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ORB WEB RECYCLING IN *ARANEUS CAVATICUS* (ARANEAE, ARANEIDAE) WITH AN EMPHASIS ON THE ADHESIVE SPIRAL COMPONENT, GABAMIDE

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ABSTRACT

The feeding of radiolabeled conspecific orb webs to *Araneus cavaticus* Keyserling clearly demonstrated the ability of this species to solubilize nearly all of the orb web, although in no instance was complete solubilization achieved. A principal component of the nonsolubilized portion is probably minor ampullate silk, as spiders fed pulled minor ampullate silk were unable to solubilize the majority of the samples. In contrast, spiders were able to completely solubilize pulled major ampullate silk. Despite the overall high percentage of web solubilization, the recycling efficiencies obtained, while variable, were never in excess of 32% (as determined using webs built by spiders fed ^{14}C-glucose).

A complete assessment of web recycling will have to consider the fate of ingested low molecular weight adhesive spiral components as well as web proteins. Of those components which have been identified, only GABamide was followed in the present study due to the labeled compound fed. On average ingested GABamide appears to be more quickly reincorporated into new web than ingested protein residues and this reutilization is, for the most part at least, in the form of GABamide. From spiders which did not build webs until several days after being fed orb webs, the indication is that GABamide can be stored for future web construction for such a length of time. Whether any storage is by physical separation from agents with metabolic activity against GABamide or by a degree of metabolic inertness is unknown.

INTRODUCTION

In previous studies the digestive fluid of *Argiope aurantia* Lucas was found to be capable of solubilizing all orb web adhesive spiral, radial, and junctional components except minor ampullate fibers (Tillinghast and Kavanagh 1977; Kavanagh and Tillinghast 1979), which commonly accompany major ampullate fibers in radii (Kavanagh and Tillinghast 1979; Work 1981). However, the method employed to make this determination, that of applying filter discs wetted with digestive fluid to plated webs, produced results which, upon reflection, could have been open to misinterpretation. The act of removing the filter disc after the incubation period could have resulted in the simultaneous removal of underlying web components which may have been only partially solubilized or otherwise weakened by the digestive fluid, rather than completely solubilized. Additionally, in no instance was web completely solubilized when immersed in a buffered solution containing digestive fluid (Tillinghast and Kavanagh 1977), and it seemed unlikely that minor ampullate fibers alone could account for all of the nonsolubilized portion. This incomplete solubilization also made the extremely

high recycling efficiency reported by Peakall (1971) seem unlikely. In an effort to resolve these inconsistencies we have examined orb web digestion and recycling *in vivo*. The ability to procure individually samples of major ampullate and minor ampullate silk also allowed us to examine more specifically the digestion and, for the former silk type, recycling of these orb web components. It should be noted that while a fraction of the ingested web components was undoubtedly used for purposes not directly related to web construction, but was nevertheless recycled in a denotative sense, in this paper the term "recycle" is restricted to the utilization of those components in subsequent webs or silks.

MATERIALS AND METHODS

Adult and penultimate female and male *Araneus cavaticus* Keyserling were collected in southern New Hampshire and Maine and kept either in cages (Tillinghast and Kavanagh 1977) or small vials, depending on whether web construction was desired or not.

To obtain radioactive major ampullate silk, spiders were fed from 4 to 20 μCi D-[^{14}C(U)] glucose (sp. act. 4.28 mCi/mmol or 329 mCi/mmol, New England Nuclear®; or 263 mCi/mmol, ICN® Radiochemicals). The silk was mechanically drawn one and/or two days after feeding as described previously (Tillinghast et al. 1984), except that a pulling rate of 1.0 cm/s was used. Again, the silking operation was monitored frequently with the aid of an Olympus®, Model X-Tr, stereo dissecting microscope, to insure as much as possible a collection of major ampullate silk free from pyriform, aciniform, and minor ampullate fibers. Since the aggregate and flagelliform glands of males degenerate shortly after adulthood is reached (Sekiguchi 1955a,b), making adhesive spiral and, thus, orb web construction impossible, adult males were only used for this purpose.

The silk pulled from each spider was cut into two portions, one roughly three times the size of the other. Each portion was desiccated over $CaSO_4$ and NaOH *in vacuo* and their dry weights were measured on a Perkin-Elmer® AD-2 autobalance. The smaller portions were hydrolyzed in 6N HCl at 110°C for 24 h, with the hydrolysates being used to determine specific activity. Radioactivity was measured in a Beckman® Beta-Mate II scintillation counter using Beckman® Ready-Solv EP as the scintillation fluid, and amino acids were quantitatively measured by the method of Moore (1968). The larger portions were assumed to have the same specific activities as their smaller counterparts.

Radioactive whole orb webs were obtained from spiders fed 10 μCi D-[^{14}C(U)] glucose (sp. act. 263 mCi/mmol, ICN®) each. After collecting the webs on 20 μL micropipettes, each was scraped off as a ring with a new razor blade and cut into one large and one small piece. These pieces were treated the same as the pulled major ampullate silk above.

Nonradioactive spiders were fed radioactive pulled silk or whole web in one of two ways. Either the spiders were offered the radioactive material while pinioned or it was placed in the spider's nonradioactive web. For the former method the spider was temporarily anesthetized with CO_2 and taped down, allowing close scrutiny of the external digestion process when the above dissecting microscope was used. For the latter method, all but two opposing frame lines were cut following placement of radiolabeled material in the unlabeled web. This was done

both to encourage web recycling and to insure that none of the radioactive material, particularly the pulled silk, would be lost during the spider's recycling of the web. The greater freedom of movement permitted by this method created a more normal situation. It was more difficult or impossible, however, to observe the movements of the spider's mouthparts. Often, two or more radioactive samples were fed to a single spider if an individual sample contained a comparatively low total amount of isotope. Spiders were not fed after ingestion of radioisotope but were given water daily. All subsequent webs built during the remainder of the experimental spiders' lives were collected for analysis. Note that spiders fed labeled whole orb web or major ampullate silk are referred to as web-fed and silk-fed spiders, respectively.

In addition to major ampullate silk, the ability of *A. cavaticus* to digest pulled minor ampullate silk was also examined, though to a much lesser extent since, in our experience, minor ampullate fibers cannot be pulled for long periods of time, as major ampullate fibers can. Also, the small amounts of minor ampullate silk obtainable made radiolabeling and partitioning of the silk impractical. Instead, digestion was only evaluated by observation of pinioned spiders under the dissecting microscope and by comparison of dry silk weights before and after feeding.

Some of the webs constructed by spiders fed radioactive material were collected intact on 20.3 cm x 25.4 cm glass plates and placed with Kodak™ SB-5 X-ray film as described previously (Kavanagh and Tillinghast 1979). The remainder of the webs were collected on micropipettes, hydrolyzed, and specific activities determined as described above. In addition, two dimensional thin layer chromatography (2D-TLC) was performed on some of the hydrolysates. Typically, 125 μg leucine equivalent amounts were chromatographed, but occasionally the amount of hydrolysate remaining after specific activity determination necessitated the use of a lesser quantity. For 2D-TLC, 20 x 20 cm Merck® precoated cellulose plates, 0.1 mm thickness, were developed using the solvent systems of Schmidt (1974); pyridine:acetone:ammonium hydroxide:water (45:30:5:20, v/v) for the first dimension and 2-propanol:formic acid (88%):water (75:12.5:12.5, v/v) for the second dimension. Development from the sample origin was 16 cm in both dimensions. Autoradiograms were prepared from the TLC plates as for plated webs, following which amines were visualized using a 11mM ninhydrin in acetone solution.

In August and September the building of orb webs, particularly by gravid female *A. cavaticus*, becomes less reliable, at least in the laboratory, than earlier in the season. Typically, these spiders instead lay down a plentiful amount of "random" fibers throughout the cage, which are presumably of major ampullate gland and, secondarily, minor ampullate gland origin, predominantly. Certainly, it seems very unlikely that any aggregate gland material is used in these constructions. Accumulations of "random" fibers produced over one or more days, as well as any orbs built by such silk-fed and web-fed spiders, were collected on micropipettes and treated the same as described above for orb webs.

