DEVELOPMENT OF MICROBODIES AND RELATED RESPIRATORY METABOLISM IN POTATO TUBER TISSUE CULTURED *IN VITRO*

Yoshio KIKUTA and Yozo OKAZAWA

Department of Botany, Faculty of Agriculture,
Hokkaido University, Sapporo, 060, Japan

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Introduction

The growth rate and extent of characteristics of callus formation in explants from potato tubers (*Solanum tuberosum* L.) depend on the concentration of auxin and kinetin in the medium on which they are grown[3,22,23]. The potato explant does not require kinetin for their intiation of callus, and produces its own cytokinin(s), and the amount synthesized varies over the growth period[3,8,21].

Metabolic change during callus formation has been previously studied.[1,2,12,13,14] Exogeneous application of auxin is indispensable for initiating callus[23], of which respiratory and glucose metabolism is very much resistant to malonate during the initiation of callus formation[1], and the oxidative pentosephosphate pathway becomes the intrinsic role of the participation in the glucose catabolism of the potato explants[2,12,13].

Change in alternative oxidase activity is found in the potato explants incubated *in vitro*[19,20,25]. A considerable increase in mitochondrial state III respiration is also found in potato explants leading to callus cultures[29]. Antimycin-A resistant respiration is recognized during the initial stage of callus formation[20,29].

Separation of the crude mitochondrial fraction on sucrose density gradients has revealed that microbodies are developed in the potato explants cultured during the first 6 days after cultures started. The potato explant excised from the central core of potato tubers contains intrinsically intact mitochondria but few microbodies[10], while MARINOS[16] pays an attention to the presence of similar structures in the buds and peripherial cells of potato tubers during dormancy. YOO *et al*[30]. also describe microbodies in field grown potato tubers.

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In this report electronmicrographs are presented in which show the morphology of the particles isolated from the potato tissue cultures and the presence of similar structures in the cultural explants. An attempt has been made to elucidate the development of mitochondria and microbodies in relation to the respiratory metabolism in potato explants cultured during the early stage of callus formation.

Materials and Methods

Plant material and tissue cultures:

Tubers of potato (*Solanum tuberosum* L., cv. Irish Cobbler) stored in a cellar at 5°C were used for culture materials. Aseptic culture technique by OKAZAWA *et al*[23]. was employed except that auxin (naphthaleneacetic acid, 10 μM) and kinetin (6-furfurylaminopurine, 10 μM) were added to the modified White's medium in order to develop malonate resistant respiration[1]. The culture conditions were detailed in the text of the previous reports[2,14].

Separation of the crude particulate fraction in sucrose density gradient system:

The cultured explants, 2.5 g, were homogenized in 20 ml of 0.5 M sucrose solution containing 0.05 M Tris buffer, pH 7.5, 0.01 M cystein 0.01 M KCl, 1 mM EDTA, 0.1 mM $MgCl_2$ and 0.1% bovine serum albumine. The explants were first vigorously ground for 60 sec with a mortar and pestle. The resulting brei was then slowly suspended in a Porter glass homogenizer and centrifuged 3,000 rpm (800×g) for 10 min. The supernatant solution was centrifuged at 20,000 rpm (47,000×g) for 20 min. and the resulting pellets resuspended in 0.5 ml of 30% (w/w) sucrose.

Portions of this crude particulate suspension containing 7 to 10 mg protein were then layered on a linear sucrose gradient contained in a Hitachi rotor SW25-3A cellulose butyrate tubes. The slope of the linear gradient consisting of 2 ml of 65% sucrose, 14 ml grading from 60% to 35% sucrose was achieved by a hand-made gradient maker. The gradients were centrifuged at 22,500 rpm for 5 hr in a Hitachi 65P ultracentrifuge and allowed to decelerate without braking. The gradients were siphoned through a Hitachi 034 UV-VIS three wave lengths effluent monitor and fractionated into thirty four 0.5 ml fractions in the order of decreasing density, and maintained at 0-5°C and assayed as quickly as possible.

Electronmicroscopy:

The peak fractions containing mitochondria and microbodies were withdrawn from the gradient and fixed for 1 hr in 3.0% glutaraldehyde. The

sucrose concentration was then lowered to 0.5 M, and the particles were pelleted. The pellet was rinsed in 0.5 M sucrose buffered at pH 7.5 with 0.1 M phosphate, and then post fixed for 2 hr with 1% OsO_4 in 0.1 M phosphate buffer, pH 7.7. All fixation steps were carried out in the cold room. The procedure namely described by BREIDENBACH *et al.*[7] was followed.

The explants were cut with a razor blade into small cubes (1 mm^3) in a small Petri-dish containing 5% glutaraldehyde in 0.1 M phosphate buffer, pH 6.8. The cubes were then transferred to fresh fixative for 3 hr at room temperature. After washing in eight changes of phosphate buffer over a period of 6 hr, the cubes were transferred to 2% OsO_4 in 0.1 M phosphate buffer, pH 6.8, for a period of 2 hr at room temperature. The fixation procedure of the intact plant tissue as was described by MOLLENHAUER *et al.*[18] was employed.

Dehydration was carried out in a series of ethanol, the sample rinsed twice in acetone and then allowed to infiltrate for 24 hr with acetone : epon mixture (v/v, 1 : 1) and then for a further 24 hr infiltration at 37°C in the complete epon mixture alone. Polymerization was completed after 48 hr at 60°C. Sections showing silver or pale gold interference colors were cut using an LKB ultramicrotome and stained with 0.4% lead citrate for 20 min, and 2% uranylacetate for 60 min and then examined in a Hitach HU-8 electron-microscope.

Enzyme assays:

Glycolate oxidase was assayed by the reaction of glyoxylate with 2, 4-dinitrophenylhydrazine. Formation of hydrazone at 25°C was measured at 324 nm by an automatic recording Hitach 356 two wave lengths spectrophotometer[27]. KCN was added to the reaction mixture when it was required for the activity.

Cytochrome C oxidase was assayed from 50 to 250 μl of enzyme pipetted into a bottom of a 3 ml spectrophotometer cuvette (diameter, 10 mm path) and 0.1 ml of 2% digitonin added, mixed with and allowed to stand for 1 min. Then 2 ml of 0.1 M phosphate buffer, pH 7.0, and 0.5 ml of 1 mM cytochrome C reduced with dithionite were added and mixed[27]. Recordings optical density at 550 nm were obtained with the Hitachi 356 spectrophotometer. The first order rate constant in the disappearance of reduced cytochrome C was calculated.

Malate synthetase was assayed in a total volume of 1 ml that contained 100 mM Tris buffer, pH 7.4; 10 mM $MgCl_2$; 0.025 mM acetyl CoA; 5 mM glyoxylate and 10 μl of enzyme. The reaction rate was recorded at 232 nm for 2 min with a blank composed of above chemicals except acetyl CoA.

Deacylase did not interfere in such a dilute enzyme concentration.

