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RESPONSES OF MERISTEMATIC CELLS IN EXCISED TOMATO ROOT TIPS TO GINSENOSIDE Rg_1 OF *PANAX GINSENG*[1]

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Abstract

Excised tomato root tips were either cultured directly in sucrose-enriched medium to establish proliferative phase meristems or, to create transitional phase meristems, they were subjected to three days of carbohydrate starvation before transferring to the sucrose-enriched medium. The effects of ginsenoside Rg_1 of ***Panax ginseng*** on mitosis and root growth were studied in the proliferative phase meristems and on the incorporation of ^{3}H-uridine, ^{3}H-leucine and ^{3}H-thymidine in both proliferative and transitional phase meristems. Our results show that Rg_1 can increase mitosis and main root length as well as the number of lateral roots before the emergence of the secondary and tertiary roots respectively. In the proliferative phase meristems, the incorporation patterns of uridine and thymidine are similar and parallel; while in the transitional phase meristems, those of uridine and leucine are similar and parallel. Ginsenoside Rg_1 has, in general, some promoting effects on the incorporation of all 3 radioactive precursors in the proliferative phase meristems throughout the 24-hour experimental period and in the transitional phase meristems from about 6 to 24 h. It has some adverse effects on the incorporation of leucine and thymidine in the first 4 to 6 h in the transitional phase meristems. The different responses between the proliferative and transitional phase meristems to Rg_1 in the early hours of the 24-h experimental period are attributed to the difference in the cellular status between these two types of meristems.

Introduction

It has been reported previously that ginsenoside Rg_1 of *Panax ginseng* can promote DNA synthesis and cell division in cultured human lymphocytes activated by phytohemagglutinin (PHA) or Concanavalin A (Con A) (Tong and Chao, 1980) and in onion root meristematic cells (Ng and Chao, 1981). Besides DNA replication,

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the syntheses of RNA and proteins also play important roles in the cell cycle. The relationship between the DNA replication and biosyntheses of RNA and/or proteins in the cell cycle has received a great deal of attention by many investigators over the past thirty years (see Yeoman, 1981).

Excised tomato root tips can grow profusely in a suitable medium. In addition, one can alter its growth by regulating the amount of sucrose in the medium (Street and McGregor, 1952). In tissue or cell culture, omitting an essential nutrient, such as sucrose or serum, can partially synchronize the cells. Thus, it has been reported by Van't Hof (1966) and Webster and Van't Hof (1970) that by means of sucrose starvation, the meristematic cells in cultured pea root tips are arrested at either G_1 or G_2 phase; this is referred to as the stationary phase. Following the provision of sucrose, such meristems can proceed to the transitional phase, that is G_1 cells enter S phase and G_2 cells enter mitosis. They reach the proliferative phase when their cells are completely asynchronous. After the establishment of the transitional phase in the cultured pea root meristems, Webster and Van't Hof (1970) then proceeded to study the requirements of RNA and protein syntheses for DNA replication at this phase.

This paper reports the results of our studies on the responses of tomato root meristems to Rg_1 (1) on the growth and incorporation of ^{3}H-uridine (^{3}H-Urd), ^{3}H-leucine (^{3}H-Leu) and ^{3}H-thymidine (^{3}H-TdR) at the proliferative phase created by culturing root tips in the sucrose-enriched medium directly after excision and (2) on the incorporation of these three radioactive precursors at the transitional phase established by transferring the 3-day starved root tips to sucrose-enriched medium.

Materials and Methods

Tomato seeds of the variety *Roma VF* (Raci Sementi S.P.A., Italy) were sterilized and allowed to germinate on moist paper in petri dishes in an incubator at 25°C. Unless otherwise stated, 10-mm root tips were excised for culture when the roots were about 20 mm long. Modified White's medium (Thomas and Davey, 1975), supplemented with 100 mg/l casamino acids, was used throughout the experiments. The concentration of the sucrose was 1.5%. The ginsenoside Rg_1 was kindly provided by Professor S. Shibata of the Meiji College of Pharmacy, Tokyo.

