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Protein Metabolism in Cultured Plant Tissues

III. CHANGES IN THE RATE OF PROTEIN SYNTHESIS, ACCUMULATION, AND DEGRADATION IN CULTURED PITH TISSUE

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ABSTRACT

During the transition of tobacco (*Nicotiana tabacum*) pith tissue to callus tissue, there were changes in the composition of the soluble amino acid pools, in the distribution of amino acids between pool and protein, and in the synthesis, accumulation, and degradation of proteins. The size of the leucine pool decreased from 90 nanomoles per gram fresh weight in fresh pith to 20 nanomoles in 24-hour cultured pith, followed by a return to 90 nmoles in pith cultured longer than 5 days. The latter value is the same as that reported for exponentially growing callus cells. Many other pool amino acids changed as dramatically. However, they always approached callus levels after 5 days of culturing. The total amino acid content of pith tissue (the sum of both pool and protein) remained unchanged during culturing. The value for total amino acid content (34 to 42 nanomoles per gram fresh weight) was also similar to that found in callus. The distribution of amino acids between pool and protein did change during culturing. The transition of pith tissue with 88% of its total amino acids free in the soluble pool to callus with 92% of its amino acids in protein was further characterized by changes in protein metabolism. Both protein synthesis and accumulation increased over the first 50 hours in culture to a maximum rate of 45 milligrams protein synthesized gram protein^{-1} hour^{-1}. After 50 hours in culture, the rate of protein accumulation decreased to equal the rate of fresh weight accumulation (10 mg g^{-1} hour^{-1}). However, protein synthesis continued at a high rate for several days, suggesting protein degradation was turned on by this time. By 5 days protein synthesis had decreased to a rate similar to that of callus.

Mature pith tissue of *Nicotiana tabacum* is generally considered to be a nondividing tissue. It appears to have little, if any, *in situ* DNA synthesis (12) and very low mitotic activity. In the absence of added hormones, cultured pith tissue shows little DNA synthesis or mitotic activity. However, when it is cultured in the presence of an auxin and cytokinin, DNA synthesis is quickly induced (12), and high mitotic activity begins after 2 to 3 days (4). The callus tissue which results appears to be made up of uniform, exponentially growing cells (6).

N. tabacum pith tissue has several advantages for a biochemical study which are lacking in other tissues. Pith tissue is abundant, easy to isolate, and is very uniform in structure and behavior (10), if consistently taken from the same site on the plant. The physiology of cultured *N. tabacum* pith tissue and the resulting callus tissue has been extensively studied (6, 10, 14), providing a sound background for biochemical studies. Thus a study of the release by hormones of the strict controls imposed upon pith tissue *in vivo* may lead to a further understanding of cellular differentiation and its control.

Methods were previously reported by us for measuring *in vivo* protein accumulation, synthesis, and degradation in callus cells (7, 8). Using these techniques we wish to report on the changes with time in rates of protein accumulation, synthesis, and degradation in cultured pith tissues. We also wish to report on the changes in amino acid composition of the biosynthetic pools and changes in the distribution of amino acids within cellular components.

MATERIALS AND METHODS

Plant Material. *Nicotiana tabacum* L. (var. Wisconsin No. 38) plants were grown in a growth chamber at 28 C with a 12-hr light cycle. Stems of plants (just before flowering) of approximately 1 m in height were excised just above the soil. The stems were defoliated and cut into four quarters with only the second quarter from the apex used in these experiments. The stem section was cut into 6 cm lengths and surface sterilized 15 min in 95% ethanol. Pith tissue was removed with a sterile cork-borer (5 mm inner diameter) and sliced into 2 mm thick cylinders. These pieces were very uniform varying by no more than 10% in fresh weight.

Tissue Culture. Pith cylinders were cultured in the dark at 28 C on Linsmaier and Skoog's medium (11). Kinetin and IAA were supplied at 1 and 11.5 μM, respectively. In some experiments varying amounts of L-leucine were supplied to the medium.

Labeling Procedure. Cultured pith tissues were labeled by carefully removing them from the tissue culture medium and placing them on fresh agar medium containing 0.7 μM L-[4,5-^{3}H]-leucine (25 c/mmole, Amersham/Searle[1]). Generally, 1.5 g fresh weight of tissue was labeled on 10 ml of medium.

For pulse-chase experiments, the tissues were transferred

[1] Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

from the radioactive medium to identical nonradioactive medium.