RESULTS

Following the digestion of web or silk by pinioned spiders, the remnant present between the endites, if any, was removed and used to estimate the percentage of

Table 1.—Solubilization of orb webs, major ampullate silk, and minor ampullate silk by pinioned *A. cavaticus*. The data from spiders fed whole orb webs have been separated into two groups to demonstrate the disparity between them (particularly with respect to the percentages determined by measuring radioactivity). Group 1 spiders were fed web on or between June 23 and July 11 and between 2130 and 0645 hours. Group 2 spiders were fed web on or between July 18 and August 23 and between 1100 and 1630 hours. Ninhydrin positive compounds were assayed using leucine as the standard.

	Percentage of Web or Silk Remaining After Feeding (Mean ±SE; Median)			
Material Fed to Spiders	Gravimetrically Determined	As Determined by Measuring Ninhydrin Positive Compounds in Hydrolysates	As Determined by Measuring Radioactivity in Hydrolysates	*n*
Orb Web Group 1	1.6 ± 0.5; 1.6	2.9 ± 1.0; 2.1	0.18 ± 0.08; 0.089	5
Orb Web Group 2	3.8 ± 0.6; 3.9	2.9 ± 0.7; 2.6	2.9 ± 0.7; 2.9	4
Maj Amp Silk Only	1.0 ± 0.4; 0.39	0.61 ± 0.22; 0.35	0.12 ± 0.10; 0.00	13
Min Amp Silk Only	97 ± 13; 97	—	—	2

material which was not solubilized (Table 1). Of the three types of measurement used to make this estimate, that of measuring residual radioactivity was probably the most accurate as it would not have been influenced by non-sample materials incorporated into the remnants; principally hairs loosened from the endites' scopulae. The higher percentages most often obtained with the other two methods support this belief. Consistent with earlier *in vitro* studies (Kavanagh and Tillinghast 1979) minor ampullate silk was found to be relatively resistant compared to major ampullate silk. Spiders fed minor ampullate silk were unable to solubilize a large majority of the samples despite digestion attempts which were typical of spiders fed major ampullate silk or whole web, both in terms of method used and time involved. The contribution of non-sample inclusions to the remnants was made apparent in one of the two minor ampullate silk feedings by the weight of the remnant exceeding that of the original sample. In the second feeding 16% of the sample, by weight, was solubilized. Again, this figure may be somewhat low due to non-sample contaminants. Additionally, the former silk sample was subsequently fed to six successive spiders, wrapped each time with a new orb web to further encourage digestion. The remnant left by the sixth spider had a weight approximately three times that of the original sample, with contributions from the orb webs no doubt accounting for much of the increase in weight. Nevertheless, the original sample, which was whiter than the rest of the remnant, could still be distinguished and was not noticeably diminished by the six spiders.

By contrast, in eight out of thirteen major ampullate silk feedings either no remnant was left at all or no significant radioactivity (< 3 SD from the mean background count) was detectable in the remnant. In all cases a very large fraction of the sample was solubilized.

Unexpectedly, radioactivity measurements made on remnants from orb web feedings indicated two significantly different groups (approximate *t*-test, Sokal and Rohlf 1981; $P < 0.05$; Table 1). An examination of the feeding conditions for each of the spiders in these two groups revealed two consistent differences; the time of day and year during which feeding took place. Group 1 spiders were fed web on or between June 23 and July 11 and between 2130 and 0645 hours.

Table 2.—Efficiency in recycling ingested ^{14}C-labeled web and major ampullate silk components. Range and mean values reflect the total isotope present in all webs and/or "random" fibers collected from a given spider subsequent to feeding, expressed as a percentage of the total amount of isotope present in the material fed to the spider.

Radioactive Material Fed to Spiders	Material Collected and Analyzed	^{14}C-Radioactivity Recycled			
		Range (%)	Mean (%)	SE (%)	*n*
Orb Web	Orb Webs	4.00-32.0	16.3	2.93	12
Orb Web	Random Fibers, Orb Webs	4.20-6.09	5.1	0.95	2
Major Ampullate Silk	Orb Webs	0.430-23.2	10.8	4.86	4
Major Ampullate Silk	Random Fibers, Orb Webs	2.91-23.7	13.2	1.85	12
Total		0.430-32.0	13.6	1.57	30

Group 2 spiders were fed web on or between July 18 and August 23 and between 1100 and 1630 hours. Whether either or both of these differences were involved in producing the different solubilization percentages is unknown. Features common to both groups include a high percentage of solubilization, although, on average, not as high as for major ampullate silk, and incomplete solubilization in all cases (radioactivity in remnants $>$ 5 SD from the mean background count).

The efficiency with which *A. cavaticus* was found to recycle ingested whole web or major ampullate silk is presented in Table 2. Total isotope present in all webs and/or "random" fibers produced by each spider after being fed radioactive material are expressed as a percentage of the total isotope present in the radioactive material fed. Considerable variability in this percentage was apparent, irrespective of the method used to feed the spiders. In no instance, however, did our recycling percentages even approach those determined by Peakall (1971) for *Araneus diadematus* Cl. Whereas we obtained a maximum recycling of 32%, which takes into account the total amount of isotope present in all webs constructed by the spider, Peakall (1971) typically found the percentage of recycled material in the first web constructed to be in excess of 90%. Earlier estimates of recycling for *A. diadematus* made by Breed et al. (1964) were more in keeping with our results, ranging from 21 to 50%. Their estimates were made from the total radioactivity present in the first two webs constructed by each spider.

The normalized specific activities of webs built by twelve spiders fed radioactive whole web and by four spiders fed radioactive major ampullate silk are presented in Figs. 1 and 2, respectively. Actual peak specific activities ranged from 2.0 to 47 CPM/μg leucine equivalents in Fig. 1 and from 1.2 to 79 CPM/μg leucine equivalents in Fig. 2. Taking into consideration the amount of isotope contained in the material fed, peak specific activities ranged from 9.8×10^{-6} to 3.3×10^{-4} CPM/(CPM in web fed $\times$ μg Leu equiv.) in Fig. 1 and from 1.9×10^{-5} to 2.7×10^{-4} CPM/(CPM in silk fed $\times$ μg Leu equiv.) in Fig. 2. For eleven of the twelve web-fed spiders, peak specific activity was present in the first web constructed, while all four silk-fed spiders attained peak specific activity in the second web built. 2D-TLC and subsequent autoradiography of hydrolysates prepared from these webs would indicate that GABamide (4-aminobutyramide) played a major role in producing this difference. GABamide is present in the adhesive spirals of those Araneidae examined thus far (Fischer and Brander 1960; Anderson and Tillinghast 1980; Tillinghast and Christenson 1984). Particularly strong support