Mitosis

Six concentrations of Rg_1, ranging from 0 to 8 mg/l, were used in this study. For each concentration, 5 root tips were cultured in a 125-ml flask with 25 ml of medium. After 24 h, roots were fixed in acetic alcohol (1:3) and Feulgen squash preparations were made for each root. For the determination of the mitotic index (MI=number of dividing cells/total number of cells counted $\times$1000), two root tips

were selected and used for each concentration and over 1,000 cells were scored in each root.

Root Length and Number of Lateral Roots

The 15-mm root tips were cultured individually in 250-ml flasks with 50 ml of medium. The same concentrations of Rg_1 mentioned above were used in this study. The length of each root was measured after 48 h and the number of lateral roots per root was counted on the 5th and 6th day. Duplicate samples were used for each treatment.

Incorporation of Radioactive Precursors

In this study, two sets of experiments were carried out. In the first set, root tips were cultured directly in sucrose-enriched medium to trace the incorporation of radioactive precursors into meristems in the proliferative phase over a 24-h period. In the second set, excised root tips were incubated first in a medium without sucrose for 72 h to establish a stationary phase in the meristem. In this connection, experiments were carried out first to determine the effect of carbohydrate starvation on mitosis. Excised root tips were cultured in the medium without sucrose for 72 h. Mitosis was found to be completely inhibited in these root tips. Following the provision of sucrose in the medium, the MI reached 43 after 8 h, and it reached 50 after 24 h. Thus, sucrose starvation for 72 h inhibited mitosis completely and cell division resumed after the provision of the sucrose. The incorporation of radioactive presursors were studied in the transitional phase meristems also for a 24-h period.

In both cases, roots were treated with Rg_1 at the concentration of 4 mg/l for 0, 2, 4, 6, 8, 10 or 24 h. For each treatment, 15 root tips were cultured in a 125-ml flask with 25 ml of medium. Roots were labeled with 1 μCi/ml of ^{3}H-Leu (sp. act. 1.0 Ci/mM), ^{3}H-Urd (sp. act. 5.0 Ci/mM) or ^{3}H-TdR (sp. act. 2.0 Ci/mM) (Radiochemical Centre, Amersham, England) for the last 2 h of treatment. All experiments were started at 9:00 a. m. For the 0-h treatment, root tips were labeled from 7:00–9:00 a. m. After the uptake of radioactive presursors, root tips were fixed in the neutral formalin or absolute ethanol at 4°C for 3 h and the fresh weight of the roots was determined before homogenization. The procedures of extraction and counting of the radioactivity of the proteins and RNA were mainly those of De Leo *et al.* (1973) and DNA those of Bloch *et al.* (1967). The percentage of incorporation was taken as the ratio of counts per minute (cpm) incorporated versus the cpm total uptake (De Leo *et al.*, 1973) per one mg fresh root weight.

