Plant Physiol. (1968) 43, 589–596

Transitory Aspects of a Single Protein in Tissues of Solanum tuberosum and Its Coincidence with the Establishment of New Growth[1]

C. A. Ryan[2], O. C. Huisman[3], and R. W. Van Denburgh
Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99163

Received November 6, 1967.

Abstract. **Qualitative and quantitative estimation of a proteinaceous chymotrypsin inhibitor in tissues of potato plants has revealed a transitory existence in all tissues except xylem, flowers, and seeds, where the inhibitor was not found. The distribution pattern in both aerial tissues and in tubers during development and senescence suggests that the concentrations of this protein in all tissues are influenced by meristematic regions of the plants. The transitory existence coinciding with breaking of apical dominance suggests that in the potato plant the protein may have some role in the process of establishing and maintaining meristematic tissue. A search for the protein in other Solanaceae species and in plants from non-related species has proven unsuccessful.**

Studies were recently initiated to acquire information concerning the origin, fate, and function in potato plant tissues of a protein that is a powerful inactivator of proteolytic enzymes (9). This protein was previously isolated and crystallized from potato juice (2, 8) and has been the subject of several reports concerning its chemical properties and inhibitory capacities (2, 3, 6, 7, 8). Recent experiments have revealed that the protein was present not only in potato tubers, where it was originally observed, but could be detected in leaflets of the potato plant (9). The protein could be demonstrated only in carefully chosen leaflets and its existence was found to be transitory (9).

The proteinase inhibitor under study is one of several now known to occur in potatoes and other plant storage organs (4). This inhibitor is specific for chymotrypsin and chymotrypsin-like enzymes, whereas almost all other known proteinase inhibitors from plants are specific for trypsin-like enzymes (4). Data concerning the *in vivo* roles of proteinase inhibitors in plants has been meager. They have been considered as control substances with a capacity to react with endogenous proteolytic enzymes or as protective agents for neutralizing proteolytic enzymes from invading insects and microorganisms. Previous to this report, no attempts have been made to study these inhibitors in tissues other than in the storage organs from which they were isolated.

The present report provides a description of the occurrence and distribution of the specific proteinase inactivator in tissues of entire plants of *Solanum tuberosum* grown in growth chambers, greenhouses, and in the field. The results indicate that the protein is present, but transitory, in all tissues of potato plants except flowers, seeds, and xylem. Its presence and accumulation in tissues consistently coincides with the establishment, or maintainance of, meristematic regions in the plants.

Materials and Methods

Chymotrypsin inhibitor from potatoes [inhibitor I (6)] was 5x crystallized according to the method of Balls and Ryan (2). Rabbit anti-inhibitor I serum was prepared by injecting subcutaneously into rabbits, biweekly, 1 mg 5x crystallized inhibitor I emulsified in complete Freund's adjuvant. Qualitative estimation of the inhibitor protein in standards or in plant extracts was determined by the method of Ouchterloney (5). This method in our hands could detect as little as 8 μg of inhibitor I per ml. Tissues of plants were ground with a mortar and pestle and the whole homogenates, or the juice therefrom, was tested for the presence or absence of inhibitor I. Although no attempt was made to quantitate exactly the precipitin line, its density was estimated in each test by comparison to known standards.

Juice was obtained by homogenizing tissues with a mortar and pestle and by subsequently squeezing the homogenate using a hand garlic press. Juice that was further clarified by centrifugation will be specified as such in the text.

Quantitative determination of inhibitor I was made using the rapid, sensitive radial diffusion

[1] This work was supported in part by United States Public Health Service Grants 1-K3-GM 17059 and GM 12505. College of Agriculture Scientific Paper #3056, Project 4791.

[2] Research Career Development Awardee of the United States Public Health Service.

[3] Present address: Department of Biochemistry, University of California, Davis, California.

method described by Ryan (7). The method will quantitate 10 μg/ml or more inhibitor I in crude potato extracts.