Citrate synthetase was assyed in the same way as malate synthetase with oxalacetate as substrate. ^{14}C-acetyl-CoA was used for the detection of the reaction products separated by paper chromatography.

Isocitrate lyase was assayed in a total volume of 1 ml that contained 100 mM Tris buffer, pH 7.4; 10 mM cystein; 10 mM $MgCl_2$; 0.1 ml enzyme preparation, and 20 mM isocitrate at 25°C 10 min. Citrate-1, 5-^{14}C was used for detection of succinate and malate.

Fumarase was assayed in a total volume of 2 ml that contained 100 mM Tris buffer, pH 7.4; 50 mM *l*-malate and 100 μl enzyme preparation. The reaction was recorded at 240 nm for 5 min at 25°C with a blank containing everything minus substrate.

Aconitase was assayed similar to fumarase isocitrate as substrate.

Protein content in enzyme preparation was assayed by method of LOWRY *et al.*[15] with bovine serum albumine as a standard.

Measurement of respiratory activity:

The rate of respiration and metabolism experiments were conducted according to the previous method described[13]. The radioactivity was counted in Bray's solution[5] with NCS solubilizer. Protein content of the particulate fraction was estimated by the method of sulfuric acid digestion followed by Nesslerization. The factor of 6.25 was used to convert nitrogen to protein value.

Results

Growth and respiration

The three types of cultures designed for the competence of growth rate and extent of characteristics of callus formation in potato explants were auxin-culture (rich in NAA 10 mg/l), kinetin-culture (kinetin 0.3 mg/l plus NAA 0.3 mg/l) and control culture (without kinetin nor NAA). The growth of cultured explants otherwise quiescent tissue slices excised from potato tubers was estimated by measuring the parameter of fresh weight during three weeks culture period. The auxin culture showed a remarkable increase in fresh weight attained to 5 folds of an initial weight and resulted a friable callus culture. On the other, the kinetin culture tended to suppress an enhanced increase in fresh weight and formed a compact callus culture. While the control culture could not increase their fresh weight, in some instances weighed up to 10%, and kept on otherwise a quiescent state. Figure 1 shows typical growth curves of the potato explants cultured under above described conditions.

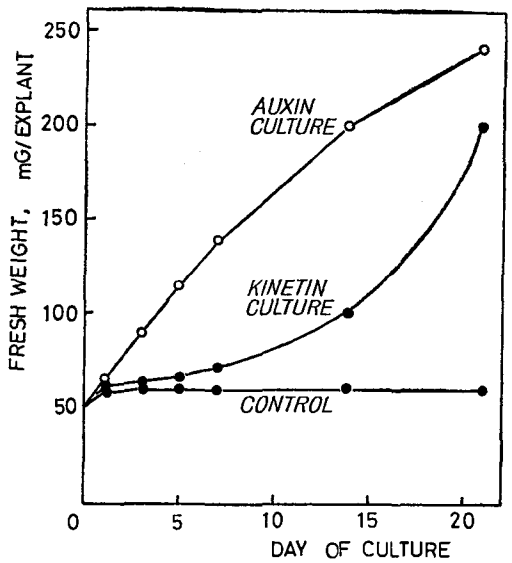

Fig. 1. Growth of explant cultured in a modified White's nutrient medium supplemented with auxin and kinetin. Auxin culture: NAA, 10 mg/l; Kinetin culture: kinetin, 0.3 mg/l plus NAA, 0.3 mg/l; Control culture: without any additions of auxin and kinetin.

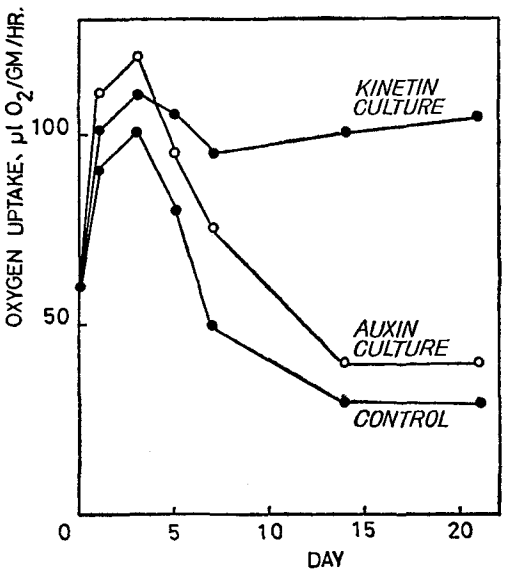

Fig. 2. Change in the rate of oxygen uptake of auxin culture and kinetin culture. Culture conditions were same as in Fig. 1.

The changes in oxygen uptake per fresh weight in auxin- and kinetin cultures are shown in Fig. 2. The initial respiratory rise during the first three days after inoculation was striking regardless of the presence or absence of exogenous application of auxin and kinetin. While the auxin culture rapidly declined the rate of respiration, the kinetin culture still kept to maintain the high level of respiratory rate.

Catabolism of glucose

Since a potato tuber stores a large amount of starch grains in cells, the respiration of potato explants is suggestive of carbohydrate catabolism during a culture period. Thus glucose-1-^{14}C and glucose-6-^{14}C were employed as the substrate in further studies on intermediary metabolism. To minimize possible differential inhibition or stimulation due to glucose, labeled glucose was supplied to explants free of added carrier or with less than 2.0 mg of unlabeled glucose per gram fresh weight of explants. Data in Table 1 show the relative incorporation of carbon-14 from specifically labeled glucose into respiratory carbon dioxide. In accordance with the respiratory studies reported previously[13], more carbon dioxide was released from the kinetin culture than from the auxin culture and control culture. However, a much larger proportion of the carbon dioxide recovered was derived from glucose-1-^{14}C in the kinetin culture as shown by the lower C6/C1 ratios. Since the labeled carbon dioxide from glucose-1-^{14}C was accelerated in the kinetin culture, the data further lend support a previous evidence of more rapid turnover of the hexose monophosphate shunt by the kinetin culture. Data in Table 1 also show that the C6/C1 ratio of potato buds is extremely low and that upon the addition of NADP, the ratio is further reduced suggesting

TABLE 1. C6/C1 Ratios determination and Catabolism of specifically labeled glucose in potato tissue cultured for 12 days and excised potato sprouts with or without NADP

Concn. of Auxin (mg/l)	Kinetin (mg/l)	$C^{14}O_2$ (%)		C6/C1
		C-1	C-6	
0	0	10.48	8.46	0.81
0.3	0	26.45	17.94	0.68
0.3	0.3	29.96	17.63	0.59
0.3	0.3	10.0	16.60	0.60
Buds		32.05	14.92	0.47
+0.2 μmol NADP		43.99	18.26	0.42

TABLE 2. Incorporation of glucose-6-^{14}C into organic acid pools of the potato explants from auxin- and kinetin cultures. Organic acids were separated by a column of ion exchange resin (Dowex 1×4, formate form) eluted with grading formic acid concentration