Results

Mitosis

The results present in Fig. 1 indicate that Rg_1 can promote mitosis (MI) in

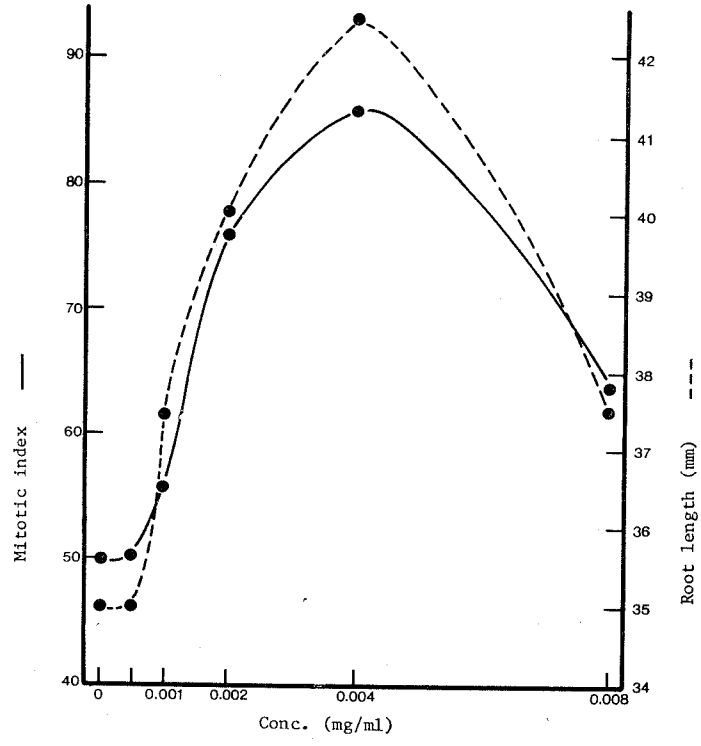

Fig. 1. Effects of Rg_1 on mitosis (24-hour treatment) and length (48-hour treatment) of cultured tomato root tips (initial length 15 mm).

the meristematic cells of cultured tomato root tips. At the concentration of 4 mg/l, the MI was about 75% higher than that of the control. The curve shows the dose-response relationship between the MI and the concentrations of Rg_1.

Root Length and Number of Lateral Roots

Rg_1 at the concentrations of 2 and 4 mg/l promoted root length significantly after two days of culture (Fig. 1). There was high correlation between the MI and root length after one and two days of culture respectively (Fig. 1), although these data were taken from different culture. The emergence of the lateral roots on the 3rd day of culture alters this correlation.

Counting of the number of lateral roots per root was done on the 5th and 6th day of culture before the emergence of the tertiary roots. On the average, the number of laterials of the Rg_1-treated roots was doubled as compared with that of the control (Fig. 2).

Incorporation of the Radioactive Precursors

In the root tips cultured in the sucrose-enriched medium directly after excision,

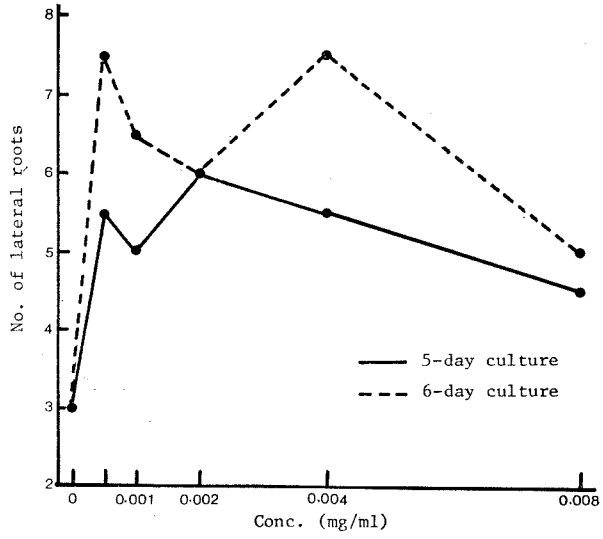

Fig. 2. Effects of Rg_1 on the No. of tomato lateral roots after 5 and 6 days of culture in White's medium.

the percentages of incorporation of ^{3}H-Urd and ^{3}H-TdR were around 50 and 60 respectively for the first 10 h of the experiments (Figs. 3 and 4). Thereafter they leveled off to about 25% and 36% respectively at the last hour (24 h). Thus, uridine and thymidine incorporation patterns are similar and parallel within the 24-h experimental period. The incorporation of ^{3}H-Leu, however, was quite different. It stayed around 55% throughout the 24-h period, although a slightly lower percentage was recorded from 2 to 8 h (Fig. 5). The percentages of incorporation of the three radioactive precursors were higher in the Rg_1-treated roots than in the controls, but significant increases were found only in the uridine incorporation at 2 and 4 h, and in the thymidine incorporation at 2, 6 and 10 h. The results may indicate that this ginsenoside can slightly promote the biosyntheses of RNA, proteins and DNA in the tomato root meristems at the proliferative phase.

