These cages and the techniques for measuring webs have been described previously (Witt, Reed and Peakall, '68). Only those spiders building webs daily throughout the course of the experiment were included in the data presented in table 1. Before the start of the experiment the amount of nitrogen per unit length of the thread of the web was determined for each spider. The total length of thread used in the web was calculated and then the web was carefully collapsed and collected on a thin glass rod. The web material was digested in selenium sulfuric acid and the amount of nitrogen present was measured colorimetrically by using Nessler's reagent (Breed et al., '64). At this point one group of spiders were given 5 μc of alanine-H^3 (330 mc/mM) orally. On the following day these spiders were removed from the cage and were not used again in the experiment. The "hot" web was measured and then a section, comprising some 10% of the total, was removed by laying a glass rod along a radius and cutting the spiral threads along the side of the rod, then the next radius was cut and the material rolled onto the glass rod. The material of three radii and the spiral material between were collected in this way (see fig. 1). The length of thread removed was calculated by multiplying the length of the middle spiral turn by the number of spiral turns and adding on the total length of the three radii. This material was then digested in 0.5 ml NCS solubilizer by refluxing for three hours, and then the radioactivity determined in a scintillation counter. The total number of counts in the web can then be calculated by multiplication. Webs were examined daily in this manner and, unless otherwise stated, spiders were maintained on water during the course of the experiments. In some experiments spiders were given cold

TABLE 1

Efficiency of re-cycling of silk proteins in successive web-buildings. The first line of numbers gives the number of radioactive counts in the web, normalized to a value of a thousand for initial web. The second line gives the calculated number of counts assuming perfect efficiency of re-cycling, and the third line gives percentage of actual over calculated. Initial value set at 1000. Figures are mean, standard error, and sample size

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Overall efficiency
No treatment, water only	1000	807 ± 56(10) 881 ± 9(10) 90.8	782 ± 35(10) 799 ± 15(10) 97.8	718 ± 54(10) 717 ± 20(10) 100.0	505 ± 32(10) 643 ± 24(10) 78.6	493 ± 47(8) 583 ± 33(8) 84.6	405 ± 42(6) 510 ± 46(6) 79.5	90.8
No treatment, flies and water	1000	886 ± 56(6) 901 ± 6(6) 98.3	791 ± 58(6) 817 ± 7(6) 96.8	700 ± 47(6) 745 ± 12(6) 93.9	692 ± 33(6) 664 ± 18(6) 104.2			98.3
Cold alanine × 10 at bar	1000	887 ± 124(4) 902 ± 24(4) 98.3	787 ± 50(4) 832 ± 32(4) 94.5	631 ± 34(4) 761 ± 40(4) 82.9	518 ± 13(4) 602 ± 29(4) 86.0			87.8
Cold web material at bar	1000	885 ± 49(5) 866 ± 18(5) 102.2	386 ± 37(5) 410 ± 33(5) 94.1	348 ± 44(5) 373 ± 30(5) 93.3	293 ± 44(5) 329 ± 27(5) 89.1			92.2
Web completely removed at bar	1000	858 ± 4(3) 907 ± 18(3) 94.9	32 ± 2(3)	27 ± 4(3)	33 ± 8(3)			