Inhibitor activities are reported as mg alpha chymotrypsin (3x crystallized from Worthington Biochemical Company) inhibited per mg nitrogen (Kjeldahl). The amount of juice, centrifuged at 2000 × *g* for 10 minutes, required to completely inhibit 1 mg chymotrypsin (calculated from the value at 50% inhibition) was determined by the method of Aldrich and Balls (1). Tyrosine ethyl ester was the substrate at pH 6.3 and 25°.

Russet Burbank potatoes were used for the study except where noted. Potatoes were grown in growth chambers under 1500 ft-c of light in a 14-hour day. The temperature range was 18 to 24°. Plants grown in a greenhouse were grown under natural light during the spring, summer, and fall of 1966. Field potatoes were grown in local soil fertilized with 200 lbs nitrogen per acre.

Results

Anti-inhibitor I serum reacted with purified inhibitor I in the Ouchterloney double diffusion assay to give a single precipitin line (9). When this serum was challenged in this assay system with homogenates or juice from potato plant tissues, a single precipitin line was also found (9). Control serum taken from the rabbit before injections of inhibitor I showed no precipitin line. The high degree of specificity of the serum in detecting the presence of inhibitor I among hundreds of proteins present was the basis for its use in the present study.

Reactivity of Anti-Inhibitor I Serum with Potato Tuber Juice. Nineteen varieties of potatoes were initially tested with the antiserum prepared against inhibitor I purified from Russet Burbank potatoes. Juice from all 19 varieties gave a single precipitin line that was, visually, fully crossreacting with inhibitor I from Russet Burbank tubers. Total inhibitory activity toward alpha chymotrypsin was also tested in the juice from all 19 varieties. The values for all varieties ranged from a high of 1.15 μg chymotrypsin inhibited per mg juice N to a low 0.23 μg/mg N. The mean was 0.51 μg chymotrypsin inhibited per mg juice N. Russet Burbank variety, used for preparation of pure inhibitor, inhibited 0.45 μg chymotrypsin per mg juice N. However, as will be discussed later, the pattern of concentration of inhibitor I within individual potato tubers depended upon the age and condition of the tubers. Thus the total inhibitory activities cannot be used with any certainty as a genetic trait of any given variety. The complete immunological crossreactivity of the proteins however did indicate that the protein among the different varieties is genetically similar.

Determination of Total Chymotrypsin Inhibitory Capacity in Tubers and Roots of the Solanaceae *Family and in Tubers, Roots, and Bulbs of Other Families.* Previous studies have shown that at least 2 proteinaceous inhibitors exist in potato tubers (6). It was considered possible that these inhibitors might be ubiquitous to underground tissues of the Solanaceae family. Juice from nightshade, eggplant, tomato, and pepper roots or stems were tested for inhibitory activity. The presence of chymotrypsin inhibitors was also tested in members of several other plant families that have fleshy underground tissues. Using inhibitor I antiserum, the presence of inhibitor I was determined in centrifuged juice from these tissues by the Ouchterloney (5) method.

Table I gives the plants of the various families and the particular tissue tested. The results in table I show that inhibitor I was found only in the potato although several of the plants contained

Table I. *Inhibition of Chymotrypsin by Juice from Various Tubers, Roots, and Bulbs*

Family	Species	Common name	Tissue	Chymotrypsin inhibited by juice (mg/mg N)	Cross reactivity with inhibitor I antiserum
Solanaceae	*S. tuberosum*	Potato	Tuber	0.45	+
	S. nigrum	Nightshade	Stem	. . .	—
	S. melongena	Eggplant	Stem	..	—
	L. esculentum	Tomato	Root	0.11	—
	C. annum	Pepper	Root	0.07	—
Convolvulaceae	*I. batatus*	Sweet Potato	Tuber	0.30	—
Musacea	*M. sapientum*	Banana	Tuber	3.85	—
Arum	*C. antiquorum*	Taro	Tuber	1.85	—
	C. esculenta	Dasheen	Tuber	0	—
Cruciferae	*B. napobrassica*	Rutabaga	Tuber	0.02	—
	B. rapa	Turnip	Tuber	0	—
Ranunculacae	*P. officinalis*	Peony	Tuber	0.02	—
Lilaceae	*A. officinalis*	Asparagus	Tuber	0	—
Iridaceae	*I. florentina*	Iris	Bulb	0	—
Amaryllidaceae	*A. cepa*	Onion	Bulb	0	—

considerable inhibitory activity toward chymotrypsin. Banana tuber juice contained almost 10 times more inhibitory activity than either sweet potatoes or white potatoes. The taro contained a high inhibitory capacity, but its close relative, the dasheen, contained none.