After labeling for the specified times, tissues were quickly weighed and immediately homogenized with a Duall tissue grinder in an equal volume (w/v) of 0.5 N sodium hydroxide. The homogenate was centrifuged for 10 min at 17,000*g* and the clear supernatant liquid was retained as the extractable protein and soluble amino acid pool fractions. As reported earlier (7), 90 to 95% of the total cellular protein of callus was extracted by sodium hydroxide and similar results were found for pith tissues.

The supernatant liquid was separated into soluble (soluble amino acid pool) and precipitated (extracted protein) fractions by the addition of an equal volume of 20% (w/v) trichloroacetic acid. After 18 hr at 4 C, the precipitated material was removed by centrifugation and washed once with 10% trichloroacetic acid. The protein fraction was hydrolyzed, evaporated to dryness, dissolved in 0.2 N sodium citrate buffer, pH 2.2, and chromatographed on a Beckman Model 120B amino acid analyzer. The soluble amino acid pool fraction was chromatographed directly. Fractions from the analyzer were collected every 2 min, and radioactivity was measured in an aliquot as described earlier (7, 8). Modifications were made in the standard long column procedure described in the Beckman Instruction Manual for operating the 120B amino acid analyzer in order to facilitate the processing of samples (8). Briefly, these modifications included equilibrating the PA-28 resin with 0.2 N citrate buffer, pH 4.25, (standard long column second buffer) at a constant temperature of 60 C, adsorbing the sample (0.2–2.0 ml) to the resin, and eluting with the pH 4.25 buffer at 60 C. The buffer and ninhydrin flow rates remained the same as the standard long column procedure. By this procedure leucine was well separated from other amino acids and eluted from the analyzer in 60 to 70 min. This procedure, however, was not designed to determine all amino acids. Therefore, for those experiments where total amino acid composition was determined, the standard long column method was used. Specific radioactivity of leucine in the pool fraction (SA pool) and in the protein fraction (SA protein) was expressed as curies of radioactivity cochromatographing with leucine per mole of leucine.

An alternative method for determining SA protein was used when large numbers of samples were encountered. This method involved dissolving the precipitated protein fraction in 0.5 N sodium hydroxide, measuring radioactivity in one aliquot and measuring protein in a second aliquot (13). Since all of the radioactivity in the protein fraction was contained in leucine and since leucine comprised 8.9% of the protein as determined by the Lowry method, a specific radioactivity could be calculated. The alternative method consistently gave results similar to those obtained by the chromatographic method.

RESULTS

Amino Acid Composition and Distribution. The amino acid composition of fresh and cultured pith tissue is shown in Table I. The compositions of protein extracted from fresh pith and pith cultured 5 days were very similar and closely resembled that reported for callus (7). The amino acid composition of the soluble pools, on the other hand, varied with time in culture and initially was very different from callus (7). The five most abundant amino acids in the pool make up over 95% of the pool in pith with glutamine being predominant.

The sum of the amino acids in both the soluble amino acid pool and protein fractions remained relatively constant (Table II) during culturing. Fresh pith contained 42 μmoles per g fresh weight of amino acids, whereas cultured pith contained from 34 to 36 μmoles. The distribution of amino acids between pool and protein, however, did vary. In fresh pith 88% of the total cellular amino acids was present in the soluble pool with only 12% in protein, whereas callus contained only 8% of the total in soluble amino acids and 92% in protein. The longer pith remained in culture the closer its distribution of amino acids approached that found in callus.

The size of the leucine pool in fresh pith is 90 nmoles per g fresh weight. When pith tissue was cultured on leucine-free medium, however, there were dramatic changes in the leucine pool with time (Table III). The leucine content de-

Table I. *Amino Acid Composition of Soluble Pools and Protein in Pith Tissue at Various Times in Culture*

Clarified cell homogenates from pith tissue cultured 0, 1, and 5 days were fractionated with trichloroacetic acid into a soluble amino acid pool and precipitated protein fractions. The fractions were chromatographed and the amino acid composition presented. Values for leucine represent the average of 10 or more runs; values for the remaining amino acids represent the average of two runs.