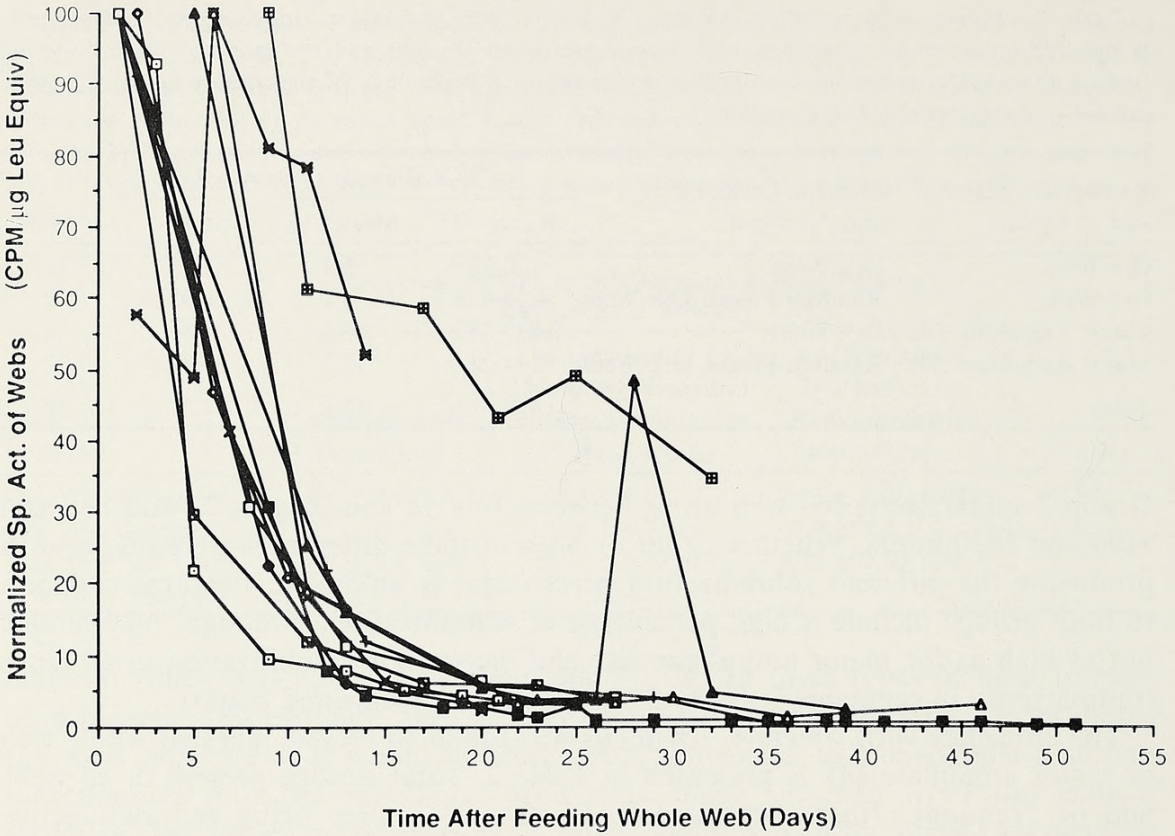

Fig. 1.—Incorporation of isotope into webs built by twelve spiders fed ^{14}C-labeled whole orb webs. The surge in specific activity at web 7 (day 28) from the spider represented by solid triangles was due entirely to isotope incorporation into UC8, as revealed by autoradiography of the 2D-TLC plate prepared from this web's hydrolysate.

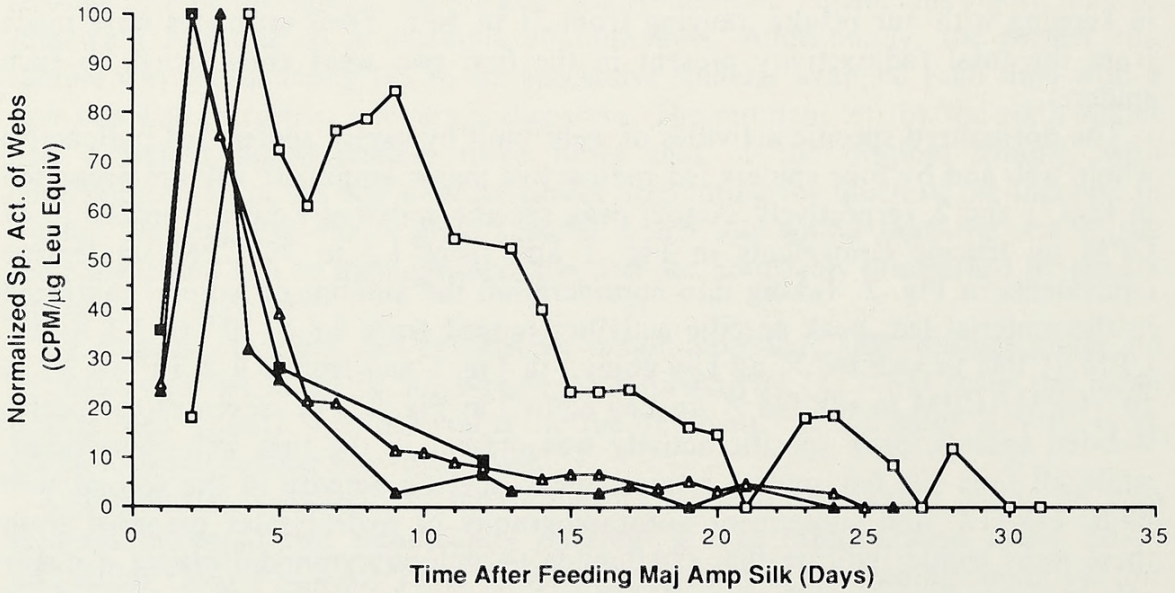

Fig. 2.—Incorporation of isotope into webs built by four spiders fed ^{14}C-labeled major ampullate silk.

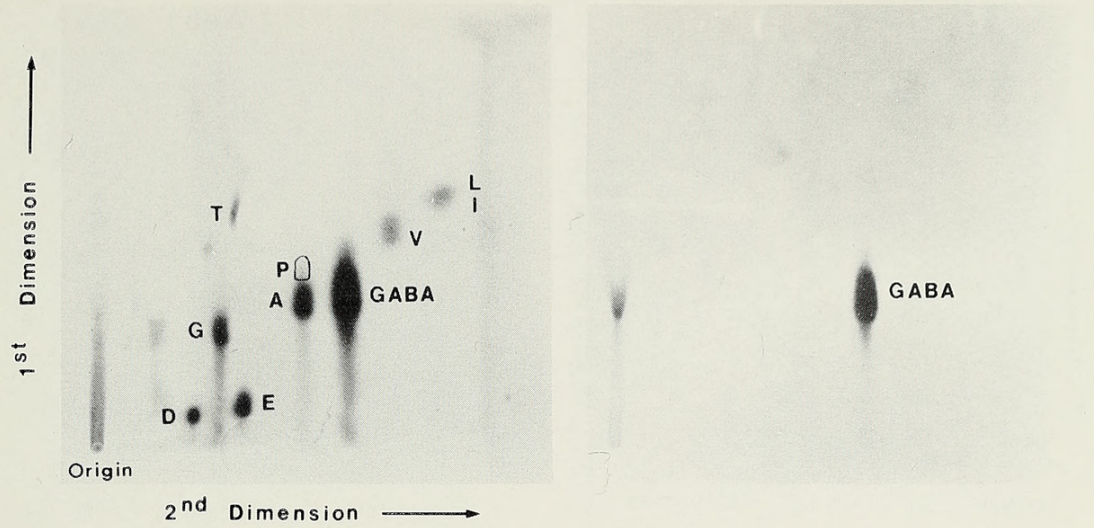

Fig. 3.—2D-TLC of three pooled web 1 hydrolysates from web-fed spiders. Of the 125 μg leucine equivalents chromatographed, 34 μg were from a web with a sp. act. of 47 CPM/μg Leu equiv. (built 1 day after feeding, represented in Fig. 1 by a solid diamond), 77 μg were from a web with a sp. act. of 30 CPM/μg Leu equiv. (built 1 day after feeding, represented in Fig. 1 by an open square), and the remaining 14 μg were from a web with a sp. act. of 43 CPM/μg Leu equiv. (built 2 days after feeding, represented in Fig. 1 by an open diamond). An exposure of 110 days was used to produce the autoradiogram (right). A = alanine; D = aspartic acid; E = glutamic acid; G = glycine; GABA = 4-aminobutyric acid; I = isoleucine; L = leucine; P = proline; T = threonine; V = valine. Proline has been circled in the chromatogram since a yellow product, difficult to see in black and white photographs, is formed when proline is reacted with ninhydrin.

for this proposal came from the first webs built by three of the web-fed spiders, two of which were built 1 day after feeding and one which was built 2 days after feeding. In these webs' hydrolysates virtually all of the isotope was restricted to GABA (4-aminobutyric acid; Fig. 3), the hydrolytic product of GABamide. Less extreme results were obtained from the other hydrolysates chromatographed (Figs. 4, 5, 6). For the spider whose webs are presented in Fig. 4, GABamide was still the major radioactive compound present in the first web built after feeding, but some amino acids and as yet unidentified compounds were also carrying label. Note that web 1 was built 5 days after feeding. In Fig. 5, GABA and an unidentified compound (UC1) can be seen to possess comparable amounts of isotope in web 1, with other unidentified compounds containing considerably lesser amounts. Web 1 of Fig. 6, built 6 days after feeding, again shows GABA dominating the autoradiogram. Thus, despite the concurrent high specific activity of UC1 in one instance, it was GABamide which appeared to be responsible for the maximum specific activities most often occurring at web 1 in web-fed spiders. The single exceptional web-fed spider produced atypical webs having few or no adhesive spiral loops. In the chromatogram prepared from this spider's first web GABA was only barely discernible and in chromatograms prepared from subsequent webs GABA was not visible at all.