Organic acid	Auxin-culture		Kinetin-culture	
	−NADP	+NADP	−NADP	+NADP
glycerate	585	623	1161	812
glyoxylate	372	338	771	435
succinate	317	455	510	360
malate	984	695	1756	900
glycolate	675	280	510	160
lactate ?	193	158	391	203

cpm/explant

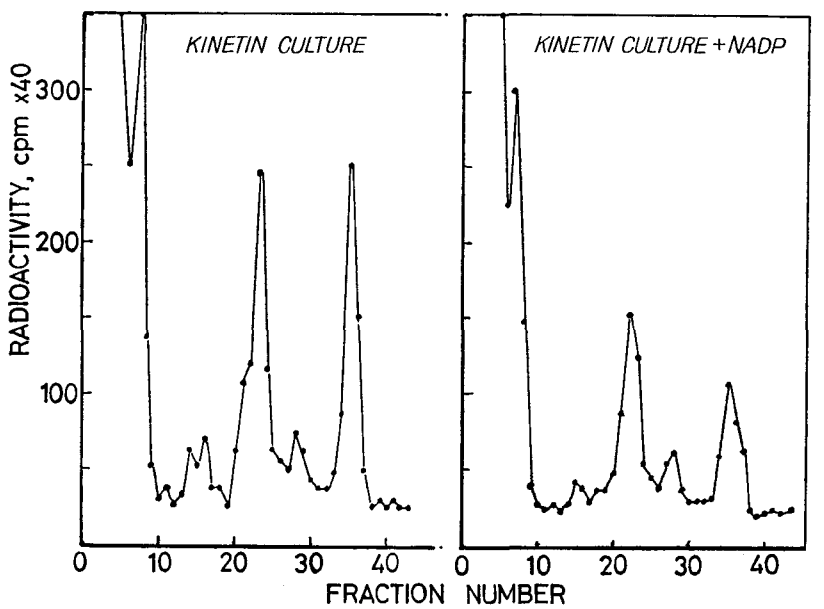

Fig. 3. Incorporation of glucose-6-^{14}C into organic acids separated by ion exchange resin column chromatography. Explants were taken from kinetin culture (left) and kinetin culture withNADP (right) during ^{14}C exposure of 2hr. Dowex 1×4, formate resin, was used for column separation and eluted with formate of grading to 6 M.

that glucose catabolism of the kinetin culture is related to that of potato buds.

Glucose carbon was found to be incorporated into all cellular fractions[14]. The largest differences occurred in pools of amino acids and organic acids where the increase in incorporation range from 10 to 50 per cent. Thus, the pathway participation and the regulatory systems concerned in synthesis and accumulation in pools of these components would appear to be intimately associated with changes in growth characteristics, although alterations in other pathway of metabolism probably contribute also to the overall growth potential of the kinetin culture.

Organic acids from the acidic fraction of ethanol soluble materials were further separated by means of ion exchange resin (Dowex 1×4, formate form) column chromatography and the radioactivity of individual acids were determined (Table 2). The levels of malate and glycerate were found to be higher in the kinetin culture than in the auxin culture. The addition of NADP during the exposure of labeled glucose also reduced accumulation of both acids (shown in Fig. 3).

Incorporation of carbon-14 from acetate-2-^{14}C into organic acids was

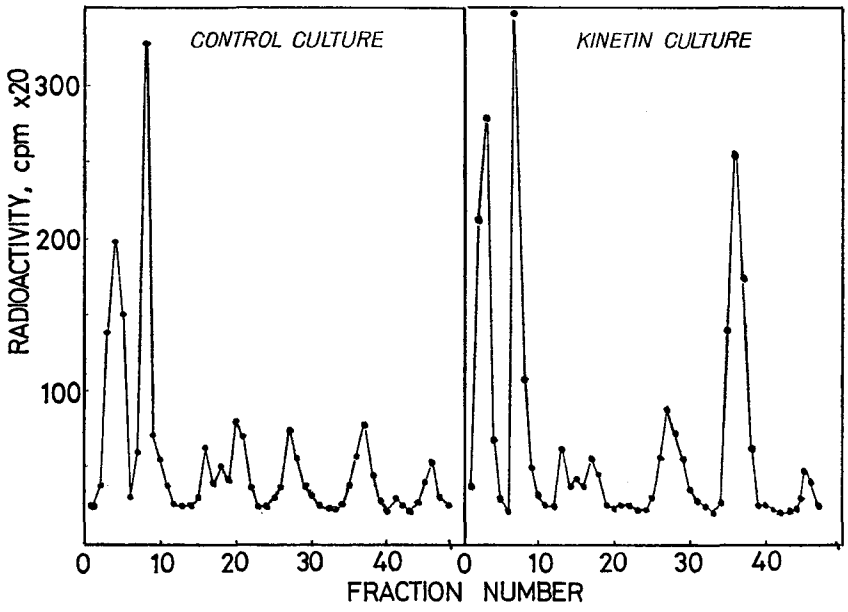

Fig. 4. Incorporation of acetate-2-^{14}C into organic acids separated by ion exchange resin column chromatography. Explant harvested were from control culture (left) and kinetin culture (right).

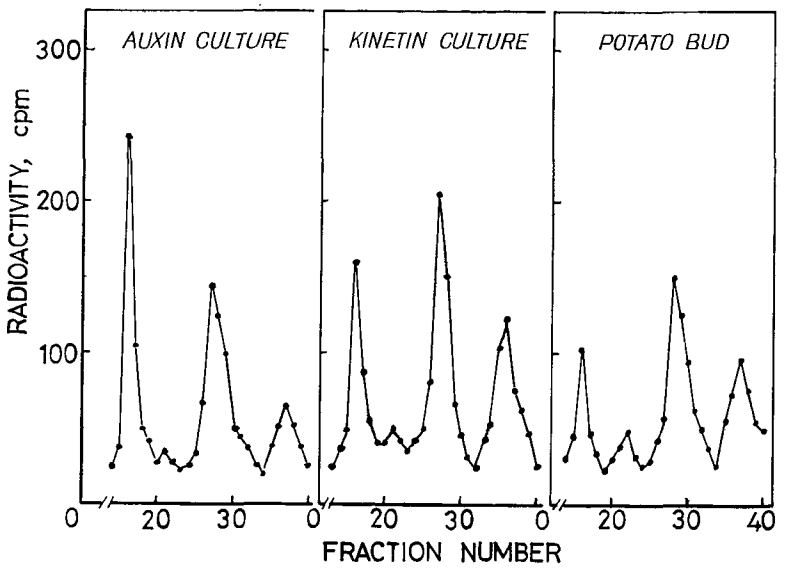

Fig. 5. Incorporation of citrate-1, 5-^{14}C into organic acids separated by ion exchange column chromatography. Explants harvested were from auxin culture (left) from kinetin culture (middle) and buds excised from potato tuber sprouts (right), and allowed to expose to citrate-1, 5-^{14}C in Warburg's flaasks for 2 hr. Dowex 1×4, formate resin, was used for column chromatography.

also examined among citrate, malate, succinate, glycolate, aspartate and glutamate detected (Fig. 4). However, malate was the most heavily labeled acid in kinetin culture. Moreover, incorporation of carbon-14 from citrate-1, 5-^{14}C in to these acids indicated that glycolate was the most labeled acid in the auxin culture while malate was accumulated in the kinetin culture, and in the potato buds minimized these acids' levels (Fig. 5).