Residue	Soluble Amino Acid Pool			Protein	
	0 day	1 day	5 days	0 day	5 days
	μmoles per g fresh wt			%	
Glutamine + Asparagine	18.8	10.3	3.6	ND[1]	ND
Glutamic acid	3.2	5.0	1.3	12.5	11.6
Aspartic acid	4.7	3.6	0.72	10.8	11.6
Threonine				5.3	5.4
Serine	0.9	0.5	0.24	5.3	6.2
Proline	8.7	4.5	0.86	5.6	5.4
Glycine	0.3	0.35	0.32	8.4	7.2
Alanine	0.13	0.58	1.26	10.0	9.5
Valine	0.06	0.05	0.05	7.4	7.4
Methionine	0.04	0.04	0.04	2.0	2.0
Isoleucine	0.06	0.04	0.03	4.6	4.9
Leucine	0.10 ± 0.01	0.02 ± 0.002	0.09 ± 0.01	8.6 ± 0.4	8.6 ± 0.5
Tyrosine	0.06	0.02	0.03	2.5	3.4
Phenylalanine	0.02	0.01	0.03	3.9	4.7
Lysine	0.04	ND	0.02	6.8	4.7
Histidine	0.18	ND	0.02	2.0	1.2
Arginine	0.04	ND	0.01	3.8	4.4

[1] ND: values not determined.

Table II. *Distribution of Total Amino Acids Between the Soluble Pools and Protein*

Pith tissues were cultured 0, 1, or 5 days, callus tissue was stock line of "tight" callus (7). Amino acids were separated as in Table I and their sum presented.

Tissue, Cultured	Cellular Fraction		
	Soluble pool	Protein	Total
	μmoles per g fresh wt (%)		
Pith, 0 day	37 (88)[1]	5.1 (12)	42 (100)
Pith, 1 day	25 (74)	9 (26)	34 (100)
Pith, 5 days	9 (25)	27 (75)	36 (100)
Callus	3.8 (8)	42 (92)	46 (100)

[1] Numbers in parentheses are percentage of total.

Table III. *Differences in the Size of the Leucine Pool in Pith Tissue Cultured on Exogenous Leucine*

Tissues were cultured for the number of hours and on the concentration of L-leucine reported. The soluble leucine pool was measured by the procedure reported in "Materials and Methods."

Time	Leucine Pool		
	0	20 μM	100 μM
hr	*μmoles per g fresh wt*		
0	0.09	0.09	0.09
2	0.09	0.09	0.11
7	0.07	0.10	
14	0.02	0.15	0.26
24	0.02	0.19	0.46
50	0.025	0.08	0.58
120	0.09	0.10	0.19

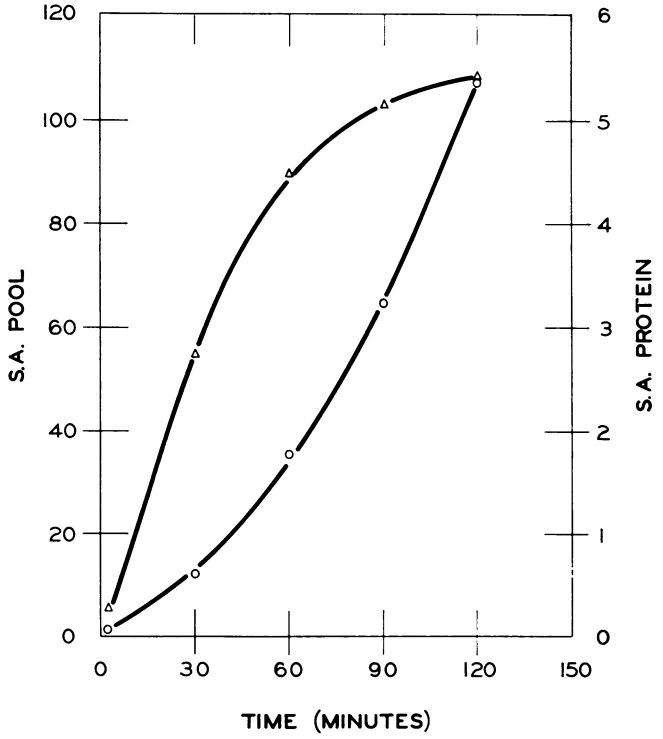

FIG. 1. Specific radioactivity of leucine in protein (○) and precursor pool (△) in cultured pith tissue after exposure to radioactive leucine. Pith cylinders were cultured 24 hr on medium containing 20 μM leucine before transferring to fresh medium containing 20 μM ^{3}H-leucine (0.9 c/mmole) for the times indicated.

creased to 20% of its initial level after 24 hr in culture and then slowly increased again to its original level after 5 days. When cultured on 20 μM leucine the pool in pith doubled during the first 24 hr, then slowly decreased to the original level. Culturing pith on 100 μM leucine had the effect of increasing the pool almost 7-fold at its maximum, and higher concentrations of exogenous leucine expanded the leucine pool to even greater levels.