On the other hand, in root tips transferred to the sucrose-enriched medium after 3-day starvation, the incorporation patterns of ^{3}H-Urd and ^{3}H-Leu were, in general, similar and parallel. Their percentages of incorporation increased greatly from the lowest level (9.0% in ^{3}H-Urd and 18.8% in ^{3}H-Leu) at 0 h to around 33% and 45% respectively within the first 4 h, thereafter both remained steady at this high level to the last hour of the experiment (Figs. 6 and 7). The percentage of incorporation of ^{3}H-TdR, however, increased very slowly in the first 6 h of the experiment (Fig. 8). It maintained this pace in the control from 6 to 24 h. A much greater increase in the incorporation of ^{3}H-TdR was observed in the Rg_1-treated roots from 6 to 24 h. Thus, the incorporation of ^{3}H-TdR was about 23% higher at 8 h, 88% at 10 h and

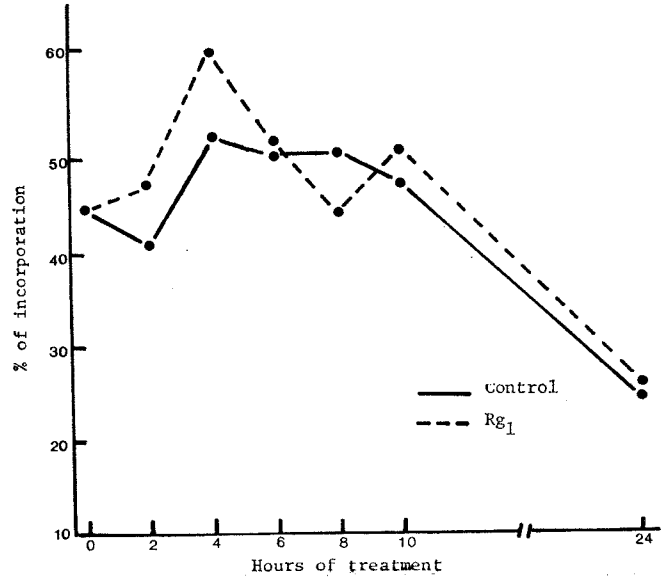

Fig. 3. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-uridine in cultured tomato root tips.

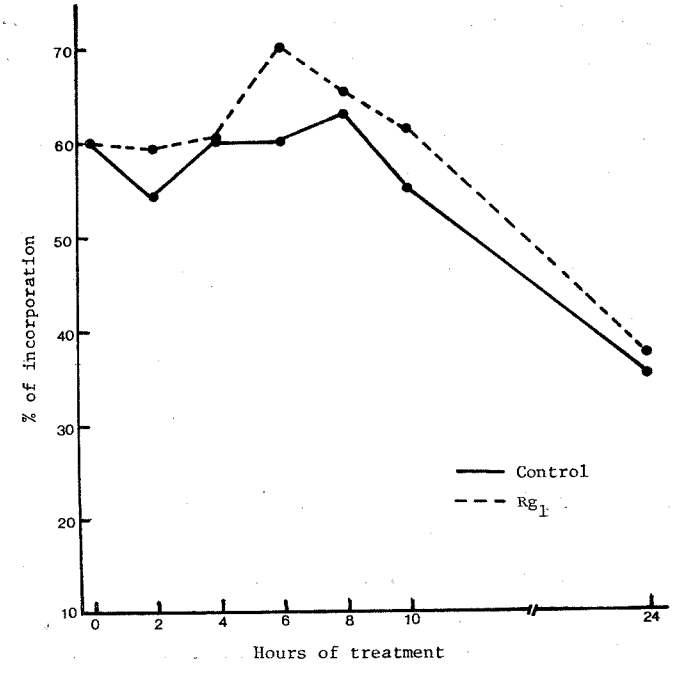

Fig. 4. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-thymidine in cultured tomato root tips.