Distribution of Inhibitor I in Potato Tubers. Potato tubers (Russet Burbank) of varying age and development were dissected into small individual sections of tissue and the juice or mascerates of individual samples were tested immunologically for the presence or absence of inhibitor I. In very young attached tubers, 1 to 3 cm in diameter, the inhibitor protein was found evenly distributed throughout all of the tissues. In older attached tubers and in freshly harvested mature tubers, the inhibitor was found to be uniformly located in the external phloem and cortex, *i.e.* the area outside the vascular ring. The concentration decreased significantly just inside of the vascular ring but increased again slightly in the center pith. In a tuber removed from cold storage after 1 year, a somewhat similar pattern was seen, but there was now in the outer phloem and cortex a decreasing gradient from the apical end toward the stem end. The inhibitor in the stem end cortex and center pith had nearly disappeared. In sprouted tubers, the overall concentration of inhibitor I had decreased even more, particularly near the sprouted eye. The sprouts themselves, however, exhibited the presence of inhibitor and the concentration was particularly high at each axil and at the apex of the new sprout. As will be described in a later section, seed pieces planted in the field lost inhibitor I protein completely as sprouts elongated and emerged from the soil. It was concluded that the inhibitor concentration in tubers was definitely changing with storage and its disappearance from the tuber closely coincided with the establishment of the growth of new sprouts.

Using the radial diffusion method, a quantitative estimate of inhibitor I in potato tubers was made. A newly harvested mature potato was selected and a longitudinal slice was removed that was about 1 cm thick and included the area of the stem attachment and the apex. A similar slice was taken from a potato stored 1 year at 4°. Each slice was dissected into the visibly defined areas comprising the tissues outside the vascular ring (cortex and outer phloem), the dense tissue just inside the vascular ring, and the less dense center pith. These tissues were then subdivided into about 30 equivalent sections. The tissue sections were mashed to a pulp with a mortar and pestle and the juice was collected and centrifuged at 2000 × *g* for 10 minutes. The juice was analyzed for total protein and total inhibitor I. The results are shown in table II. Whereas the total biuret protein remained relatively constant among all of the tissue sections, the differences in percent inhibitor I between the 2 potatoes were striking, especially at the stem end and center pith.

In order to find if the results from the slices tested typically represented new and stored potatoes, 4 typical tubers were selected from each age group and sections of the stem and apical cortex and of the center pith were removed and the juice recovered and tested as described above. In addition, 4 very young tubers 2 to 3 cm in diameter were detached from parent plants and similarly tested. The cumulative results are shown in table III. Again, with age, the striking change in concentration at the stem end and center pith were seen. The decreasing ratio of percent inhibitor I in the stem versus apical cortical regions is shown in the right-hand column of table III. Coinciding with this ratio was the concentration of inhibitor I in the center pith, which decreased markedly with the age of the tuber. It was also noted that in the very young tuber, the cortical areas exhibited a uniform but lesser concentration than in the new mature tuber. Although inhibitor I in individual tubers can vary considerably, the cumulative results are consistent with the earlier observations that the levels of the inhibitor are undergoing constant change during aging, decreasing near the stem and center pith but remaining in the highest concentra-

Table II. *Summary of the Distribution of Inhibitor I in Tissues of New and Aged Potato Tubers*