Distribution of Radioactivity. Cultured pith tissues were labeled for as long as 2.5 hr on an agar medium containing ^{3}H-leucine as described in "Materials and Methods." The distribution of radioactivity in the soluble amino acid pool and in the protein fraction was much the same as callus (7). More than 95% of the radioactivity in the soluble protein fraction cochromatographed with leucine and less than 0.2% of the radioactivity was associated with the other amino acid peaks. Approximately 75% of the total radioactivity in the soluble amino acid pool fraction was coeluted from the analyzer with leucine. The remaining 25% of the radioactivity was eluted with unidentified, ninhydrin-negative material.

Synthetic Rates. Fresh pith was placed in culture for 24 hr on medium containing 20 μM ^{1}H-leucine and medium containing no added ^{1}H-leucine. As reported in Table III there was a 10-fold difference in the size of the two leucine pools at the end of the culture period. The specific radioactivity of leucine in protein (SA protein) and pool (SA pool) was also measured for tissues labeled various times on ^{3}H-leucine medium in the presence (Fig. 1) and absence (Fig. 3B) of added ^{1}H-leucine. In both cases SA pool increased sharply over the first 60 min of labeling, with a slower increase over the next 90 min. SA pool became relatively constant after 150 min of labeling. The shape of the SA protein curve was predictable from the SA pool curve, assuming the only source of radioactivity for incorporation into protein came from the leucine pool.

Equation 1 was previously defined and integrated between the limits $t = o$ and $t = T$ (7).

$$\frac{d(\text{SA protein})}{dt} = R \times (\text{SA pool}) \qquad (1)$$

Since SA pool for cultured pith tissue was not constant over the entire labeling period a general derivation of equation 1 was necessary where integration was performed between the limits $t = T_1$ and $t = T_2$.

$$R = \frac{(\text{SA protein})_2 - (\text{SA protein})_1}{(\text{SA pool})(T_2 - T_1)} \qquad (2)$$

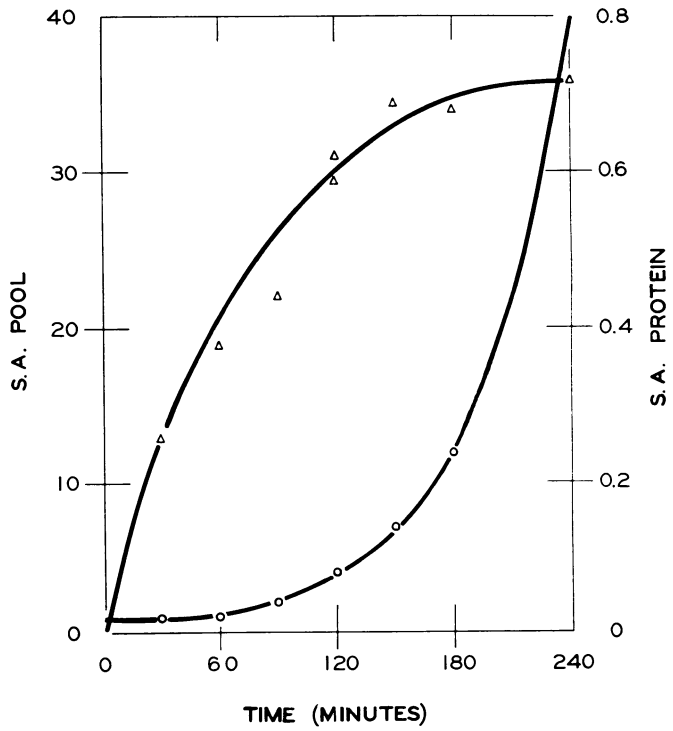

FIG. 2. Specific radioactivity of leucine in protein (○) and precursor pool (△) in fresh pith tissue immediately after exposure to radioactive leucine. Pith tissue was taken from a tobacco plant and cut immediately into cylinders. The cylinders were placed directly on medium containing 0.8 μM ^{3}H-leucine (25 c/mmole) for the times indicated.