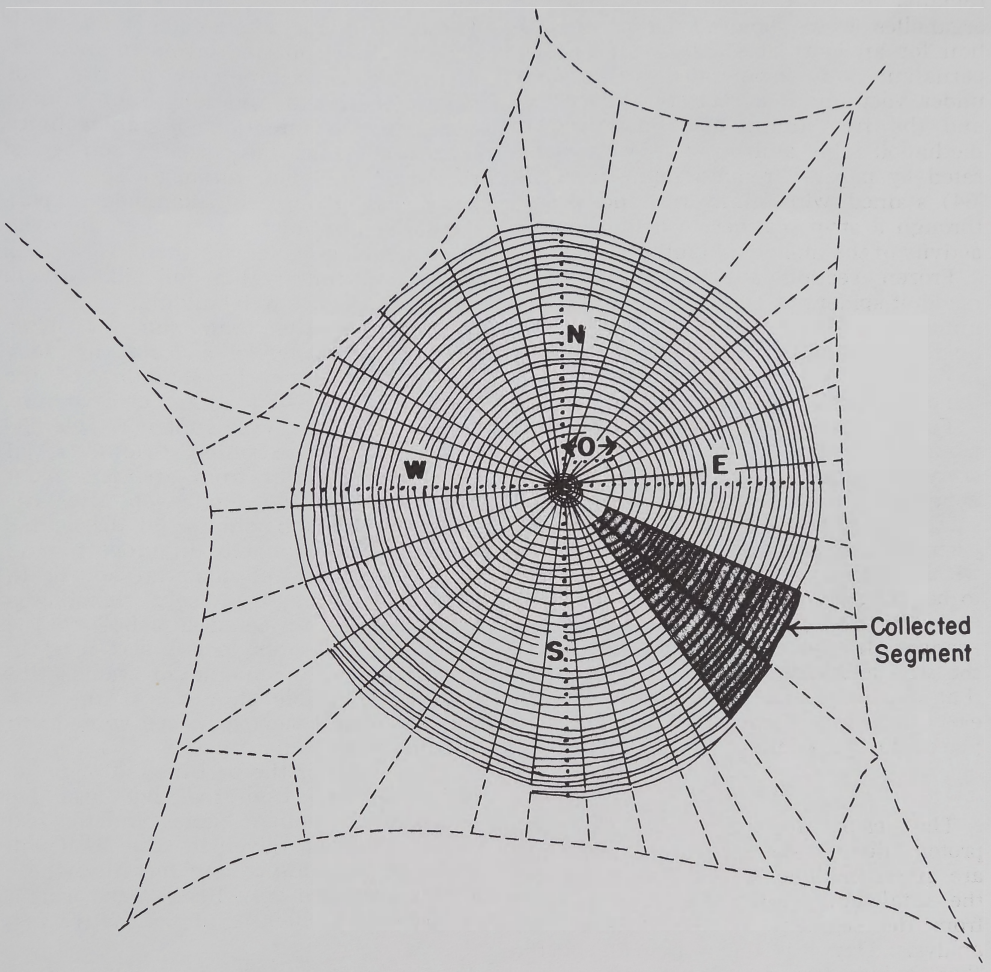

Fig. 1 Diagrammatic representation of web sampling method.----------, thread not included in calculation of length. R (average length of radii), (N + S + E + W)/4; n, number of radii; k, number of spiral turns on S radii; total length is given by formula nR + k (a + R).

alanine, cold web material, or flies. Some spiders were dissected at the end of the experiment and their ampullate glands removed. These glands were washed several times in 0.9% saline and then digested in NCS solubilizer before counting. The rest of the spider, including the washings of the ampullate gland, were also digested for counting. Some spiders were fixed, sectioned, and autoradiographs were prepared.

For the experiments on the amino acid pools in the ampullate gland the spiders were maintained in small jars. The spiders were fed 10 μc C^{14} protein hydrolysate (640 μc/mg) a day before the start of the experiment. Thread was reeled from the spider onto a glass rod rotated by a small motor. The ampullate glands were dissected out of the spider at fixed time after the silk glands had been emptied by reeling out the silk. The glands were removed intact and washed rapidly three times in 0.9% saline. The glands were then broken up by mild sonification; the silk in the lumen of the gland (if any)

remains intact. Cellular debris and cell organelles were removed by centrifugation for an hour at 105,000 xg. The supernatant was evaporated to dryness under vacuum in a rotatory evaporatory and the free amino acids dissolved in methanol. The amino acids were separated by paper chromatography (Peakall, '64) stained with ninhydrin and passed through a strip scanner to determine the activity of the individual amino acids.

Frozen section autoradiographs were made of spiders at 10, 20, 30, 60 and 120 minutes after ingesting a "hot" web. Spiders were frozen in isopentane cooled in liquid nitrogen and then sectioned by the procedure of Appleton ('68).

Field studies on web renewal were made on a total of thirty spiders for a six week period during August and early September near Dryden, New York. A small dab of colored paint was placed on each female *Araneus diadematus* found within the study area to allow individuals to be recognized. The area was examined early in the morning and late in the evening. In the evening the contents of the web and damage done was noted so that it was possible to determine in the early morning if the web had been replaced during the night.

RESULTS

The results of the recycling of silk protein during successive web buildings are given in table 1. The first line gives the total cpm's of the web calculated from the segment of web collected for analysis. Day 1 is the web made by the "hot" spider and day 2 onwards by the "cold" spider. The initial count is set to a thousand and all other counts reduced in the same ratio. The actual range of counts on day 1 was 1745 to 7470. The figures in line 2 are the theoretical maximum number of counts that could be in the new web if all the radioactive material available to the spider was in fact used. This figure is calculated by subtracting the actual number of counts removed from the system by sampling from the number of counts in the previous web. All figures are normalized to an initial value of a thousand. The third line gives the percentage of counts utilized. There are considerable day-to-day fluctuations in the total number of counts. These variations are greater than would be expected from the errors of measurement; i.e. measurements of the total length of thread and length of sample, and the determination of radioactivity. It appears that the specific activity of thread is variable, although this possibility has not been examined experimentally. Despite this variation the overall conclusion is clear, there is a high degree of conservation of silk protein during successive web-building.