Tissue tested	Area tested	New potato		Year old potato, 4°	
		mg Protein per ml juice	Inhibitor I % of juice protein	mg Protein per ml juice	Inhibitor I % of juice protein
Cortex	Stem end	12.19	5.17	10.90	0.71
	Medial area	10.77	2.89	10.65	0.62
	Apical end	9.31	1.56	9.31	3.00
Inner phloem area	Stem end	8.71	0.45	12.46	0
	Medial area	7.52	0.60	11.85	0
	Apical end	8.66	0.51	10.56	0.14
Pith	Stem end	7.22	0.61	10.47	0
	Medial area	7.13	0.52	11.16	0.03
	Apical end	8.21	0.68	8.44	0.07

Table III. *Distribution of Inhibitor I in Cortex and Pith of Potato Tubers in Various Developmental States*

Tubers	Inhibitor I % of juice protein	Ratio % $\frac{\text{stem}}{\text{apical}}$
Very young potato tubers		
2–3 cm diameter		
Stem cortex	0.74	
Center pith	0.86	1.00
Apical cortex	0.74	
New mature potato tubers		
Stem cortex	1.37	
Center pith	0.75	0.90
Apical cortex	1.53	
Year old mature potato tubers		
Stem cortex	0.42	
Center pith	0.27	0.40
Apical cortex	1.05	

tions near the region of potentially new growth, *i.e.* the apex.

Distribution of Inhibitor I in the Vegetative Potato Plant. The distribution of inhibitor I in vegetative tissue varied depending upon the physiological state of the potato plants. At particular times during development of plants, the protein could be demonstrated qualitatively by the Ouchterloney method in every tissue or organ except in the xylem of the main stem, in flowers and in seeds. The concentration in leaflets, petioles, stem, and roots was generally highest just preceding or during the establishment of new growth, receding or disappearing thereafter.

Inhibitor I was observed quantitatively in leaflets from a young potato plant grown in the growth chamber from true seed and from a young plant also grown in the growth chamber, but from a sprouted potato tuber. When tested, both plants were still developing new rhizomes. Tubers had not yet begun to form. The seedling plant had not visibly begun to break apical dominance whereas the plant from the seed piece was breaking apical dominance at the lower 4 or 5 axils. The small leaflets from the 3 petioles near the apex of both plants were not assayed. These leaflets were small and in all plants tested consistently exhibited the presence of inhibitor I. From the fourth petiole down from the apex to the base of the plant selected, leaflets were assayed for inhibitor I concentration by the radial diffusion method. The crude juice from the terminal and from the leaflet twin nearest the stem on each petiole was tested for inhibitor I. The concentration of inhibitor I in these leaflets at each node are shown in figure 1. A comparison of leaflet inhibitor I between plants shows that the plant grown from the seed piece, just breaking apical dominance at nodes 13 through 16, has much higher levels in most leaflets than the plant grown from true seed. The distribution with respect to leaflet and petiole position is also different. In the seedling plant, not yet breaking apical dominance, inhibitor I is absent or nearly so in the lower leaflets in contrast to the high concentration in the larger plant near the middle lower leaflets. Although the 2 plants described are from different origins, *i.e.* 1 from true seed and 1 from a seed piece, it appears that the stage of development of