Separation of crude mitochondrial fraction

Five bands were usually formed after the gradient centrifugation of the crude mitochondrial particulate fraction. The top was a fluffy layer 1.16 g/cm^3; the second thick band with a peak density of 1.19 was composed mainly of mitochondria; the third small band with a peak density of 1.22 was made chiefly of membraneous aggregates of broken microbodies and intact proplastids. The fourth thick and dense band with a peak density of 1.25 contained microbodies. Electron micrographs of these bands were shown in Figure 6. The last thin band with a peak density of 1.28 was composed mainly of small starch grains and protein bodies, both of which were not studied. Nuclei and cell-wall fragments were found in some in-

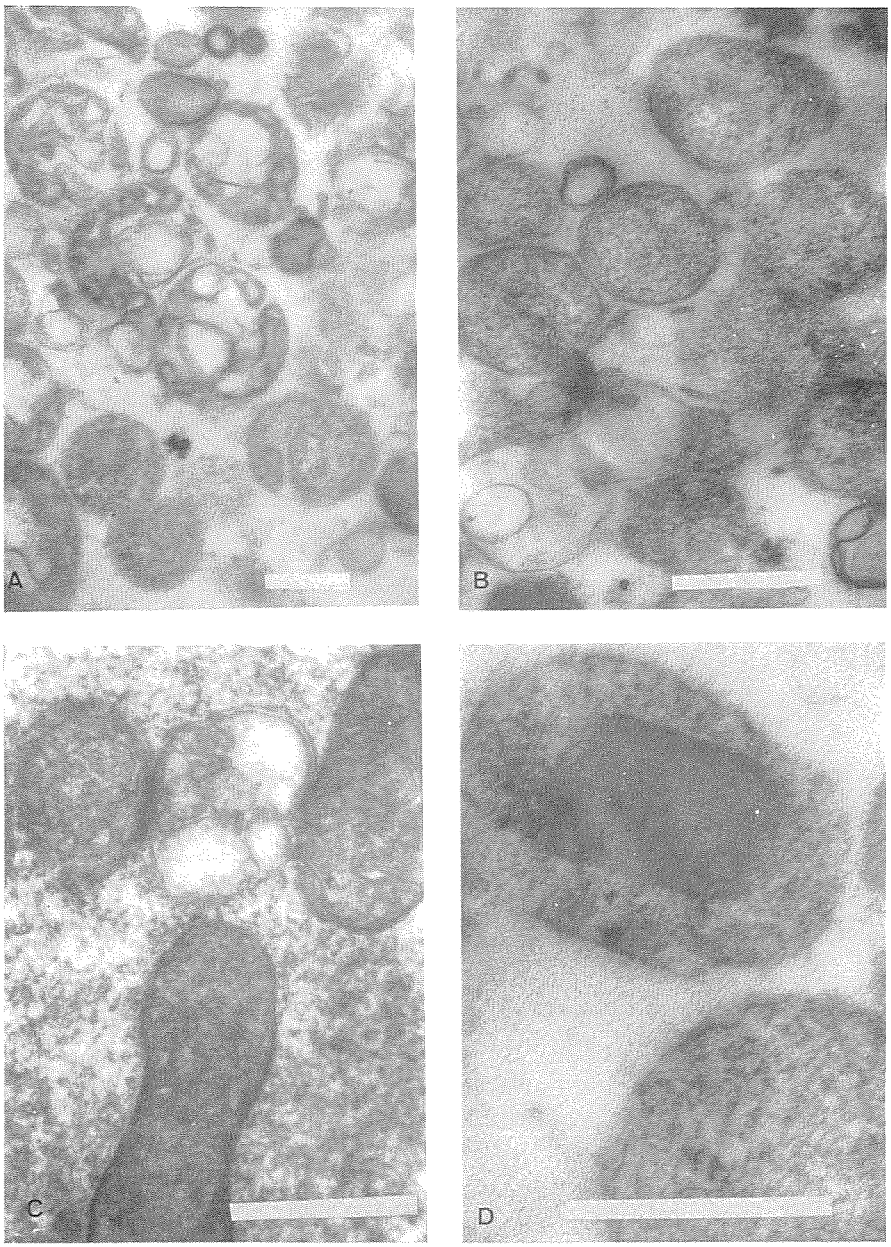

Fig. 6. Electronmicrographs of organelles isolated from potato explants. A: Mitochondria isolated from a peak of 1.19 gcm^{-3} density; B: Microbodies isolated from a peak of 1.25 gcm^{-3} density; C: Mitochondria observed in intact cell; D: A crystalline containing microbodies isolated from kinetin culture. The bar at the base of all figures represents 0.5 μ.