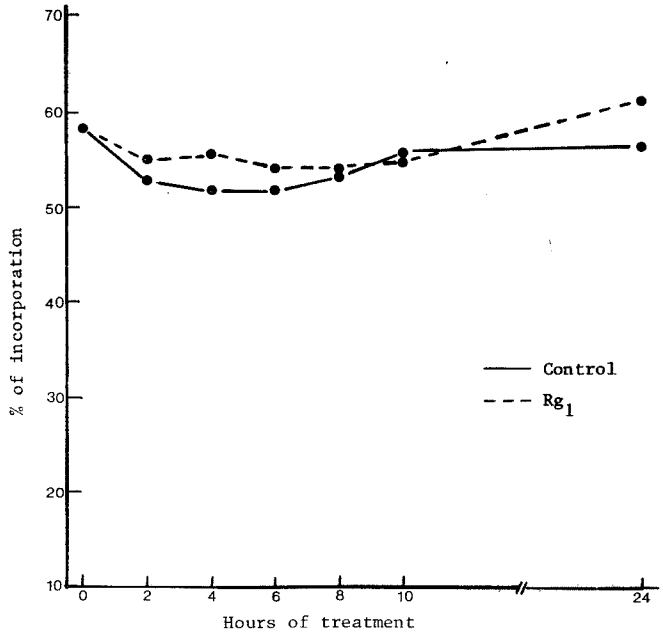

Fig. 5. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-leucine in cultured tomato root tips.

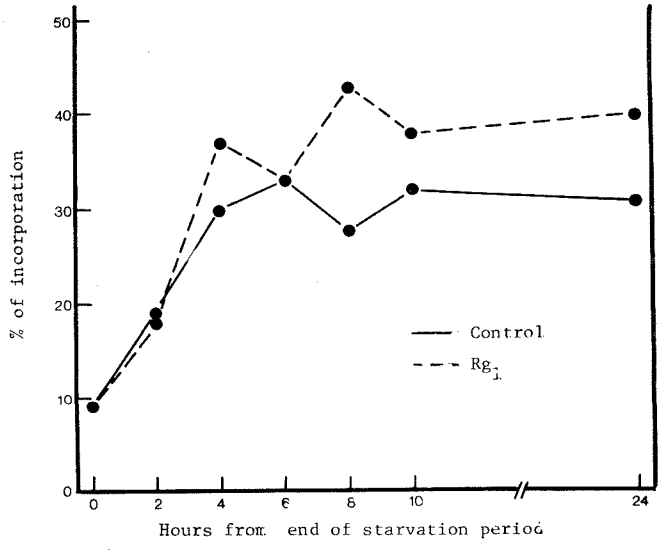

Fig. 6. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-uridine in cultured tomato root tips after starvation of sucrose for 3 days.

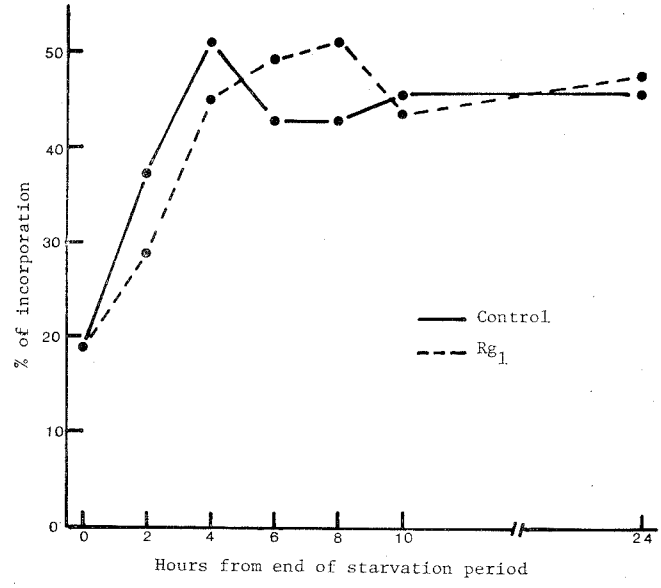

Fig. 7. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-leucine in cultured tomato root tips after starvation of sucrose for 3 days.