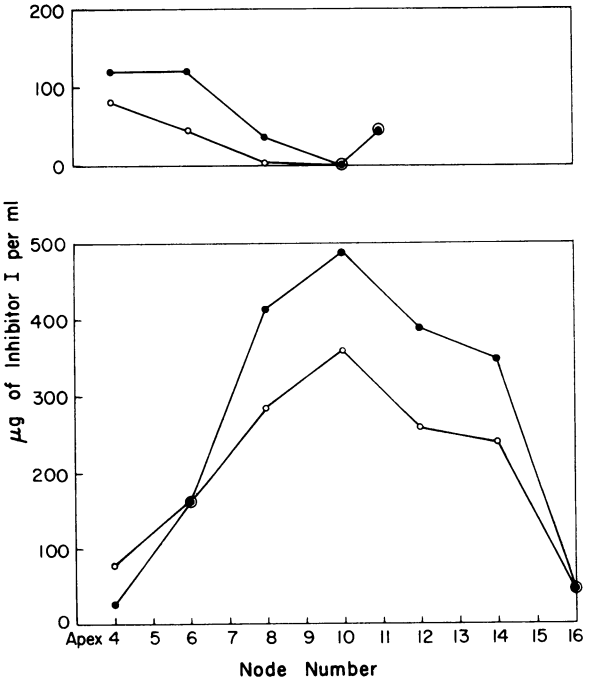

FIG. 1. Quantitative determination of inhibitor I in leaflets of potato plants grown from true seed (top) and from a sprouted tuber piece (bottom). The solid circles (●) represent the μg inhibitor I in juice from leaflets nearest the main stem on petioles of the indicated nodes. Open circles (○) represent μg inhibitor I in juice of the terminal leaflet from each petiole.

the plants, rather than origin, determines the levels of inhibitor I in the tissues.

The youngest leaflets on each petiole, nearest the stem, contain the highest levels of inhibitor as compared to terminal leaflets (fig 1). It has already been mentioned that the youngest leaflets near the apex usually contain inhibitor I. In addition, lateral buds consistently contained inhibitor I no matter what stage of growth or environmental condition the plant was in, except late senescence.

A pattern of transient existence of the inhibitor I in leaflets of very young seedling plants has previously been described (9). In those experiments, inhibitor I appeared in leaflets just preceding new rhizome growth and nearly disappeared after several rhizomes were established (about 50 days after planting). The interesting observation was made that decapitating very young plants before rhizome formation promoted the accumulation of inhibitor I protein in leaflets. In rapidly growing plants where the protein had appeared and subsequently receded, the removal of rhizomes and apex simultaneously caused a striking increase in the numbers of leaflets containing inhibitor I within 48 hours. These results were the first indications that new growth centers were exerting a control upon the accumulation of inhibitor I protein in leaflets. It is now known that seedling plants maintained in the growth chamber beyond about 60 days exhibit an increase in inhibitor I in most leaflets and the plants generally tend to break apical dominance. If the plants are transferred to a greenhouse under natural light in the short-day period of February to April within 60 days of planting they tend to grow with a single stem and apex. Under the short day conditions inhibitor I remains in low concentration throughout most of the tissues of the plants.

Quantitative observations of the accumulation of inhibitor I in seedling plants, induced by excising apices and rhizomes, are reported here using potato plants grown from seed in growth chambers. The plants possessed several actively growing rhizomes but had not yet begun to form tubers (beyond 50 days after planting). Table IV shows quantitatively a typical response of inhibitor I 48 and 96 hours after excision of both the apex and rhizomes. The areas of the plants tested were A) the apical area of the control plant, B) the leaflets from the 3 upper-most petioles nearest the apex, C) the terminal leaflets from the remaining 3 to 4 lower petioles, D) the upper stem corresponding to the upper leaflets, E) the lower stem, F) the roots, and G) the stolons of the control plant. The removal of the apex and stolens caused an increase in the concentration of inhibitor I in the upper leaflets with time and also induced the lower leaflets and lower stem to accumulate inhibitor I (table IV). The progression of the accumulation is apparently from the top of the plant down. The roots did not exhibit the presence of inhibitor in these experiments. The pattern of the accumulation of inhibitor I in these younger plants is somewhat different than the pattern found in older, more mature plants. In young plants, inhibitor I could be observed in stem tissue under the same conditions that it was found in leaflets. In older plants the removal of apices and rhizomes caused an increase in inhibitor I in stem tissue even before an increase could be observed in leaflets. In either case, the energy for the protein synthesis is probably derived from photosynthesis since removal of all leaflets caused an immediate disappearance of inhibitor I in the stems.

In plants just previous to, or during the breaking of apical dominance or establishment of new rhizomes, inhibitor I could sometimes be detected in juice from roots. Under other conditions, it was not possible to demonstrate its presence in root extracts.