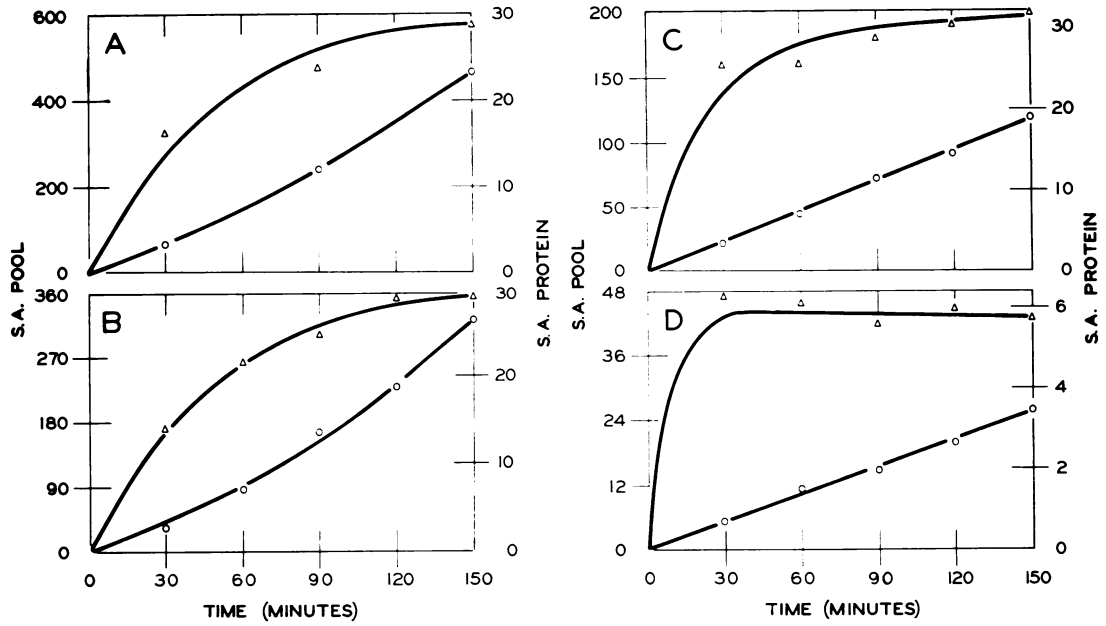

FIG. 3. Specific radioactivity of leucine in protein (○) and precursor pool (△) for pith tissue cultured various lengths of time prior to exposure to radioactive leucine. Pith cylinders were cultured a total of 14 hr (A), 24 hr (B), 50 hr (C), and 145 hr (D), during which time they were transferred from nonradioactive to ^{3}H-leucine medium for the times indicated.

Equation 2 still requires that SA pool be constant between some time limits. However, the limits can be quite short. An alternate method for calculating the rate of protein synthesis involved measuring an instantaneous rate of change of SA protein by drawing a tangent to the SA protein curve at a particular time, and estimating synthesis by dividing the instantaneous rate of change by the value for SA pool at that time.

As an example of calculating rates, protein synthesis for pith cultured 24 hr in the presence of leucine (Fig. 1) was estimated from SA protein values of 3.2 and 5.3, and limiting values of SA pool of 102 and 109 at 90 and 120 min, respectively. Substituting these data into equation 2, two rates of synthesis were calculated representing the limits. These rates were 39 and 41 mg protein synthesized g protein^{-1} hr^{-1}. The instantaneous change in SA protein method gave rates from 35 to 40. In the absence of added leucine (Fig. 3B) 24-hr cultured pith had almost an identical rate of protein synthesis (43 mg g^{-1} hr^{-1}) as calculated from equation 2.

Rates of synthesis for pith cultured various lengths of time in the absence of added leucine were calculated from incorporation data presented in Figures 2 and 3. Because the rate of protein synthesis increased rapidly over the first several hours in culture, the instantaneous rate method was used to estimate synthetic rates between 0 and 3 hr in culture, and equation 2 was used for the remaining times. The rate of protein synthesis in pith reached a maximum of 45 mg protein synthesized g protein^{-1} hr^{-1} after 30 hr in culture and then slowly decreased, eventually approaching a rate measured for callus (7). The change in rate of protein synthesis for pith cultured various lengths of time is summarized in Table IV.