In contrast, while GABA could clearly be seen in 2D-TLC autoradiograms prepared from the second webs built by silk-fed spiders, it certainly did not carry the majority of the label (Figs. 7, 8). Rather, several of the amino acids prevalent in web proteins were evidently responsible for the peak specific activities in the second webs of these spiders.

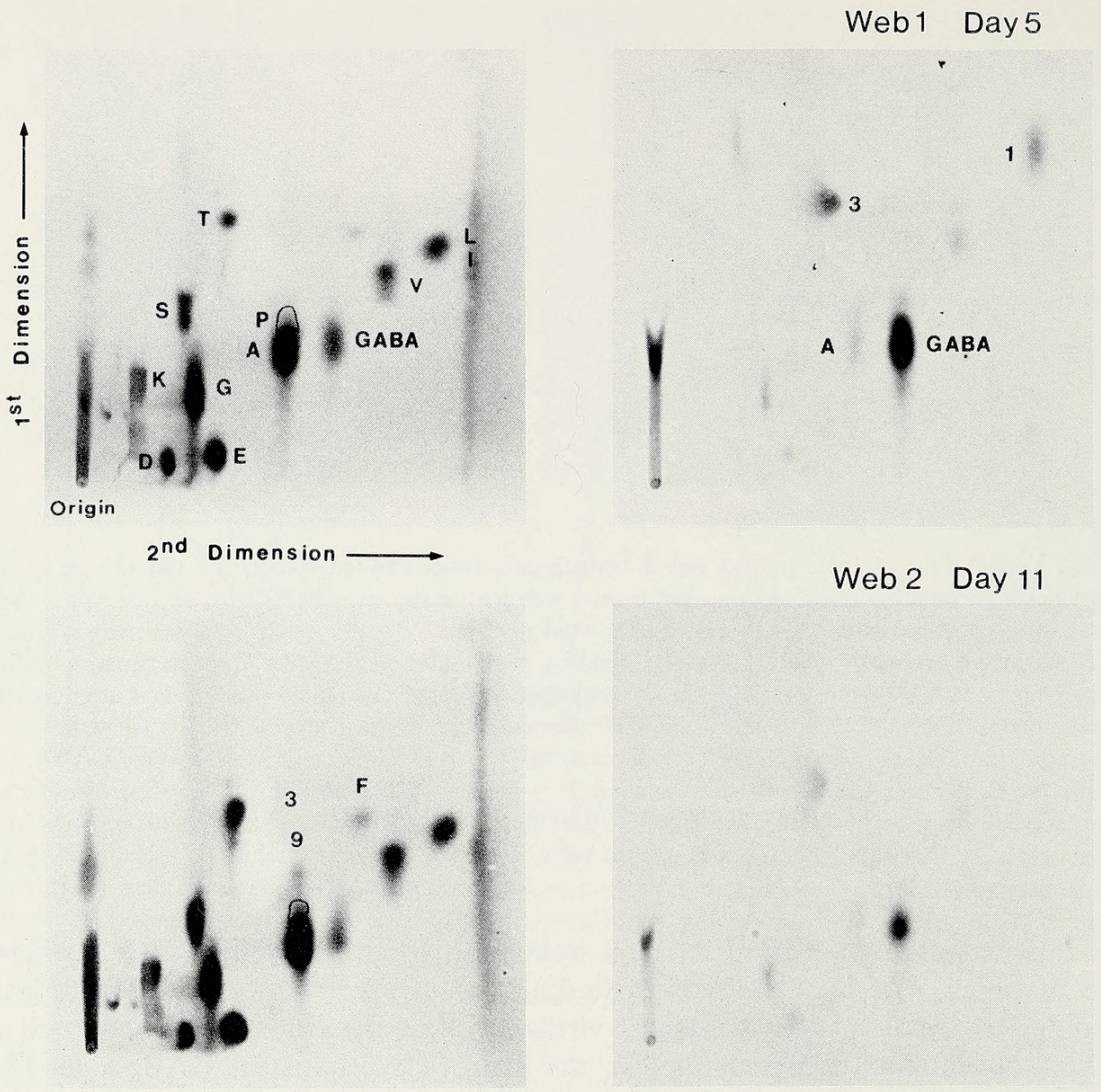

Fig. 4.—2D-TLC of hydrolysates (125 μg Leu equiv.) made from the first two webs built by a web-fed spider. These webs are represented in Fig. 1 by solid triangles. Autoradiograms form the right side of each pair. Sp. act. (CPM/μg Leu equiv.): web 1, 14; web 2, 3.6. X-ray film exposures (days): web 1, 119; web 2, 122. A = alanine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; GABA = 4-aminobutyric acid; I = isoleucine; K = lysine; L = leucine; P = proline; S = serine; T = threonine; V = valine. Numbers designate unidentified compounds (UC).

Autoradiograms prepared from plated webs were consistent with the TLC results. Thus, the first webs built by three web-fed spiders had adhesive spirals which were much more intensely labeled than radii or hub spirals. The adhesive coverings were particularly dark, especially considering the extent to which they were smeared during preparation for autoradiography. Subsequent webs had less intense adhesive coverings and were apparently less labeled overall. In the second webs built by two silk-fed spiders, the radii and adhesive spirals were of roughly equal intensity and the adhesive spiral core fibers appeared to contain the majority of the adhesive spirals' isotope. Peak activity was apparently possessed by these second webs.

Also instructive were the results obtained when collections of "random" fibers, in addition to any webs constructed, were analyzed. The normalized specific activities of such collections and webs, produced by two web-fed spiders, are presented in Fig. 9. Likewise, those produced by ten silk-fed spiders are shown in