Similar results were obtained when the spider was given a "cold" fly daily immediately after the sampling of the "hot" web. Nor did giving cold alanine (c 10 times the web content) alter the efficiency of re-cycling. However, if cold web material from another spider was added to the "hot" web after the sample was taken, dilution of the counts occurred approximately in proportion to the amount of web material added. In this case the length of "cold" thread was measured before being attached to the "hot" web and this length was used in calculating the amount of radioactive material available (line 2). If the web was completely removed, then subsequent webs were not radioactive.

The results of the partitioning of radioactive material from the "hot" web between the ampullate glands and the rest of the spider are given in table 2. It will be seen that, despite the fact that these glands comprise only 10% of the weight of the spider, 80-90% of radioactive ma-

TABLE 2

Partitioning of radioactivity between the silk glands and the remainder of the spider. Source of radioactivity is alanine-H ^{3} from "hot" webs

Total cpms in ampullate silk glands	Total cpms in remainder of spider	Ratio
3200	931	0.291
2705	300	0.111
2532	273	0.108
1209	122	0.101
1180	184	0,156
2847	729	0.256
2187	243	0.111
1484	117	0.079
2043	287	0.140
	Mean with standard error	0.150 ± 0.024

terial is located there. This conclusion is supported by autoradiographic studies which show appreciable localization only in the silk glands.

The quality of the frozen section autoradiographs was not good but even so showed clearly that labeled material was present in the ampullate silk glands thirty minutes after ingestion of a "hot" web.

The results of the determination of the composition of the amino acid pools of the body fluids and the silk glands are shown in figure 2. The tracings clearly show an increase of aspartic, serine, glycine and alanine in the resting gland as compared to either the active gland or the body fluids.

A total of 384 webs involving thirty individual spiders were examined in the field over a six week period. Except for five webs, involving three spiders, webs were renewed daily. One spider built a new web every other day over a six-day period before laying eggs and ceasing to build altogether. Two spiders ceased to build for two and three-day periods before a molt. Otherwise new webs were produced daily and successive webs were almost always in essentially the same position. The only exception was a web with an eight foot mooring thread, a feat that the spider was unable to reproduce. Abandonment of the web was always associated with the disappearance of the spider. Thus for *Araneus diadematus* in my study area it can be stated that the old web is removed and a new web placed in essentially the same place each day. Observations on six webs for three nights showed that webs were removed and eaten within an hour before starting to build the new web.

DISCUSSION

The overall amino acid flow sheet for the web building spider is shown diagrammatically in figure 3. The various pathways will be considered in order of the numbering in the diagram. Digestion of food of the spider takes place, largely at least, out of the spider. Digestive en-