Table IV. *Rate of Protein Synthesis for Cultured Pith Tissue*

Time in Culture	Rate of Synthesis
hr	*mg protein g protein^{-1} hr^{-1}*
1	1
2	3
4	19
14	20
18	30
24	43
30	45
50	41
72	43
120	28
144	28

Protein Accumulation. Fresh weight and protein content per piece of cultured pith are shown in Figure 4. Fresh weight did not begin accumulating in pieces of cultured pith until after 48 hr in culture, whereupon it began increasing exponentially at a rate equal to a doubling every 3 days. Protein, on the other hand, began accumulating almost immediately with a maximum rate attained between 12 and 50 hr in culture. After 50 hr the rate of protein accumulation decreased to approximately the rate of fresh weight accumulated (doubling every 2.6 days). The maximum rate of accumulation (12–50 hr) equaled 40 mg protein accumulating g protein^{-1} hr^{-1}, the same as the measured rate of synthesis.

If one assumes there is *no* protein turnover occurring in the tissue, then the rate of protein synthesis should equal the rate of protein accumulation. Using the values for rate of protein synthesis presented in Table IV and the total protein content of fresh pith (24 μg protein per piece), the amount of protein which should accumulate (assuming no turnover) in a piece of cultured pith tissue was calculated and compared to the actual measured values plotted in Figure 4. This comparison, represented by the dotted line in Figure 4, revealed that the calculated and measured values were identical through 50 hr in culture. However, after 50 hr the calculated values were increasingly higher due to a rapid decrease in the rate of accumulation (possibly due to turnover).

Pulse-Chase. The comparison of the rates of protein synthesis and protein accumulation during the first 50 hr pith tissue was cultured strongly suggested little protein turnover. After 50 hr, however, the rate of synthesis was considerably greater than accumulation, suggesting considerable protein turnover. If this were the case, then during a chase period SA

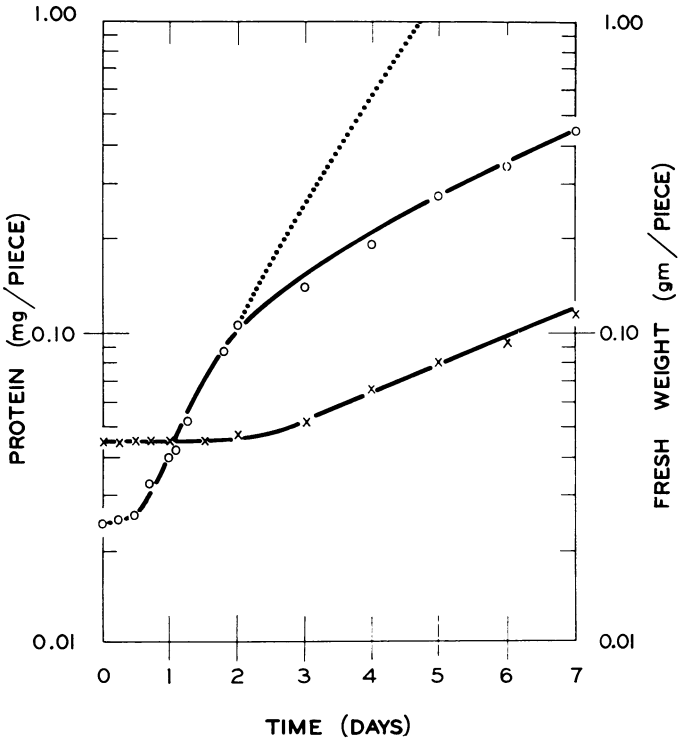

FIG. 4. Growth and protein content of cultured pith tissue on medium containing 88 mM sucrose, 11.5 μM indoleacetic acid, and 1.0 μM kinetin. Fresh weight points (×) represent the average fresh weight of 15 pieces. Protein (○) represents the total NaOH-extractable protein. Protein accumulation (dotted line) was calculated from rates of synthesis summarized in Table IV. The calculated accumulation is coincident with the measured accumulation (○) through 50 hr.

pool for ^{3}H-labeled, 24-hr cultured pith should chase to a level of zero while SA pool for 72-hr cultured pith should chase to a level near the value for SA protein. A pulse-chase experiment was performed by labeling tissues cultured 24 hr and 72 hr for 2.5 hr, then transferring them to fresh, nonradioactive medium. The specific radioactivity of both leucine pool and protein was measured at the times reported in Figure 5. Radioactivity was rapidly chased from the leucine pool of both pith tissues. In both cases SA pool appeared to decrease exponentially, but to different levels. The level for pith cultured 24 hr was well below its SA protein, whereas the level for pith cultured for 72 hr was above its SA protein. The changes in SA protein should give a good indication of whether radioactivity was chased from SA pool into protein. If this were not the case, then SA protein should be reduced by the amount of new, nonradioactive protein accumulating during the chase period. For the 24-hr cultured pith the change in SA protein would be from 22 to 10. On the other hand, assuming that all of the radioactivity lost from SA pool appears in SA protein, then the change should be from 22 to only 15. The experimentally determined change was 22 to 16 (Fig. 5), suggesting that SA pool was the precursor pool for protein synthesis. A similar argument was made for pith cultured 72 hr and the same conclusion was reached.