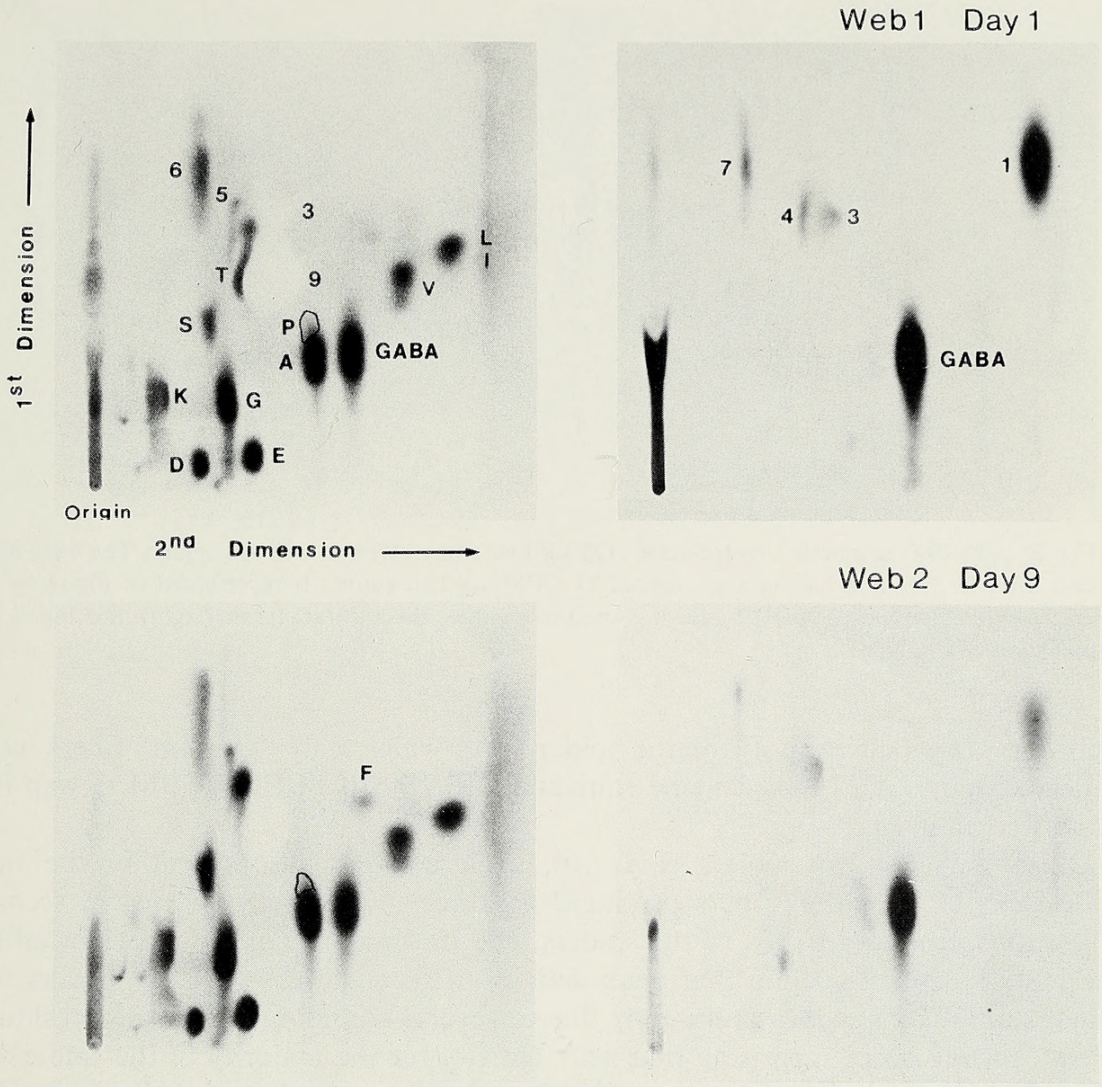

Fig. 5.—2D-TLC of hydrolysates (125 μg Leu equiv.) made from the first two webs built by a web-fed spider. These webs are represented in Fig. 1 by solid squares. An exposure of 111 days was used to produce the autoradiograms (right side of each pair). Sp. act. (CPM/μg Leu equiv.): web 1, 38; web 2, 12. See Fig. 4 for explanation of symbols.

Fig. 10. Unlike the trend observed in Fig. 1, the maximum specific activity was not present in the first collection of "random" fibers from either of the web-fed spiders (Fig. 9). Assuming the construction of "random" fibers does not involve aggregate gland secretions, it would seem that this difference was due to the lack of an outlet for ingested radioactive GABamide as GABamide. Note that one of the web-fed spiders built nine webs following the collection of the first "random" fibers and that the first web built possessed the highest specific activity. 2D-TLC and autoradiography of this first web revealed that while radioisotope was clearly present in glycine, alanine, glutamic acid, aspartic acid, and serine, GABA and two unidentified compounds (UC2, UC3) contained the majority of the isotope (Fig. 11). GABA's intensity on the autoradiogram was particularly striking considering the relatively low amount of GABA demonstrated by the chromatogram. Apparently, with the building of the first web, an outlet for GABamide was provided, resulting in peak activity. Actual peak specific activities in Figure 9 were 0.28 CPM/μg leucine equivalents [3.8×10^{-6} CPM/(CPM in

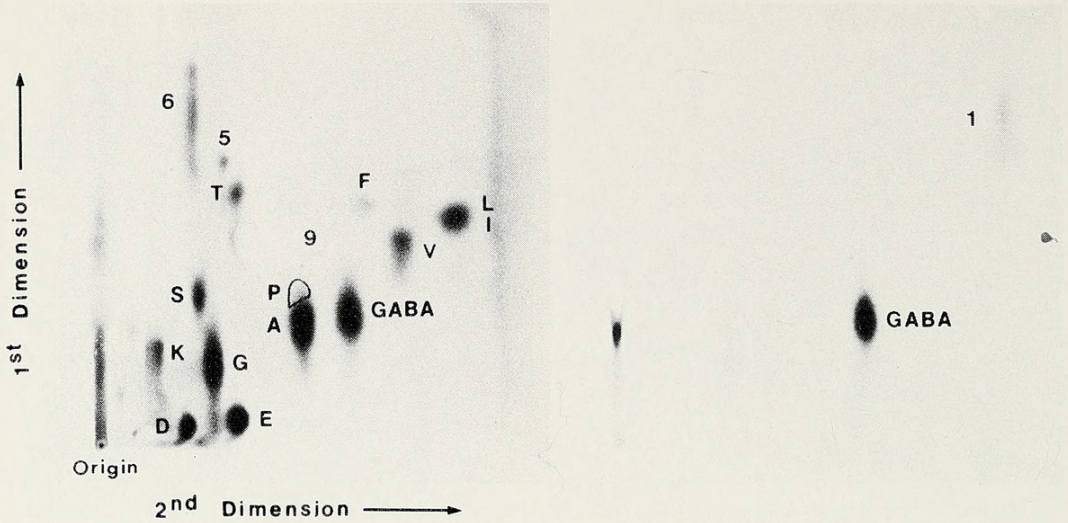

Fig. 6.—2D-TLC of a web 1 hydrolysate (125 μg Leu equiv.) from a web-fed spider. The web, built 6 days after feeding and having a sp. act. of 11 CPM/μg Leu equiv., is represented in Fig. 1 by an open triangle. An exposure of 119 days was used to produce the autoradiogram (right). See Fig. 4 for explanation of symbols.

web fed × μg Leu equiv.)] for the spider from which only "random" fibers were collected and 12 CPM/μg leucine equivalents [5.2×10^{-5} CPM/(CPM in web fed × μg Leu equiv.)].

Of the ten silk-fed spiders (Fig. 10), peak activity was present in the first collections of "random" fibers produced by three of these spiders, in the second collections produced by six of the spiders, and in the third collection produced by the tenth spider. Recalling that peak activity in second webs built by spiders fed major ampullate silk was apparently due to several common web protein residues (Figs. 7, 8), it is not surprising that peak activity for the majority of the spiders in Fig. 10 occurred in the second or third "random" fiber collections. Such protein residues are obviously utilized in "random" fibers as well as in webs. The occurrence of peak activity in first and third collections may simply reflect the variability inherent in the time between the synthesis of progenitive silk components and their inclusion in drawn silk fibers. Alternatively, while it was our intent to obtain comparable amounts of "random" fibers during each collection, we found that similar appearing fiber accumulations in the cages sometimes possessed deceptively and considerably different weights. Most probably, both of these sources of variability contributed to the observed results. In contrast to the single case in Fig. 9, no silk-fed spider produced a web with a specific activity significantly higher than the "random" fibers produced just prior to it, indicating that ingested radioactive GABamide was the source of most of the radioactive GABamide seen in the first web of Fig. 9. Actual peak activities in Fig. 10 ranged from 0.077 to 15 CPM/μg leucine equivalents [3.2×10^{-6} to 3.4×10^{-5} CPM/(CPM in silk fed × μg Leu equiv.)]