Inhibitor I was determined in stem and root

Table IV. *Accumulation of Inhibitor I in* Solanum tuberosum *Seedlings Induced by Excising Apexes, Stolons, and Lateral Buds*

Tissues examined[2]	Inhibitor I, % of soluble protein following excision[1]		
	0 Time control	48 hrs after excision	96 hrs after excision
Apical leaflets	0.21	...	...
Upper leaflets	0.23	0.28	0.48
Lower leaflets	0	0.31	0.26
Upper stem	0.69[3]	0.40	0.31
Lower stem	0	0	0.23
Roots	0	0	0
Stolons	0	...	...

[1] 90,000 × *g* supernatant of tissue juice. Plants were 6 to 11 cm in height, having 9 or 10 petioles and 5 to 9 stolons.

[2] Apical leaflets were from 3 small leaflets surrounding the apex. Upper leaflets were the terminal leaflets of the next 3 petioles proceeding down from the apex. Lower leaflets were the terminal leaflets from the lowest 3 or 4 petioles of the plants. The entire stem was equally divided after removing all lateral growth.

[3] Included apical stem, initially high in inhibitor I.

Table V. *Accumulation of Inhibitor I in Stem of Tuberizing* Solanum tuberosum *Plants Induced by Removal of Stolons, Tubers, and Apexes*

Plants grown from cuttings had an average height of 46 cm.

Tissues examined	0 Time control	Inhibitor I, % of soluble protein 144 hrs after removal of stolons and apexes 1[1]	2[2]	3[3]
Petioles	0	0	0	0
Upper stem	0	0	0	0.3
Middle stem	0	0	0.8	0.5
Lower stem	0	0.9	1.1	0.7
Underground stem	0	1.8	0.8	0.7
Stolons	0.5	...	...	...
Roots	0.1	0.4	0	0
Tubers	1.4	...	...	...

[1] Plant had broken apical dominance, 6 new stolons.
[2] Plant had broken apical dominance, 3 tubers, no stolons.
[3] All new growth, *i.e.*, lateral buds and stolons removed as they appeared during experiment.

tissues of tuberizing plants grown from cuttings in the greenhouse. In these plants the stolons and attached tubers contained appreciable inhibitor I and it could be demonstrated in the roots (table V, control plant). Removal of the apex and stolons of 3 plants of equivalent age and size induced a considerable accumulation of inhibitor I in the stem after 144 hours. This was especially so near the base of the plants. From 1 of the plants (plant No. 3), the lateral buds and new stolens were continually removed as they grew or reformed. In this plant, the inhibitor could be detected higher up into the stem than in the 2 plants where new lateral growth, stolons or tubers had been allowed to grow. In none of the plants was it possible to detect inhibitor I in the petioles or in leaflets except in close proximity to the apex of the control plant.

Observations of Inhibitor I in Tissues of Field-Grown Potatoes. Because the distribution and concentration of inhibitor I was apparently dependent upon both the age of the plants and the environmental conditions of growth, it was of interest to study inhibitor I in plants in their natural environment under normal growing conditions. A field 50 feet square in the Washington State University Agronomy Farm, Pullman, Washington, was planted with several rows of sprouted Russet Burbank potato seed pieces spaced in the rows about 24 inches apart. The plants were regularly irrigated and were weeded, fertilized, and sprayed with an insecticide at approximately 2-week intervals. The plants grew at what was considered a normal, healthy rate. Seed pieces were planted May 12, and the first plants emerged June 6. Potato plants were analyzed qualitatively for the presence of inhibitor I in tissues every 7 to 10 days from June 8 through senescence. Entire young plants were dissected into individual leaflets, petioles, stem(s), rhizomes, roots, and seed pieces and tested separately. As the plants grew larger, it became im-

Table VI. *The Presence of Inhibitor I in Juice from Tissues of Field Grown Potatoes*

Values were assigned from the densities of precipitin lines formed by the juice with anti-inhibitor I serum in double diffusion assays in agar gels. The precipitin lines were compared to those formed by known inhibitor I standard solutions.