Assuming (*a*) there is no protein turnover at 24 hr in culture, (*b*) all the radioactive leucine chased from the pool appears in protein, and (*c*) there is an exponential decrease in SA pool during a chase period, then it is possible to calculate the rate of protein synthesis from the following equation which relates the rate of synthesis to an SA pool chase rate:

$$\frac{d(\text{SA pool})}{dt} = -\frac{R}{P}(\text{SA pool}) \quad (3)$$

where R = rate synthesis, P = μmoles leucine in soluble pool/μmole leucine in protein.
Integrating equation 3,

$$\frac{\ln (\text{SA pool})_2}{(\text{SA pool})_1} = -\frac{R}{P}\Delta t \quad (4)$$

If $(\text{SA pool})_1 = 2(\text{SA pool})_2$ and $\Delta t = t_{1/2}$ then,

$$R = \frac{0.69}{t_{1/2}} P \quad (5)$$

P equals 0.04 for tissue cultured 24 hr and $t_{1/2}$ ranges from 0.7 to 1.0 hr (Fig. 5). Therefore, the rate of protein synthesis calculated from chase experimental data ranges from 28 to 40 mg protein synthesized g protein^{-1} hr^{-1}. These calculations fit well with those from uptake and incorporation data, and again suggest no protein turnover at 24 hr in culture.

DISCUSSION

The amino acid composition of both fresh and cultured pith protein appeared to be similar to one another and the same as that reported for callus (7). Other investigators (5) also have found little difference in the amino acid composition of the proteins, not only from cells of different organs of the same species, but also from cells of different plant families. The amino acid composition for tobacco cells reported by us also appears to be indistinguishable from those re-

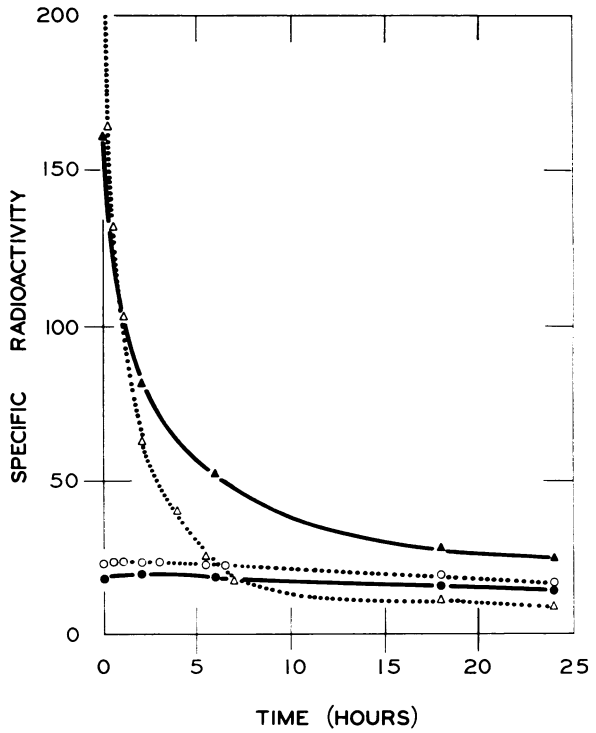

FIG. 5. Specific radioactivity of leucine after transferring labeled pith tissue to nonradioactive medium. Tissue was labeled 2.5 hr on ^{3}H-leucine (25 c/mmole) medium after 24 hr (open symbols) and 72 hr (closed symbols) in culture. Tissues were harvested and prepared as described in "Materials and Methods," and specific radioactivity of the leucine pools (triangles) and protein (circles) was determined.

ported by Gamborg and Finlayson (5). This is not to say, however, that the proteins themselves are the same. Stafford and Galston (14) reported changes in oxidase isozymes patterns during culturing of pith, and we have observed changes in protein staining patterns on polyacrylamide disc gel electrophoresis (unpublished results). These results seem to suggest that although new proteins are synthesized their average amino acid composition remains unchanged.