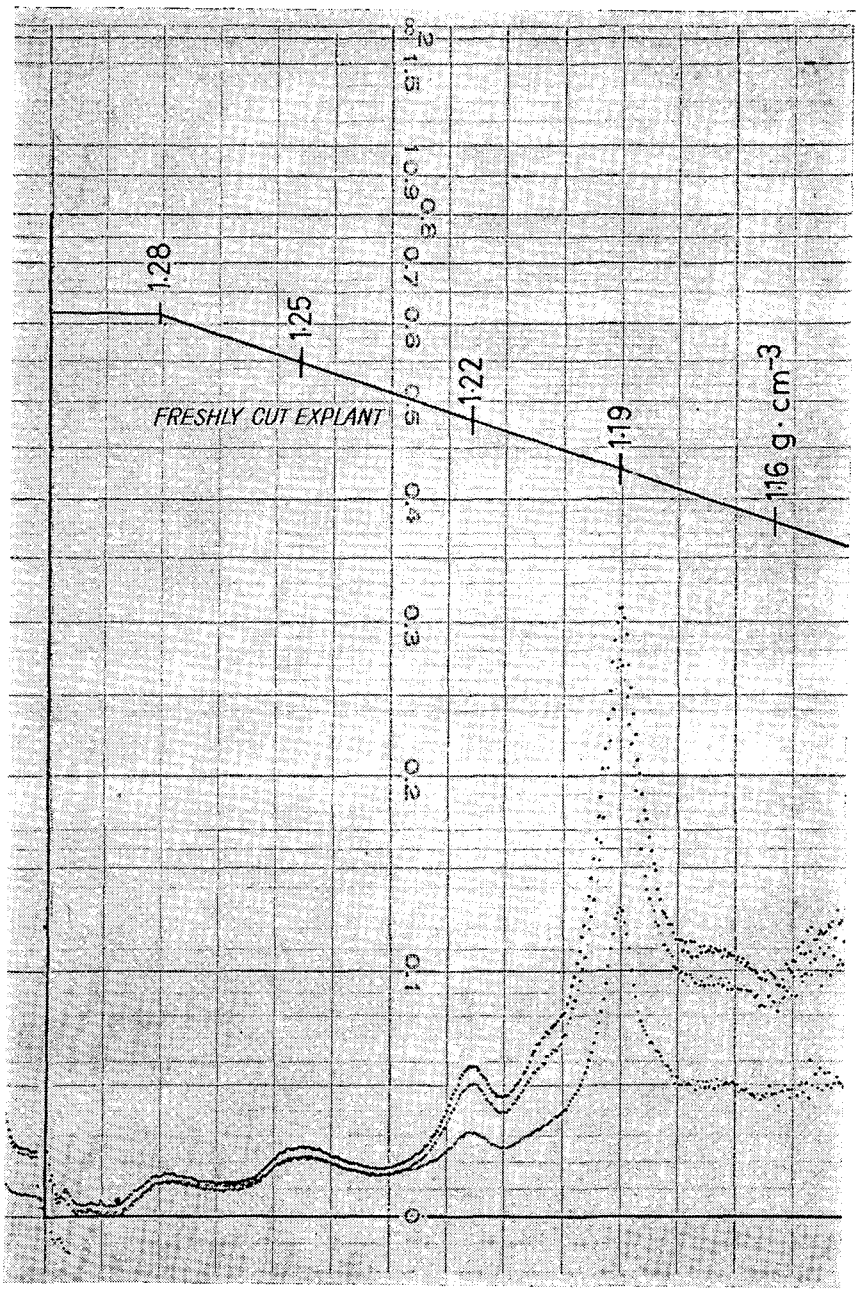

Fig. 7. Distribution of the particles of the crude mitochondrial fraction after sucrose density gradient separation. The crude mitochondrial (precipitate of 1,000×*g* to 34,000×*g*) fraction isolated from 4.0 grams of potato tuber tissue.

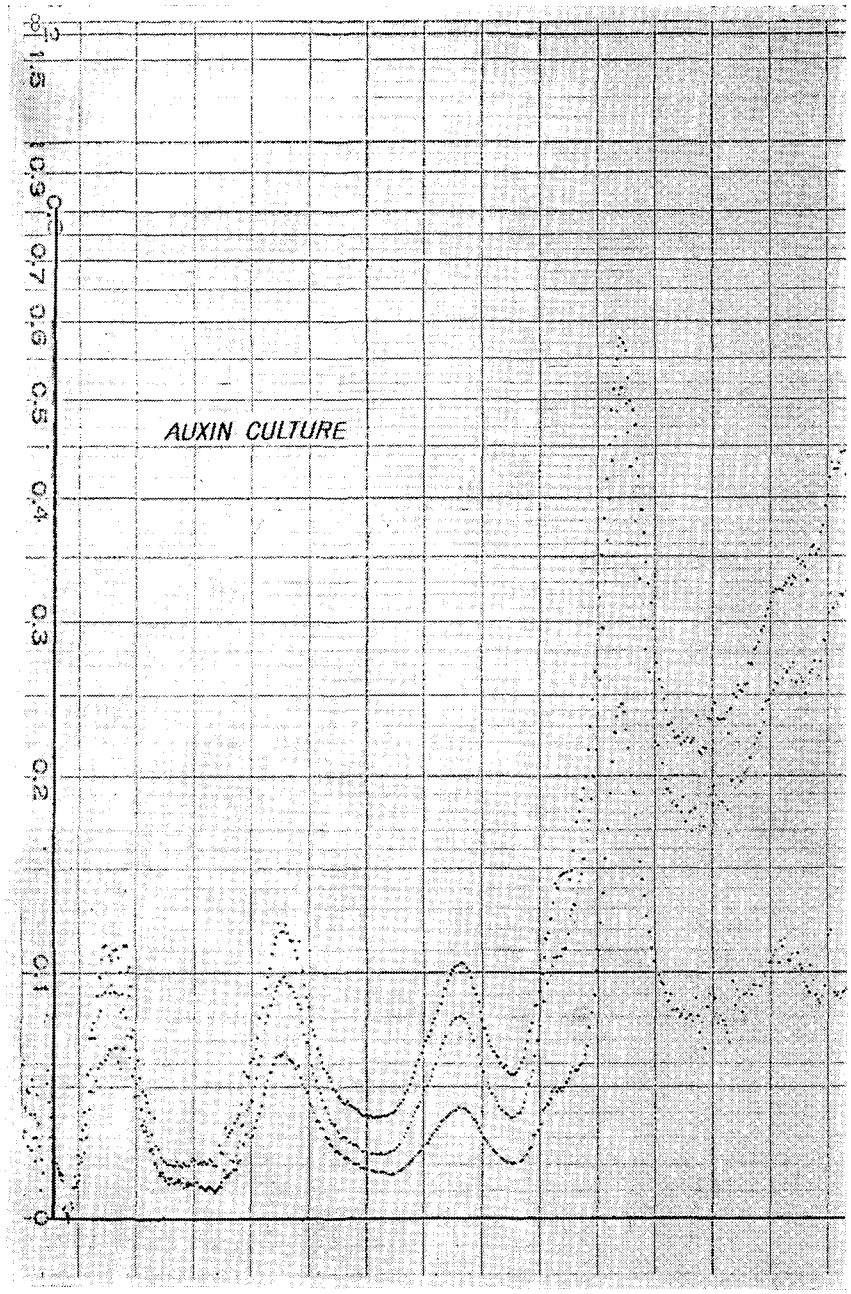

Fig. 8. Distribution of the particles isolated from auxin culture after sucrose density gradient separation. The particulate fraction was isolated from 2.6 grams of 12 explants from auxin culture.