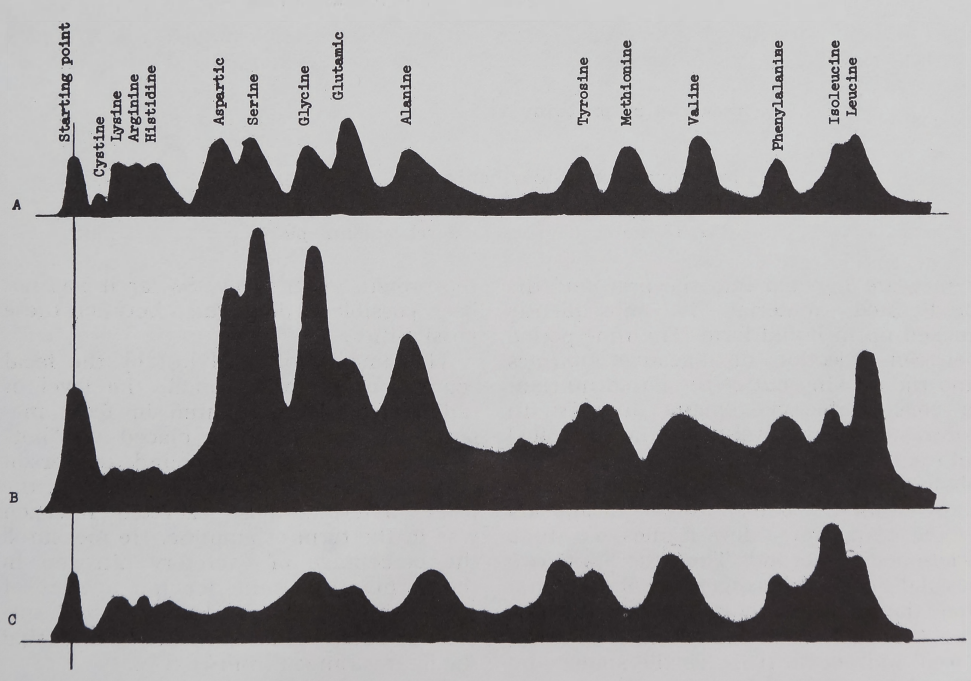

Fig. 2 Composition of free amino acid pool of (A) body fluids, (B) resting ampullate gland and (C) ampullate gland during active protein synthesis.

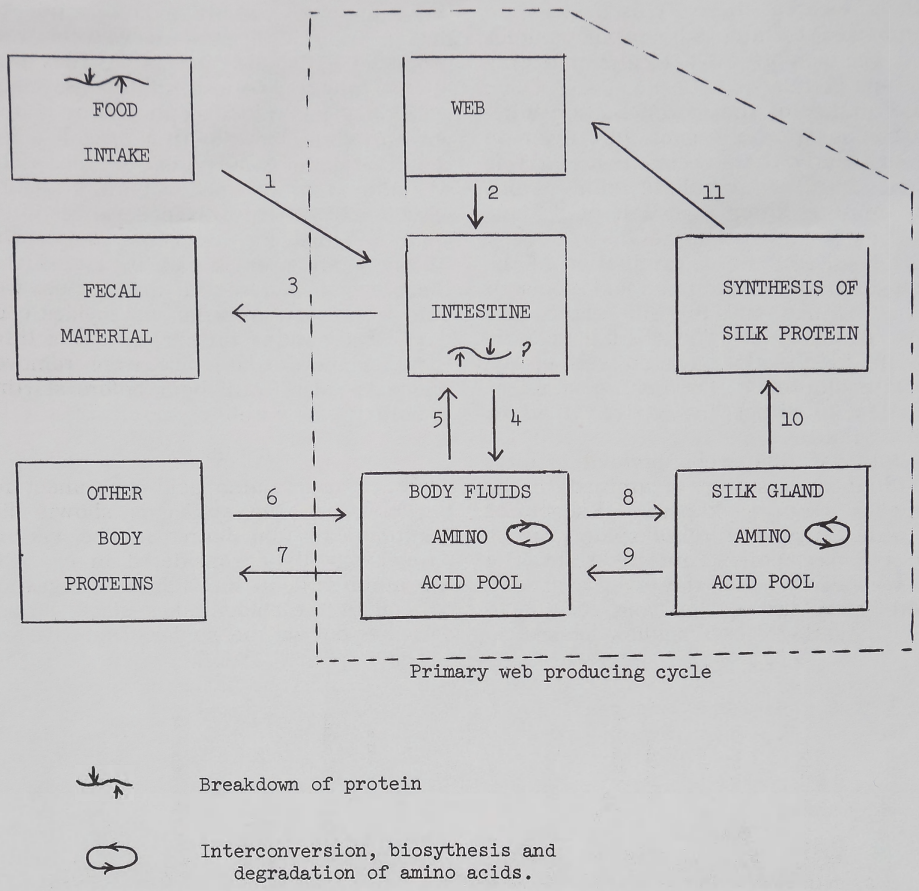

Fig. 3 Amino flow sheet for a web building spider.

zymes are injected into the prey and the predigested material is subsequently sucked up in liquid form. The time period between injection of digestive enzymes and the sucking out of the liquid nutrient is considerable, i.e. many minutes. In contrast to this, web material is rolled into a ball and rapidly swallowed. Movie photographs (Witt, unpublished) have shown that the ball of thread disappears in the course of a few frames, i.e. in a fraction of a second. Thus there are two possibilities for the digestion of silk, first that the digestive enzymes are injected into the ball of thread before it is swallowed and act on it inside the spider and second that the thread is broken down by a different set of peptidases than is the protein of the prey. So far it has not been possible to distinguish between these possibilities.