On the other hand, the amino acid pool composition changed dramatically along with the distribution of amino acids between pool and protein. The high levels of glutamine, asparagine, glutamic acid, and aspartic acid probably reflect the role played by these amino acids in the control of nitrogen metabolism and storage. The loss of these four amino acids from the pool in the 1st day of culture was more than enough to account for all the carbon and nitrogen in the newly synthesized protein. Aside from proline and alanine, the remaining pool amino acids make up a small portion of the total pool and are probably not significant as a storage pool. The total amino acid content of fresh pith tissue, cultured pith, and callus was about the same, 34 to 46 μmoles per g fresh weight. The distribution of these amino acids between pool and protein, however, changed dramatically. This change from a tissue with most of its amino acids free in the soluble pool (pith) and predominantly glutamine to one where most of the amino acids were bound in protein (callus) may be a further expression of the developmental change from a nondividing possibly storage tissue to a rapidly growing tissue.

Exogenous leucine was rapidly concentrated by cultured pith tissue and could expand the leucine pool many times. The rate of protein synthesis calculated from total measurable pool and protein specific radioactivities, however, were the same for pith cultured on medium containing 20 μM leucine (expanded) or in the absence of leucine (nonexpanded). These results suggested that the total measurable amino acid pool, whether expanded 10-fold or not, was the precursor pool for protein synthesis. The results are in contrast to those found for *Candida utilis* (3), *Lytechinus* (1), mammalian cells (9), and plants (2, 15) where more than one pool for a particular amino acid apparently exists. If a large, second pool did exist in cultured pith, it would have to be in equilibrium with the biosynthetic pool or at least equally available for protein synthesis.

The observed rate of protein synthesis was near zero when pith tissue was initially placed in culture. Over the next several hours, however, the rate increased rapidly to 19 mg protein synthesized g protein^{-1} hr^{-1}, followed by a slower increase to about 40. Whether the initial rate of zero was indicative of *in vivo* pith tissue or whether it reflected a wound response is not known at this time. The maximum rate of synthesis was reached by 30 hr, and the rate remained high through 70 hr where it then gradually decreased to that of callus.

Early protein accumulation showed a response similar to synthesis. For the first few hr there was little accumulation, but by 20 hr the rate had increased to 40 mg g^{-1} hr^{-1} and remained at that rate until 50 hr of culture whereupon it rapidly decreased to 10. These results suggested two separate events were taking place. The first event occurs within hours after the tissue was placed in culture and was characterized by a rapid increase in protein synthesis with a concomitant increase in protein accumulation but no increase in fresh weight. During this time (0–50 hr) degradation must have been very low if it existed at all. At about 50 hr a second event occurred which was characterized by a gradual decrease in the rate of protein synthesis, a rapid decrease in the rate of protein accumulation, and the increase of fresh weight at a rate equal to protein accumulation. Also associated with the second event was the presence of protein degradation which was inferred from the difference between the calculated and measured protein accumulation (see dotted line in Fig. 4). The relatively high rate of protein degradation was estimated at 30 mg protein degraded g protein^{-1} hr^{-1}.

Independent experimental evidence suggesting that protein degradation was turned on during the second event was provided by the pulse-chase experiments performed using tissue cultured 24 hr and 72 hr (Fig. 5). During the chase period SA pool decreased rapidly. The observed SA pool chase level for 24-hr culture pith was below SA protein and for 72-hr pith above SA protein. The latter result was similar to that found for callus, where SA pool leveled off at 3 to 5% of initial SA pool above SA protein. Assuming that 5% of the *radioactivity* associated with pool leucine was not part of the biosynthetic precursor pool and, therefore, subtracting it from the SA pool data in Figure 5, then SA pool for 24-hr cultured pith chases to zero and SA pool for 72-hr pith to SA protein. These results then strongly suggest that at least 95% of the measured SA pool was the precursor pool for protein synthesis, and that protein degradation occurred after 72 hr in culture, but not after 24 hr in culture.

In conclusion, there appear to be at least two measurable events occurring as pith tissue is converted to callus tissue in culture. The first event involves a turning on of protein synthesis ultimately to rates in excess of 40 mg g^{-1} hr^{-1} with no increase in fresh weight or no protein degradation. The second event begins after 50 hr in culture and is characterized by a turning on of protein degradation to rates approaching 30 mg g^{-1} hr^{-1}, an accumulation of fresh weight, and a slow decrease in the rate of protein synthesis.

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