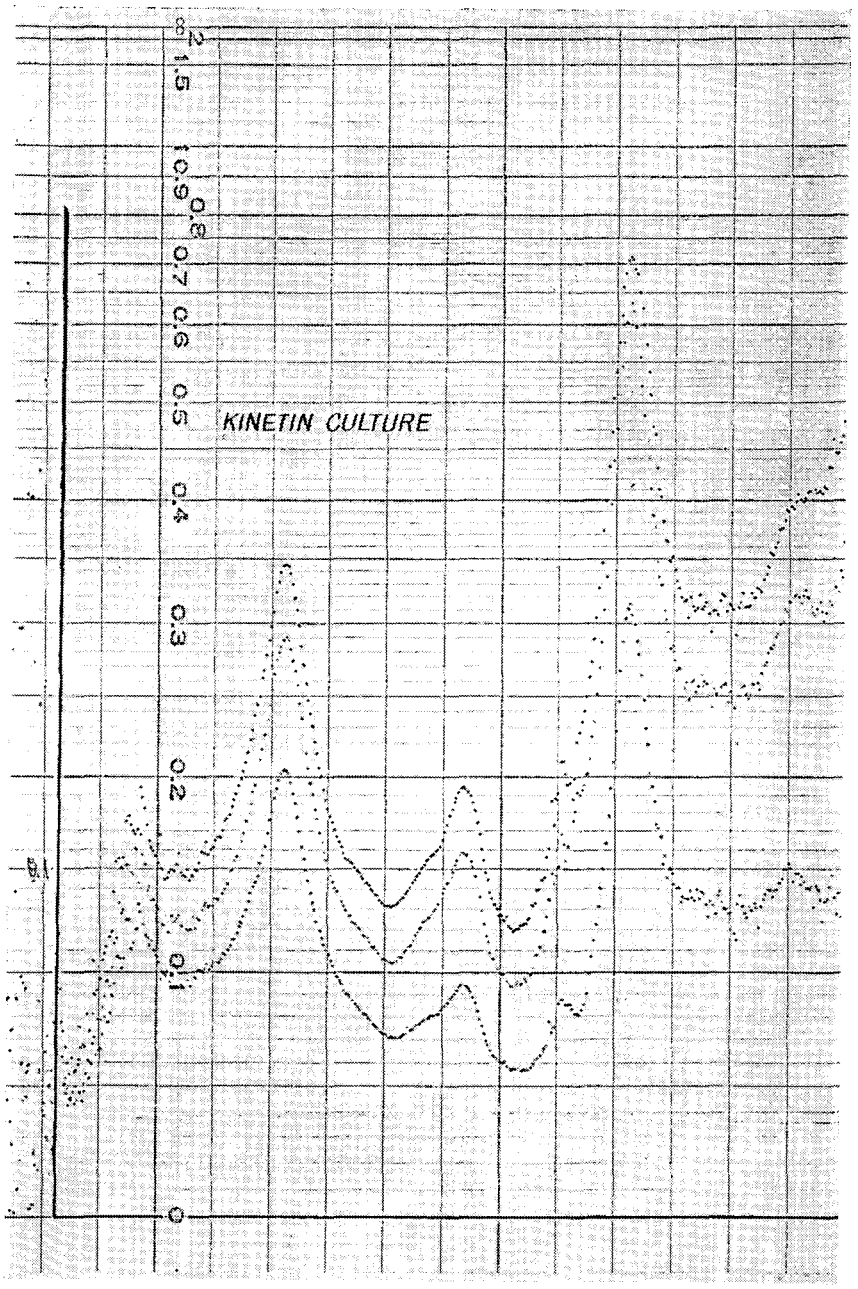

Fig. 9. Distribution of the particles isolated from kinetin culture after sucrose density gradient separation. The particulate fraction was isolated from 2.23 grams of 12 explants from kinetin culture.

stances in the crude pellet by routine works but they were sedimented in the bottom of the gradient tubes.

Figures 7, 8 and 9 illustrate the particle distribution profile for the typical gradient separations of the freshly cut potato explants, the auxin culture and the kinetin culture, respectively. In potato tuber tissue, the intrinsically intact mitochondrial band is seen in the chart of an effluent monitor (Fig. 7). While the mitochondrial band and the microbodies band were recognized to be increased in the kinetin culture (Fig. 9). The microbodies were also found to be present in the sprouted buds of potato tuber and also in the control culture in some extent.

The activities of cytochrome C oxidase and glycolate oxidase were assayed after the fractionation of the gradient, both of which were the marker enzymes of mitochonria and microbodies, respectively. Figure 10 shows the distribution of cytochrome C oxidase and glycolate oxidase activities in the

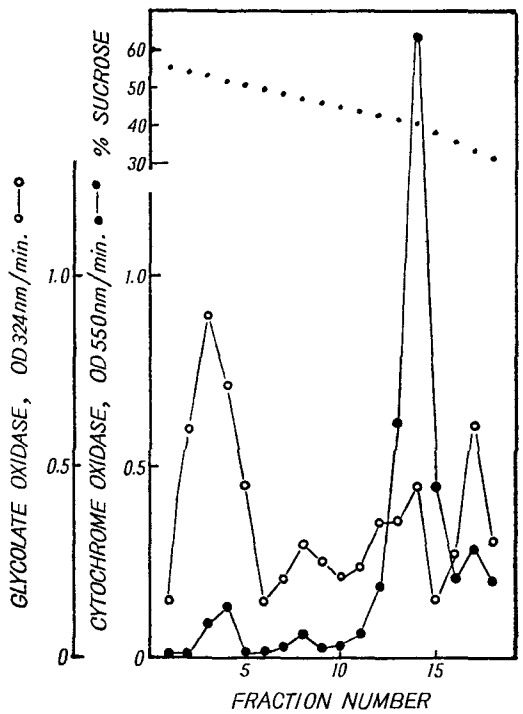

Fig. 10. Distribution of cytochrome oxidase and glycolate oxidase in particle fractions isolated by sucrose density gradient centrifugation. Data in graph show 1/10th of each fraction.

TABLE 3. Citrate and malate synthesis from acetate-1-C^{14} by the particulate fractions isolated from cultured potato tissues and sprouts

Substrate Products	Auxin-culture		Kinetin-culture		Buds
	Mt	Mb	Mt	Mb	Mb
Glyoxylate					
Citrate	3020	980	2610	1460	1440
Malate	2760	4540	1740	4280	3420
Others	6100	3600	7360	5200	5520
Isocitrate					
Citrate	540	1780	640	720	1560
Malate	1520	4060	1240	4680	3600
Others	5420	4340	5500	3300	5460

Others; glycolate, glyoxylate, ketoglutarate succinate.
Additions; GSH, CoA, ATP, $MgCl_2$, 0.05 M Tris pH 7.0 and Acetate-C^{14}.
Particles; 0.5 gm FW equiv., (0.05 gm FW equiv. buds).
Mt: Mitochondrial particles.
Mb: Microbodies particles.

separated mitochondrial and microbodies fractions. The microbodies band contains 50-60 per cent of the total glycolate oxidase activity found in the original homogenate, and the mitochondrial band contains 60-70 per cent of the total cytochrome C oxidase activity detected. The reminder is in the microbodies fraction and in the supernatant. Fumarase is specifically associated with the mitochondria and is eventually absent from the microbodies.

In Table 3, malate and citrate synthetase activities by these particulate fractions isolated from the auxin- and the kinetin cultures and from the potato buds are shown. The microbodies produced more malate than citrate when glyoxylate or isocitrate was present in the reaction mixtures.