The amount of label lost by the fecal route appears to be small; the level of radioactive material found in fecal material of "cold" spiders placed on "hot" webs is near to background. Anderson ('66) did not detect amino acids in the fecal material; most excreted nitrogen was in the form of guanine. He measured the percentage of excretory nitrogen in the form of guanine for ten species of spider, including the closely related species, *Araneus solitarius*, and found that the figures ranged from 76-87%.

There is little information on transport across the intestinal mucosa and subse-

quent uptake into the silk gland. Frozen section autoradiographs, taken at short time intervals after ingestion of labeled web proteins, it can be shown that a period of 30 minutes is adequate for the label to reach the silk gland. Witt ('63) has carried out a number of experiments on the effect of food deprivation on web building and weight of spiders. In one experiment spiders had their webs eliminated daily for six days while fed only water. No significant change in the web was noted but the amount of silk that could be pulled decreased from 0.49 mg to 0.39 mg and the weight of the spiders decreased by 12%. In the experiments described in this paper only 10% of the web was removed daily, the weight loss was 8%. In another experiment Witt deprived spiders of food for 20 days and noted significantly smaller and wider meshed webs by the end of the period. But web building continued despite the loss of 49% of body weight; the amount of thread that could be reeled was down to 47% of the original figure. Thus, it is clear that a great deal of body substance is used up before web building is abandoned. This is not unexpected since without web building it is impossible for the spider to obtain any food. However, the high degree of conservation of web protein demonstrated in the experiments described in this paper show that normally there is little need for exchange between body proteins and the web proteins.

The free amino acid pool composition of the silk gland is quite different from that of the body fluids (fig. 2). Also, the composition of the gland pool varies considerably with the stage of the protein synthesis cycle. The silk gland has been shown to have a high capacity for producing short chain amino acids, i.e. rapid pyruvate to alanine conversion. There is no information on transport of amino acids out of the silk gland into the body pool. In view of the high rate of protein synthesis and the storage of short chain amino acids during the time of low rate of synthesis of silk it is likely that this back transport does not occur.

The process of silk synthesis within the silk gland and its extrusion into the lumen of the gland have been extensively studied previously (Witt et al., '68; Bell and Peakall, '69; Peakall, '69). The conversion of a soluble protein in the lumen of the gland to an insoluble protein of considerable physical strength involves a whole set of problems. Increase of molecular weight indicates polymerization of the silk at some stage. If this change is under enzymatic control, then the most likely site of secretion would be junction of the sac and the duct. Electron microscopy shows that some secretion does occur at this point (Bell and Peakall, '69) and that beyond this point the duct is acellular. In any event these changes, although vital to the production of a strong web, do not materially effect the amino acid balance of the spider.

The re-cycling experiments described in this paper lead to the conclusion that the web producing cycle, involving arrows 2, 4, 8, 10, 11 of diagram 2, is normally largely independent of the other body constituents. The existence of this closed circuit within the spider raises several problems. Two main possibilities exist, (1) that the "old" silk protein is incompletely broken down by the digestive system before absorption into the silk gland and thus can be "recognized" or (2) that the amino acids are so rapidly absorbed by the silk glands that little is diverted into other proteins.

Theory (1) would require either further breakdown of the polypeptides within the cell or a novel method of protein synthesis. The relationship of time of web digestion to silk synthesis time may be critical to theory (2). Detailed studies on the time of eating the web have not been made. Witt (per. comm.) states that in the laboratory "that this happens directly before the building of the new web. It could be half an hour or an hour before, but certainly never occurs in the evening nor throughout the day." Limited observations in the wild, described in this paper, support the above observation. The timing of web-eating and web-building may well be critical to the re-cycling process. The maximum demand for amino acids for making web proteins is very shortly after web building. Previous work (Peakall, '69) has shown that protein production comes into high gear soon after the silk is removed from the lumen.

Thus, if the silk is reduced to its individual amino acids, thus destroying any identity to the original protein, the only explanation for the fast absorption is that these amino acids are available at a time when demand from the silk gland is high and at a time when few other amino acids are available.

Under natural conditions the web recycling process occurs daily irrespective of the degree of damage to the web. These findings are in disagreement with McCook ('89). However, McCook does not give the species involved, merely discusses the "orb-web weavers" in general. There is no evidence that the above findings apply to other species. In fact laboratory studies show that the closely related spider, *Araneus sericatus*, builds less frequently than *A. diadematus*. In view of the high efficiency of re-cycling web proteins a case can be made that it is better to rebuild daily than to decide" if re-building is necessary.

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