Tissue	Days after planting								
	0	*27*	*35*	*40*	*48*	*61*	*77*	*84*	*90*[2]
Upper leaflets	A[1]	+	+	+	±	±	±	±	—
Lower leaflets	A	A	+	+	—	—	—	—	—
Upper stem	A	A	+	+	—	—	—	—	—
Middle stem	A	A	+	+	±	—	—	±	—
Lower stem	A	A	+	+	±	—	±	—	—
Underground stem	A	+	+	+	+	+	+	+	—
Rhizomes	A	+	±	±	+	+	+	±	—
Tubers	A	A	A	A	+	+	+	+	+
Roots	A	+	±	±	±	±	±	—	—
Seed piece	+	+	±	±	—	—	A	A	A

[1] "A" indicates tissue absent; + represents approximately 50 μg or more inhibitor I per ml juice; ± between 8 and 50 μg per ml; and — indicates not detectable.
[2] Senescent plants.

possible to test the entire plants. Therefore, selected leaflets, stem areas, roots and stolens were tested as representative tissues. The juice from the selected tissues was tested in the Ouchterloney method. Upper leaflets were chosen from petioles near the apices of plants and they contained inhibitor I until late senescence (*i.e.* 90 days after planting). Lower leaflets were sampled randomly from the middle or lower portion of the potato plants. The upper stem was selected near the apex, and the lower stem near the soil level. Table VI shows representative cumulative results of the entire growth cycle of the field-grown potatoes.

The results show a definite pattern of the presence of inhibitor I in the potato plants. The seed piece when planted, and the new growth contained appreciable quantities of the inhibitor. As the plants developed rhizomes, inhibitor I in the seed piece diminished rapidly. The initially high concentration of inhibitor I in aerial tissues receded rapidly and nearly disappeared after 48 days, remaining mainly in the apex and lateral buds. The disappearance coincided with the establishment of new tubers. Underground stems and rhizomes exhibited inhibitor I until late senescence.

In a plant representative of about 70 days growth (when inhibitor I was present in appreciable amounts in underground stems) a transverse section of stem was dissected and the various differentiated tissues were tested for inhibitor I. An interesting pattern was observed that paralleled the distribution in tubers. The outer parenchymal tissue and outer phloem contained relatively high concentrations of inhibitor I. The inner phloem and pith contained a lesser amount. No inhibitor I was detected in the xylem. Its absence in the xylem was confirmed in a separate experiment in which xylem contents, from a stem segment that contained inhibitor I, were extruded through a cut end by exerting a positive hydrostatic pressure on the other end. No inhibitor I could be demonstrated in the extruded xylem juice whereas it could still be demonstrated in the parenchymal tissue of the stem segment.

During growth of the field-grown plants, inhibitor I accumulated in the newly formed tubers. After the tubers matured and the plant leaflets became yellow with senescence, inhibitor I disappeared entirely from the plant except in the mature tubers. Thus a cycle of occurrence of inhibitor I from the tubers to plants to new tubers had taken place.

Discussion

The data establish that inhibitor I is present in high concentrations in potato tubers and can be demonstrated in almost all aerial vegetative tissues. Of interest was the finding that its existence in the tissues was transitory. In the tubers themselves, a striking shift of inhibitor I concentration in individual tissues during development and maturation suggested that this protein was involved in a dynamic aspect of the protein metabolism of the potato plant. The similar but more profound changes in concentration of inhibitor I in aerial vegetative tissues also reinforced the opinion that the protein is somehow involved in the protein metabolism of new growth centers. In all tissues the concentration of inhibitor I was highest just preceding or coincidental with establishment of new growth, be it in the tubers or in vegetative tissues, or induced by removing growth centers. The subsequent recession of its concentration in aerial tissues as new growth centers became established suggests that the new growth centers can influence the levels of inhibitor I in these tissues.

Inhibitor I may be an important factor in the establishment of the new growth. Whether its properties are unique in this respect is not yet known. Other proteins not yet discovered may also exhibit the behavior observed here. The protein itself is apparently not a normal constituent of the other Solanaceae family species tested. However the potato plant shows a lack of inhibitor I during certain stages of development and therefore the other members of the Solanaceae family could possibly possess the gene for producing the protein but its expression may be under strict controls in the plants. Under different conditions of testing, inhibitor I may yet be found in other plant species.