DISCUSSION

It is clear from the web feeding trials that *A. cavaticus* is able to solubilize the vast majority of the orb web, a fact which was not revealed by the *in vitro* studies

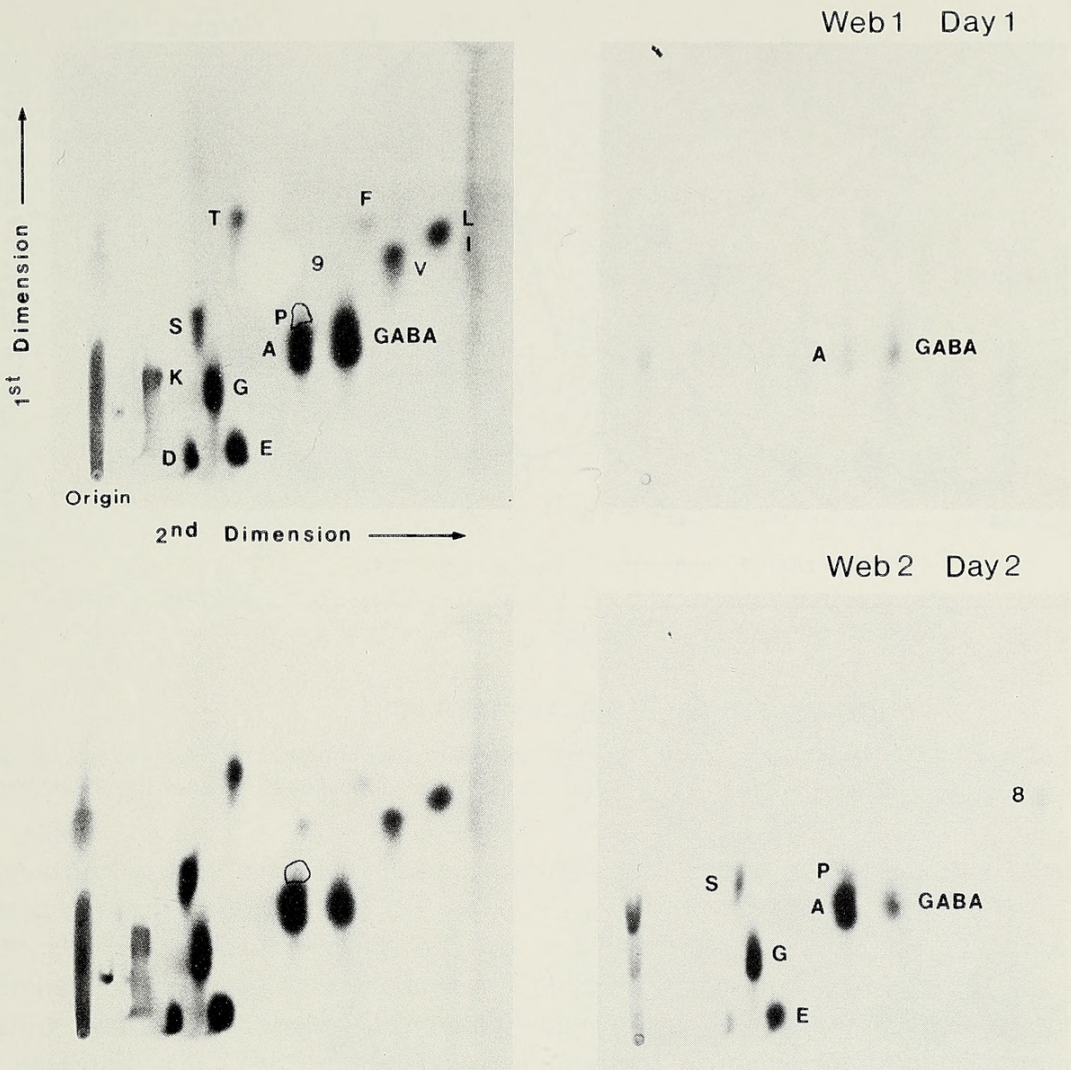

Fig. 7.—2D-TLC of hydrolysates made from the first two webs built by a silk-fed spider. The amounts chromatographed were 125 μg Leu equiv. from web 1 and 140 μg Leu equiv. from web 2. These webs are represented in Fig. 2 by open triangles. An exposure of 111 days was used to produce the autoradiograms (right side of each pair). Sp. act. (CPM/μg Leu equiv.): web 1, 3.1; web 2, 12. See Fig. 4 for explanation of symbols.

on rod-wound web (Tillinghast and Kavanagh 1977). Not only was a greater percentage of the web solubilized *in vivo*, but at a clearly greater rate, such that more digestion occurred *in vivo* within 20 min than occurred *in vitro* within 24 h. Certainly these differences must have been in part a result of the digestive fluid dilution made during the *in vitro* studies; a factor which may have been important not just because of the lowered protease concentration. As proposed earlier (Kavanagh and Tillinghast 1983), digestion may also require or be facilitated by non-enzymatic components in the digestive fluid, such as surfactants, which would also have been diluted. In addition, observations on pinioned spiders indicate that the contribution of mastication and digestive fluid replenishing to orb web digestion is considerable. Spiders frequently rotated, pierced, and compressed web or silk samples using their fangs and endites, and, often at very short intervals, ingested the digestive fluid already surrounding a sample, only to regurgitate more digestive fluid immediately thereafter. These

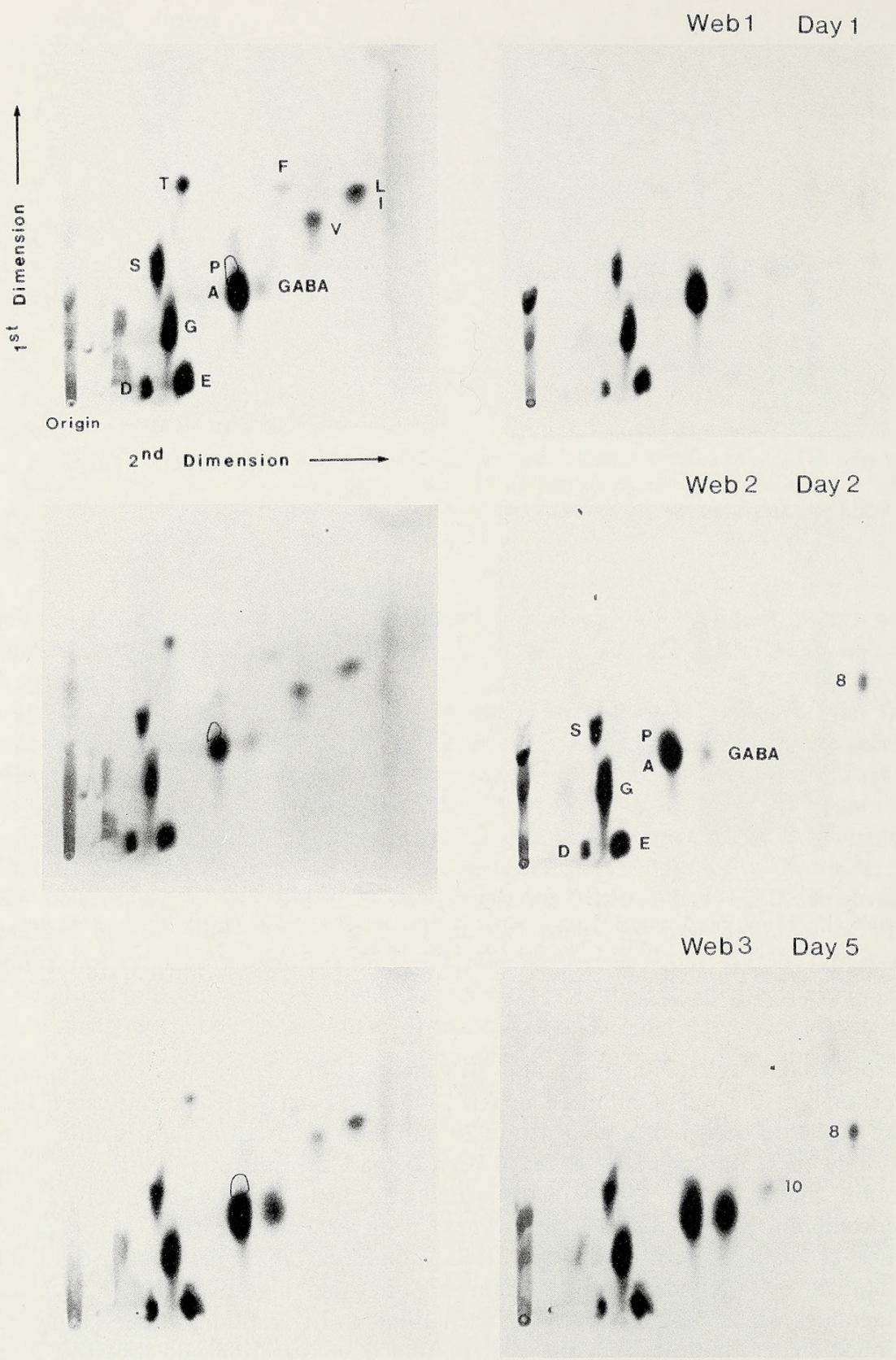

Fig. 8.—2D-TLC of hydrolysates made from the first three webs built by a silk-fed spider. The amounts chromatographed were 125 μg Leu equiv. from webs 1 and 3, and 60 μg Leu equiv. from web 2. These webs are represented in Fig. 2 by solid squares. An exposure of 122 days was used to produce the autoradiograms (right side of each pair). Sp. act. (CPM/μg Leu equiv.): web 1, 28; web 2, 79; web 3, 22. See Fig. 4 for explanation of symbols.