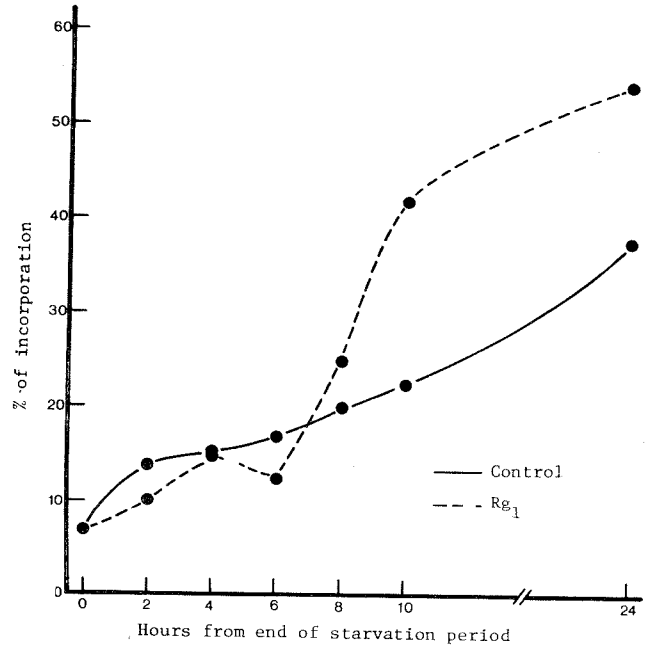

Fig. 8. Time course effect of Rg_1 (4 mg/l) on the incorporation of ^{3}H-thymidine in cultured tomato root tips after starvation of sucrose for 3 days.

50% at 24 h in the Rg_1-treated roots than in the controls (Fig. 8). The significant increase of the percentage of uridine incorporation was also observed from 4 to 24 h (Fig. 6) and of leucine incorporation from 6 to 8 h in the Rg_1-treated roots (Fig. 7). However, the percentages of leucine incorporation at 2 and 4 h (Fig. 7) and of thymidine at 2 and 6 h (Fig. 8) ware lower in the Rg_1-treated roots than in the control ones. Thus, a general conclusion is that in the transitional phase meristems, Rg_1 can promote uridine incorporation throughout the 24-h period and it also promotes leucine and thymidine incorporation at the later hours of the experiment (6–8 h in leucine and 8–24 in thymidine). In the early hours of the experiment, Rg_1 has some adverse effects on leucine (2–4 h) and thymidine (2–6 h) incorporations.

Discussion

The results of this study confirm our previous reports that Rg_1 can promote mitosis and DNA replication (Tong and Chao, 1980; Ng and Chao, 1981). It is interesting to note that the most effective concentration of Rg_1 in promoting mitosis in both onion and tomato root tip cells is at 4 mg/l. Our results also reveal that the response curves of MI and root length determined after 24- and 48-h treatments respectively are very similar (Fig. 1), indicating that in the first two days of culture, there is high correlation between the rate of root growth and the rate of cell division.

The number of laterals per root determined on the 5th and 6th day of inoculation showed that Rg_1 can increase the number of laterals in the cultured tomato roots (Fig. 2). Lateral roots are endogenous in origin, being initiated mainly from the mature cells of the pericycle and stimulated by auxins (Esau, 1977). Butcher and Street (1960) and Blakely *et al.* (1972) reported that both gibberallic acid and I-naphthalene acetic acid can increase the number of lateral roots in the cultured tomato roots, especially when the concentration of the sucrose in the medium is low (about 0.75%). The promoting effect of Rg_1 on the number of laterals in our study indicates that this ginsenoside may have auxin-like action which can stimulate more potential initials in the pericycle to divide and to form laterals.

In general, the results of our study indicate that Rg_1 can enhance the incorporation of all 3 radioactive precursors. However, in the early hours of our experiments, some different responses to this ginsenoside were observed between the meristems at the proliferative phase and those at the transitional phase. In the former, some positive effects of Rg_1 on the incorporation of all 3 precursors were observed while in the latter some negative effects on the incorporation of ^{3}H-Leu and ^{3}H-TdR were recorded. This difference may be attributed to the difference in the status of cells in these two types of meristems.