Development of microbodies

A developmental pattern of mitochondria and microbodies in the potato explants cultured with the addition of auxin and kinetin to the medium is demonstrated in Figure 11 and 12. Not only did the total quantity of these organelles as measured by protein content increase with the advancement of the cultures, the respiratory rates of individual culture was accordingly related (see also Fig. 1 and 2). In the later stage of culture, the respiratory activity of the auxin culture was reduced as biogenesis of these organelles were exhausted. Only the kinetin culture, in contrast, kept to maintain

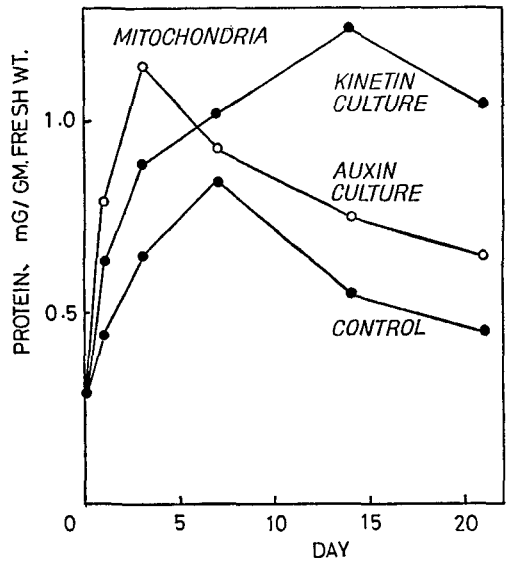

Fig. 11. Increase in mitochondrial protein content during culture perion. The culture conditions were the same as in Fig. 1.

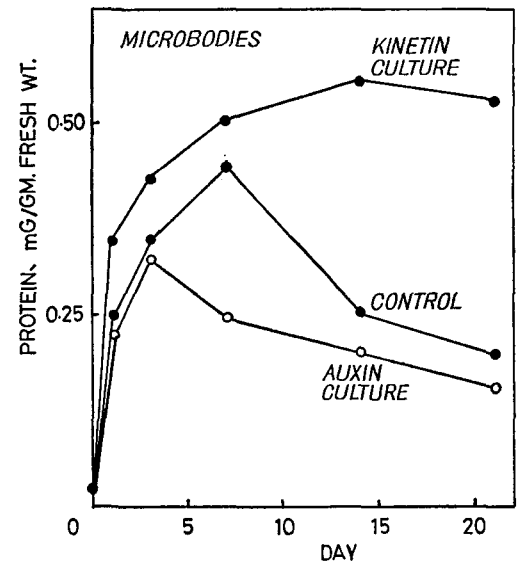

Fig. 12. Increase in microbodies protein content during culture period. The culture conditions were the same as in Fig. 1.

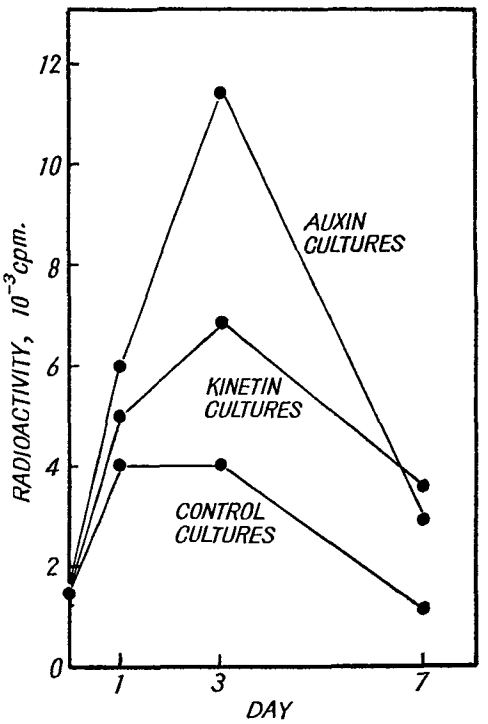

Fig. 13. Incorporation of leucine-1-^{14}C into mitochondrial protein of the cultured explants. The explants harvested were allowed to expose to leucine-^{14}C for 2hr and isolate mitochondria by the sucrose density gradient centrifugation.

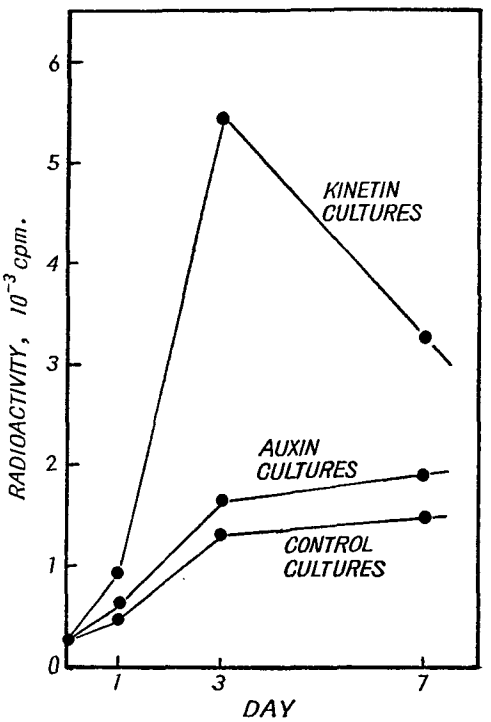

Fig. 14. Incorporation of leucine-1-^{14}C into microbodies protein of the cultured explants. The explants harvested were allowed to expose to leucine-^{14}C for 2 hr and isolate microbodies protein after the sucrose density gradient centrifugation.

the elevated levels of both organelles and the respiratory rate of the explants. From the developmental pattern of the organelles *in vivo* and leucine-1-^{14}C incorporation into these organelles shown in Figures 13 and 14, the results are suggestive that the biogenesis of microbodies in the potato explants during the culture period is probably stimulated at the time of callus initiation by the one of growth regulators, such as kinetin, added to the medium. While for mitochondrial development, auxin and kinetin were required in the explants. In Figure 15, the changes in the specific radioactivities are illustrated on the microbodies, the mitochondrial and the cytosol proteins isolated from the explants in the kinetin culture during two hour exposure of leucine-^{14}C followed by the chase of these activities of the above mentioned

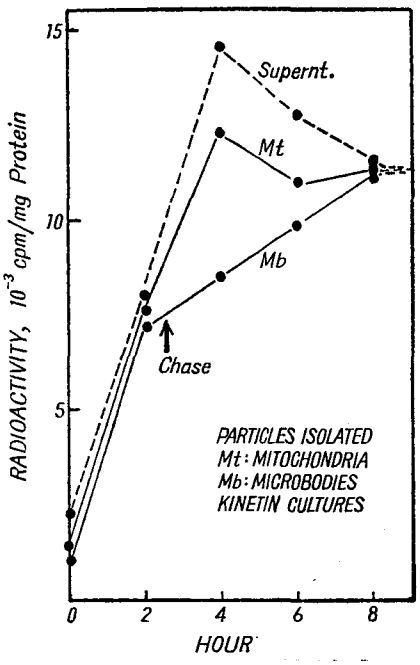

Fig. 15. The pulse-chase experiment of *l*-leucine-1-^{14}C incorporation into the proteins of mitochondria, microbodies and cytosol of potato explants in kinetin culture. The explants harvested 2 days after inoculation were allowed to expose to leucine-^{14}C for 2hr pulse followed by chasing 6 hr with 1000 fold of cold leucine. The specific radioactivities of peak fractions were determined after the sucrose density gradient centrifugation.

fractions. These organelles are different in the change of the specific activity of their protein components that the microbodies proteins are labeled independently from the labeling pattern of the mitochondria, of which the change of the specific activity is dependent on the withdrawal of leucine-^{14}C from the medium, while the microbodies are slowly labeled during the chasing period. These suggest that the origin and biogenesis of microbodies are completely different from those of mitochondria.