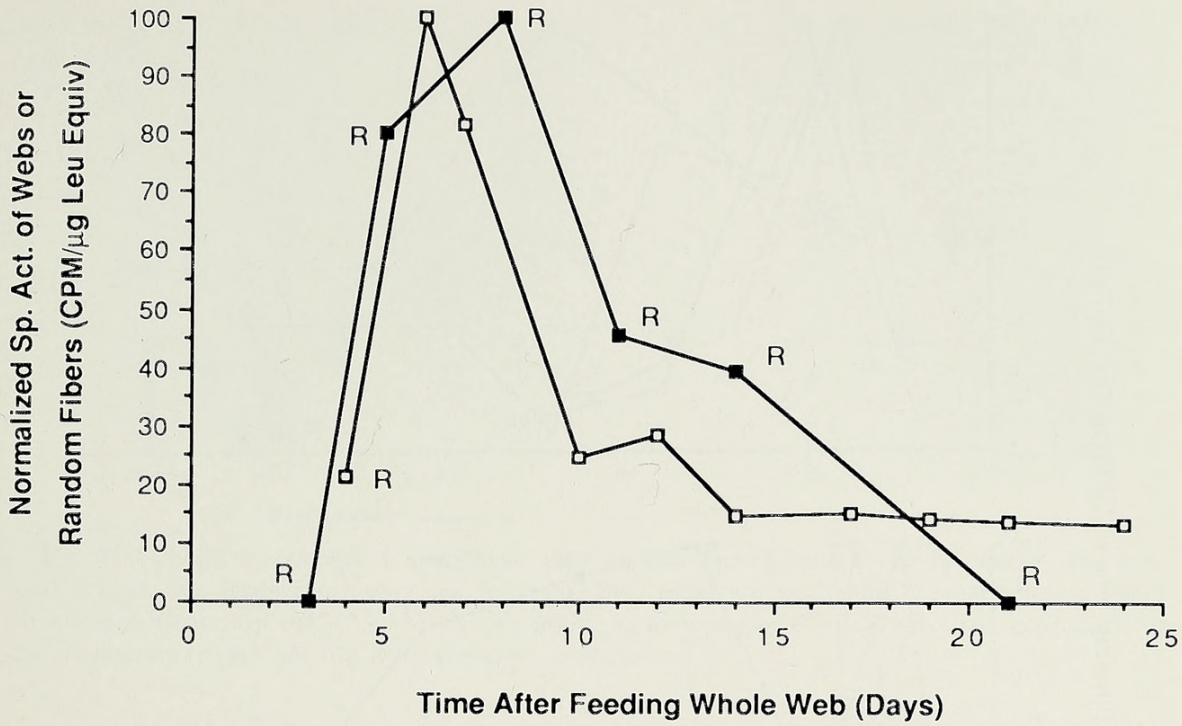

Fig. 9.—Incorporation of isotope into webs and "random" fibers produced by two spiders fed ^{14}C-labeled whole orb webs. R = collections of "random" fibers.

actions presumably helped to hasten and maintain exposure of the entire sample to active enzyme.

Despite the greater extent of orb web solubilization *in vivo*, complete solubilization was still never achieved. Unlike the situation *in vitro*, however, the small percentage of nonsolubilized web which remained *in vivo* does not preclude the possibility that the remnants were composed primarily of minor ampullate silk. In fact, the percentage of web remaining after some feedings was so small as to indicate that minor ampullate silk must be at least partially digestible. The possibility that these webs may simply have contained very few or no minor ampullate fibers cannot be excluded, but seems unlikely based on observations of minor ampullate fiber occurrence in orb webs (Kavanagh and Tillinghast 1979; Work 1981). Moreover, evidence for partial digestion was also obtained in one of the two minor ampullate silk feedings. Kovoor (1972) has demonstrated the composite nature of minor ampullate fibers from *A. diadematus* through a comparison of the distal and proximal minor ampullate cell types. The granules secreted into the lumen by these two cell types were found to be histochemically distinct. This raises the possibility that partial digestion could result from the selective digestion of one or more of the component species of minor ampullate fibers.

At present we cannot explain the large discrepancy between the recycling efficiencies we obtained and those of Peakall (1971). A number of differences in the materials and methods used could have contributed to this discrepancy. These differences included the species of *Araneus* used, the radiolabeled compound fed, and the method used to estimate the total amount of isotope in the ingested web. Also, Peakall considered the time between web recycling and new web construction to be critical to efficient recycling; a time which in *A. diadematus* was reportedly not more than one hour. As a consequence of the methods we