As mentioned in the previous section, in our starved tomato root tips, cell

division in the meristematic cells was found to be completely inhibited, and in our autoradiographs made by labeling the DNA with ^{3}H-TdR, no labeled interphase meristematic cells were observed for the first 4 h after the transfer. Thus, in accordance with the hypothesis of Gelfant (1962, 1963) and the observations of Webster and Vant' Hof (1970), these meristematic cells are also assumed to be arrested either at G_1 or G_2 phase and are at the stationary phase. Following the transfer, they will enter the transitional phase while the meristematic cells in the tomato root tips cultured directly into the sucrose-enriched medium after excision are at the proliferative phase. These two types of cells must be different in certain respects. This is reflected in the different incorporation patterns of the three radioactive precursors. In the meristems at the transitional phase, incorporations of both ^{3}H-Leu and ^{3}H-Urd increased rapidly from 0 to 6 h, then they were at a constant high rate to 24 h. Their patterns of incorporation were similar (Figs. 6 and 7). This implies that, at this stage, no influence between protein and RNA syntheses occures. The ^{3}H-TdR incorporation, however, increased very slowly from 0 to 6 h following the transfer (Fig. 8). While in the meristems at the proliferative phase, incorporation patterns of uridine and thymidine were similar (Figs. 3 and 4) but that of leurine was different (Fig. 5).

As for the relationship between the DNA replication and RNA and protein syntheses in the early transitional stage, Webster and Van't Hof (1970) found that in pea roots, increased protein synthesis is required for the initiation of DNA synthesis and mitosis, and increased RNA synthesis is not needed initially for the progression of the cell cycle. In the study of the early protein, RNA and DNA syntheses in the meristematic cells of the primary roots of germinating broad beans, Jakob and Bovey (1969) reached the same conclusion that some protein synthesis is necessary for the initiation of DNA replication.

Our results of incorporation study indicate that at the early transitional phase, initiation of DNA replication may also depend on some protein molecules, since in the root tips following the provision of sucrose, Rg_1 has some inhibitory effects on both ^{3}H-Leu and ^{3}H-TdR incorporation but not on ^{3}H-Urd. In addition, in the meristems at the proliferative phase, the patterns of ^{3}H-Urd and ^{3}H-TdR incorporation are similar and parallel, indicating in that they may be no direct influence on each other. This is also true in the first 50 h period in the primary roots of the germinating broad beans (Jakob and Bovey, 1969; Jakob, 1972).

Different effects of Rg_1 on the incorporation of ^{3}H-Leu and ^{3}H-TdR between the meristems at the early transitional phase and those at the proliferative phase also indicate that the action of this ginsenoside may depend on certain cellular substance(s) which are lacking in the former but present in the latter. In this connection, it should be mentioned that we have already reported that Rg_1 can promote DNA replication and mitosis in the cultured human lymphocytes activated

by PHA or Con A but it cannot stimulate division in resting lymphocytes (Tong and Chao, 1980).

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培養蕃茄根尖生長組織對人參皂甙 Rg_1 之反應的研究

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蕃茄幼苗之根尖，以下列二種方法培養：(1)根尖切下後，直接培養於含有蔗糖之培養液內，使其生長組織處於增殖狀態。(2)切下之根尖，置於無蔗糖之培養液內三天後，再移植於有蔗糖之培養液內，使其生長組織在過渡狀態。所用之人參皂甙 Rg_1，係由白參中提取，以之分別處理增殖期與過渡期之生長組織，而測定其對有絲分裂，根之生長，與去氧核醣核酸等三種物質合成之影響，結果簡述如下：

1. Rg_1 有促進有絲分裂與增加根的長度與側根數之效用。
2. 在24小時實驗過程中，Rg_1 能促進增殖期分生組織內蛋白質，核醣核酸與去氧核醣核酸的合成。
3. 此人參皂甙亦能促進過渡期生長組織內蛋白質等三種物質之合成，但在實驗過程第6至24小時期內。在實驗初期之6小時內，Rg_1 對蛋白質與去氧核醣核酸的合成，稍有抑制作用。由此推斷蛋白質與去氧核醣核酸之合成，可能有密切的關係。
4. Rg_1 在實驗初期對二種生長組織內蛋白質與去氧核醣核酸合成作用的差異，推測是由於二種生長組織細胞的某種含有物有所不同之故。