Discussion

The first communication in 1965 draws an attention to the presence of a new plastid like structures about 1 μ in diameter, bounded by a single membrane and containing a crystal with lattice spacings of 125-160 Å, in the bud and peripherial cells of potato tubers during dormancy. MARINOS[16]

observed that in potato peripherial cells the crystals were formed within structures bounded by a single membrane which eventually the similarity of the membrane around the crystal with the profiles of the endoplasmic reticulum raised an attractive possibility that the crystals were formed within vesicles of the endoplasmic reticulum. The crystal containing bodies were also found in tobacco culture cells[17] and in sycamore cell suspension culture[9], that the bodies was in contact with a rough surfaced endoplasmic reticulum in earlier developmental stage of culture and that these bodies with crystaloid core were a predominant feature of stationary phase cells.

The similar structures are now designated as microbodies. BEEVERS[4] and TOLBERT[26] recently reviewed the research development of structures and function in this field since original isolation of microbodies from fatty seedlings as glyoxysomes from caster bean endosperm[6,7]. and shortly after from leaves as peroxisomes[27,28]. BEEVERS[4] mentioned three types of plant microbodies *i. e.*, glyoxysomes and leaf-peroxisomes both of which recognized their functions in the cells, and the third to be isolated were those from a variety of plant tissues other than fatty seedlings and vegetable leaves. These unknown functional organelles are classified as non-specialized microbodies. The microbodies found to be present in cultured cells of potato tubers are one of non-specialized microbodies.

The preparations of microbodies from potato tuber lacked glyoxylate reductase and transaminase, both of which were the typical enzymes of leaf peroxisomes and the distinctive enzymes of glyoxysomes were missing[10]. They were few in number and comprised less than 1 per cent of the particulate portein of the parent tissue cells[4]. In contrast, the potato explants cultured in medium containing kinetin increased in the numbers of microbodies to approximately 50-fold during the initial stage of callus formation. The major enzyme constituents were apparently glycolate oxidase and catalase, and citrate and malate synthetases were yet minor activities found in the isolated microbodies. From *in vivo* studies of metabolites analysis, glycolate or malate was accumulated in the explants which were not responded to malonate[1] nor antimycin A[29].

Of the tissue slices from a dormant potato tuber, respiration proceeds at a low rate in order to provide the energy needed to maintain the differentiation potentials and metabolite concentrations which are essential to the morphological structure. But upon the potato explant cultured, the respiration rate increases because growth and differentiation of explants require large supplies of energy[12]. It is an interesting problem whether the increased respiration rate is the result of an enlargement or intensification of the

respiratory system that was already producing energy in the dormant tuber or whether, when cell division and expansion is resumed, new energy producing systems arise in addition to those of active systems.

The four respiratory systems named after their terminal oxidases can be distinguished by differences in their sensitivities to specific inhibitors and their affinity to oxygen in tissue from potato tubers[19,20,25] except that a specific inhibitor for ascorbic acid oxidase system is still unknown. Antimycin A specifically inhibits the electron transport via the cytochrome C oxidase pathway, salicylaldoxime that via the phenol oxidase system, while acryflavine specifically blocks the glycolate oxidase pathway[20].

The glutathione content of both reduced and oxidized forms in the dormant potato tuber tended to increase at the end of the dormant period, especially in cortex with an apical bud[24], but there was no rise in ascorbic acid content in the commencement of sprouting. However, it was found that cyanide always had an inhibitory effect but to a high degree after the end of dormancy, while Salicylaldoxime or Dieca severly inhibited oxygen uptake during dormancy but hardly at all during sprouting[19]. The relevalent findings led to the conclusion that the phenol oxidase pathway might be the main system for electron transport during dormancy only, and the cyanide sensitive pathway would be the main during sprouting. The phenol oxidase system was probably replaced by some flavoprotein pathway of the glycolate oxidase system provided by the much greater inhibition of oxygen uptake by acryflavine at the end of dormancy[20].

NISHIYAMA and TAGAWA also found that when the explants freshly cut from dormant tubers were incubated in water for 48 hr at 13°C, they became as sensitive to acriflavine as freshly cut explants from sporuting tubers.

Electron micrographs of potato microbodies are shown in Fig. 6. The particles are characterized as peroxisomes or glyoxysomes as containing a dense granular stroma surrounded by a single membrane. In most cases a denser area are visible within the particles. The shape of the potato microbodies appears spherical and about 0.5–1.0 μ in dia-meter. The membranes of the particles were often broken by shearing forces during the preparation process. Thus the trouble with that the functional characterization of the particles made it defficult to clarify enzyme components after the separation of the particles on sucrose density gradient.

The significance and function of microbodies from potato explants are yet unknown, but have to be carefully considered. One possibility is that these microbodies function as a site of electron transport via the glycolate

oxidase and catalase. In considering the function of leaf peroxisomes[26], glycolate is a major product of CO_2-photosynthesis. In contrast to leaf microbodies, in the potato explants a major substrate for the microbodies may also be glycolate or malate and respiration may be attributable to these microbodies and may be differentiated *in vivo* from mitochondrial system. The present conclusion is tentative and does not assign a clear function to the glycolate oxidase pathway and these microbodies.

Summary

Microbodies have been isolated from the potato explants cultured *in vitro*. By grinding explants in 0.5 M sucrose, the crude mitochondrial fraction was prepared by differential centrifugation. After sucrose density gradient centrifugation, the microbodies banded in 1.25 gcm^{-3} density were separated from the mitochondria banded in 1.19 gcm^{-3} density. The particles, 0.5–1.0 μ in diameter, contained a dense granular stroma surrounded by a single membrane. The potato microbodies contained glycolate oxidase, catalase and some malate synthetase. These microbodies increase in potato explants when cultured in White's nutrient medium supplemented with kinetin and auxin. Although a parallel development of mitochondria has been observed in the explants, upon the inoculation to the kinetin culture microbodies increase to approximately 50 fold during the initial stage of callus formation. The functions of these microbodies are discussed with a role of an electron transport system as glycolate oxidase pathway during the initial respiratory rise of the culture period related to the respiratory change during the end of the dormancy followed by the sprouting of potato tubers.

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