In potato plants containing tubers, the storage organs as a whole always showed the highest levels of any tissues tested. As shown in table II, the stem end of the cortical region of 1 tuber contained over 5 % of the total protein as inhibitor I. Individual small localized areas have been assayed that exceed this figure. Yet, with time, the inhibitor disappears from these areas (table III). The continued change in concentration of inhibitor I in the tissues of potato tubers during maturation and storage suggests that the protein may be closely associated with the internal changes in the tuber components that are necessary for the initiation of new growth. These changes may also reflect the presence of substances that can control the expression of genes in cells of these tissues. It is significant that inhibitor I concentration consistently remains high in the apical portion of the tubers, in the area where new sprouts usually appear. In the field-grown potatoes (table VI) inhibitor I entirely disappeared from seed pieces within a few days after the new plants emerged. The seed pieces at this time were still visibly in good condition. The new stem and new aerial growth contained appreciable inhibitor I and continued to exhibit its presence until new stolons and tubers were established. The new tubers then accumulated inhibitor I as the concentration in aerial tissues diminished.

The cumulative data suggest that inhibitor I is present or absent in tissues depending upon the

physiological state of the plant. The levels of inhibitor I are influenced by both environment and age as well as the state of development. It is concluded that the biosynthesis and degradation of this protein are regulated within the plant and this regulation to a large extent, and in an unknown way, is influenced directly by the meristematic tissue of the plants. The fact that the protein is found associated with meristematic tissue and that its appearance in other tissues coincides with the breaking of apical dominance strongly suggests a role for the protein in the establishment or maintainance of meristematic tissue.

Experiments to further understand the physiological and biochemical role of this protein, and the nature of the substance or substances involved in the control of its transient existence, are currently in progress.

Acknowledgments

The authors gratefully thank Mr. Charles R. Oldenburg for technical assistance. We also thank Drs. R. Kunkel, W. Hoymen, G. Easton, and S. Locke for samples of potatoes, potato seeds, and potato plants. Our appreciation is extended to Dr. Robert Nilan for the generous use of greenhouse and field space.

Literature Cited

1. ALDRICH, R. L., JR. AND A. K. BALLS. 1958. The reversibility of inactivation of chymotrypsin. J. Biol. Chem. 233: 1355–58.
2. BALLS, A. K. AND C. A. RYAN. 1963. Concerning a chymotryptic inhibitor from potatoes and its binding capacity for the enzyme. J. Biol. Chem. 238: 2976–82.
3. DEEDS, F., C. A. RYAN, AND A. K. BALLS. 1964. Pharmacological observations on a chymotryptic inhibitor from potatoes. Proc. Soc. Exptl. Biol. Med. 115: 772–75.
4. LASKOWSKI, M., SR. AND M. LASKOWSKI, JR. 1954. Naturally occurring trypsin inhibitors. Advan. Protein Chem. 9: 203–42.
5. OUCHTERLONEY, O. 1949. Antigen-antibody reactions in gels. Acta Pathol. Microbiol. Scand. 26: 507.
6. RYAN, C. A. 1966. Chymotrypsin inhibitor I from potatoes: Reactivity with mammalian, plant, bacterial, and fungal proteinases. Biochemistry 5: 1592–96.
7. RYAN, C. A. 1967. The quantitative determination of soluble cellular proteins by radial diffusion in agar gels containing antibodies. Anal. Biochem. 19: 434–40.
8. RYAN, C. A. AND A. K. BALLS. 1962. An inhibitor of chymotrypsin from *Solanum tuberosum* and its behavior toward trypsin. Proc. Natl. Acad. Sci. U.S. 48: 1839–44.
9. RYAN, C. A. AND O. C. HUISMAN. 1967. Chymotrypsin inhibitor I from potatoes: A transient component in leaves of young potato plants. Nature 214: 1047–49.