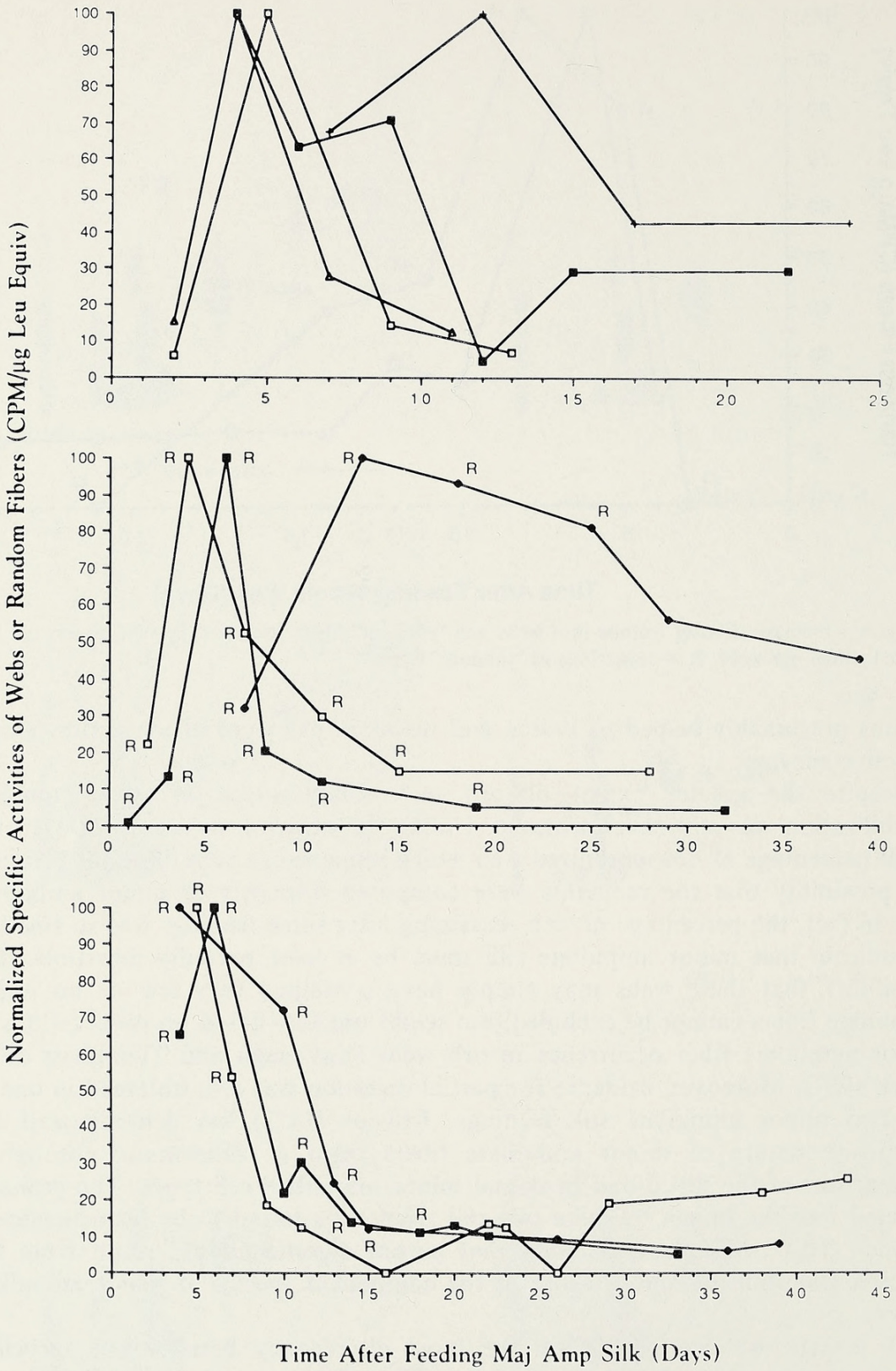

Fig. 10.—Incorporation of isotope into webs and "random" fibers produced by ten spiders fed ^{14}C-labeled major ampullate silk. All data points in the top graph were obtained from "random" fiber collections. In the center and bottom graphs, an R designates "random" fiber collections. The ten spiders were separated into three graphs merely for the sake of clarity. Note the different ranges of the abscissas.

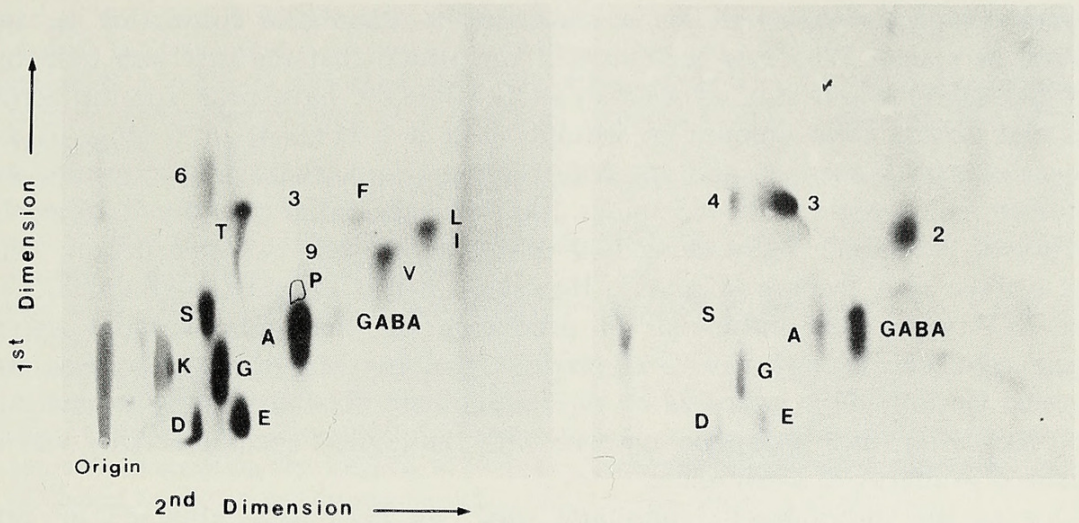

Fig. 11.—2D-TLC of a web 1 hydrolysate (125 μg Leu equiv.) from a web-fed spider. The web, built 6 days after feeding and after one "random" fiber collection was made, is represented in Fig. 9 by an open square. Sp. act. 12 CPM/μg Leu equiv. An exposure of 117 days was used to produce the autoradiogram (right). See Fig. 4 for explanation of symbols.

used to feed web, this interval was either over ten hours or under ten hours but unknown in our experiments on recycling efficiency. Thus, additional experiments in which this interval is shortened will be required to better evaluate Peakall's claim. Solely on the basis of the digestibility of the orb web as determined *in vivo*, and contrary to the previous *in vitro* findings, the high recycling efficiency reported by Peakall is at least plausible.

From the specific activities of the successive webs built by spiders fed radioactive web or silk, along with the chromatograms and autoradiograms prepared from those webs' hydrolysates, it would appear that on average ingested GABamide is reutilized in new web more quickly than ingested protein residues. Thus, the first web constructed by a spider fed whole web usually had a higher specific activity and more total isotope than webs produced subsequently, and the relatively high specific activity of GABamide in the first web was apparently responsible for this trend. In contrast, for spiders fed major ampullate silk, peak activity and the largest total amount of isotope were present in the second webs constructed, and protein residues, particularly alanine, glycine, glutamic acid, and serine, possessed a large majority of the isotope in these webs. The results from web-fed spiders which produced constructions lacking GABamide (i.e., "random" fibers; Fig. 9) were more similar to those from silk-fed spiders and lend further support for GABamide's more rapid reutilization. As radiolabeled GABamide was present in the webs of spiders fed major ampullate silk (Figs. 7, 8), it is reasonable to assume that non-GABamide web components were also used to synthesize some of the labeled GABamide present in webs built by web-fed spiders. However, since GABamide was responsible for peak specific activity only in webs built by web-fed spiders, it is also reasonable to assume that most of the radioactive GABamide in these webs must have come from radioactive GABamide in the ingested web.

The results also indicate that a sizable fraction of the ingested GABamide may remain available for incorporation into new web for at least several days, should web construction be forgone for such a period. Whether this is due to an actual

sequestration of GABamide or a resistance to metabolic conversion or both cannot be stated. Whatever the cause, it was found that the first web built by a web-fed spider could still, as a result of GABamide, have peak specific activity and the largest total amount of isotope even if 5 (Fig. 4) or 6 (Fig. 6) days elapsed between feeding and its construction. Somewhat similar results were obtained from another web-fed spider despite a substantial quantity (1.16 mg Leu equiv.) of "random" fibers being laid down before web 1's construction; which was 6 days after feeding (Fig. 11). However, in this instance GABamide, UC2, and UC3 were each influential in producing the maximum specific activity. Again, that the majority of GABamide's label in these three webs was from ingested GABamide is indicated by the results from silk-fed spiders; in particular, the observation that isotope in the web with the highest specific activity was not localized primarily in GABamide.

Due to the radiolabeled compound used, no data were obtained on other known components of the adhesive spiral's covering, such as the inorganics, KH_2PO_4 and KNO_3 (Schildknecht et al. 1972). This was also true for taurine, obtained by acid hydrolysis from the adhesive spiral's taurine derivative(s) (Fischer and Brander 1960; Anderson and Tillinghast 1980), since ^{14}C-labeling of this compound was meager at best in spiders fed radiolabeled web or silk. Thus, it is not known if GABamide's behavior is shared by other low molecular weight adhesive spiral components.

During the course of the 2D-TLC, several unidentified compounds have repeatedly been encountered and designated UC1-UC10 (Figs. 3-8, 11). UC1-UC4, UC7, UC8, and UC10 are ninhydrin negative but can incorporate isotope when spiders are fed ^{14}C-glucose. UC5 and UC6 are ninhydrin positive but have not been found to incorporate isotope. UC9 is neither ninhydrin positive nor does it become radioisotopically labeled by ^{14}C-glucose. However, the cellulose support of the TLC plates fortuitously takes on a light purple background hue with ninhydrin visualization, which UC9 inhibits. Thus, within about 1 day after application of the ninhydrin spray, UC9 makes its presence known by the white spot it leaves on the chromatogram. UC3 behaves similarly to UC9 in this respect.

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