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DISTRIBUTION OF PEROXIDASE IN REGENERATING ROOT SEGMENTS OF *TARAXACUM OFFICINALE* WEB.

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Abstract

Qualitative and quantitative measurements of the activity of peroxidase was carried out in the regenerating and non-regenerating parts of *Taraxacum officinale* Web., root cuttings. High peroxidase activity was found in the differentiating tissues as compared to the non-regenerating ones. A relationship between the peroxidase activity and the formation of new tissues was observed.

Introduction

In the past no attempts were made to evaluate the differences in the level of enzymes in the regenerating and non-regenerating parts of the root cutting. However, various enzymes have been assayed only in the regenerating or growing primary roots by a number of workers (Mehrlick, 1931; Ball & Hale, 1934; Dore, 1954; Galston & Dalberg, 1954; Van Fleet, 1947, 59; Surrey, 1956).

During regeneration of *Taraxacum* root segments, proteins and nucleic acids increased much more in the regenerating portions as compared to the non-regenerating ones (Khan, 1972a). Although there was no significant quantitative difference in protein levels at the two ends of the cuttings, (Proximal and Distal) it was though that there might be some qualititative differences in view of the anatomical differences between the two ends. The present study was, therefore, undertaken to study the enzyme peroxidase qualitatively as well as quantitatively in regenerating and non-regenerating tissues of *Taraxacum* root segments. The main purpose of this study was to see if there was any correlation between the enzyme activity and the differentiating and un-differentiating tissues.

Materials and Methods

Root pieces of *Taraxacum officinale* Web., 2 cm long and 7-10 mm diameter, were excised and grown as described earlier (Khan, 1972a). Duplicate samples, of five roots each, were taken every 12 hr upto a regenerating period of 84 hrs. Two consecutive slices, 2.1 mm in thickness, were cut from both the proximal and distal ends of the

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root pieces using the specially designed razor cutter (Khan, 1972b). The proximal end slices PA, PB and the distal-end slices DA, DB were used for the extraction and estimation of peroxidase. Anatomical studies of regenerating *Taraxacum* root segments (details to be published elsewhere) has shown that regenerating tissues are located about 100 μm beneath the cut ends. Thus segments PA and DA contains the regenerating tissues while PB and DB contained non-regenerating tissues.

In another experiment, effect of IAA application on the activity of peroxidase in regenerating root segments was studied. The root segments were placed upright, on their proximal end, in Petri dishes containing washed sand irrigated with 20 ml of distilled water, the dishes being maintained in a humid box at 25°C. After a regeneration period of 12 hr, only the outer slices (PA and DA) were excised and used for the extraction and estimation of peroxidase. Each sample was of five roots and triplicate estimations were made. After sampling at 12 hr stage, half of the remaining roots were transferred to other Petri dishes containing sand moistened with 20 ml of 100 ppm IAA solution while the rest remained in the original dishes. Samples of control and IAA-treated (Proximal end treated only) roots were then taken for enzyme assay at 24, 36, 60 and 84 hr of regeneration.

Enzyme extraction:

The slices in which enzymes were to be estimated were crushed in an ice-chilled mortar together with 5 ml of cold (5°C) 0.02 M Tris-HCl buffer, pH 7.4, using acid washed sand. The slurry so obtained was filtered through eight layers of muslin and the filterate centrifuged at 4000 x g for 20 min at 5°C. The supernatent solution was decanted and kept surrounded by ice chips and immediately used for the estimation of the enzyme peroxidase.

Estimation of peroxidase:

Peroxidase was estimated quantitatively by the method of David & Murray (1965). Crude enzyme extract, 0.2 ml, was mixed with 2.5 ml of 0.2 M phosphate buffer, pH 7.0, and 0.2 ml of 1% (W/V) guaicol solution in a 1 cm wide silica cell, the later solution being absent from the reference cell. The cells were left for several minutes to equilibriate in a temperature-controlled SP- 800 spectrophotometer at 30°C and then 0.1 ml of 0.3% H_2O_2 solution was added and stirred quickly. Maximum absorption was found at 470 nm and the optical density (O.D.) increased linearly for only 40 seconds (Fig. 1). The specific activity of the enzyme was measured as the increase in O.D. at 470 nm per mg protein per 30 second.

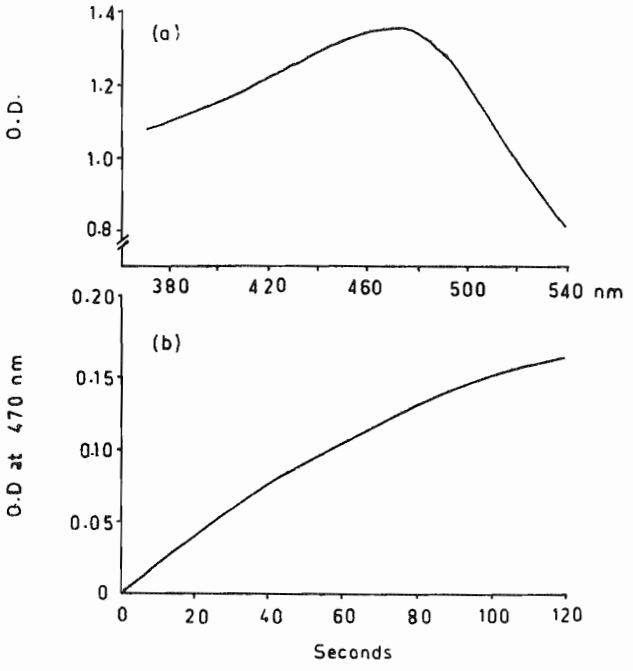

Fig. 1. Peroxidase estimation by guiacol method.
(a) Absorption curve.
(b) Progress of colour reaction.

Temperature and pH optimas of this enzyme was also studied. The results presented in Fig. 2 indicates the optimum temperature to be 30°C and pH as 7.0.

Histochemical localization of peroxidase:

Peroxidase was localized in the tissues by the method of Issac & Winch (1947) as described by Jensen (1962).

After appropriate periods of regeneration, 2 cm long, 7-10 mm diameter root pieces were cut transversely into half to obtain a proximal and a distal piece. Each piece was then immediately sectioned with a freezing microtome and 100 μm thick sections obtained. After a brief rinse in water, the sections were incubated for 2 min

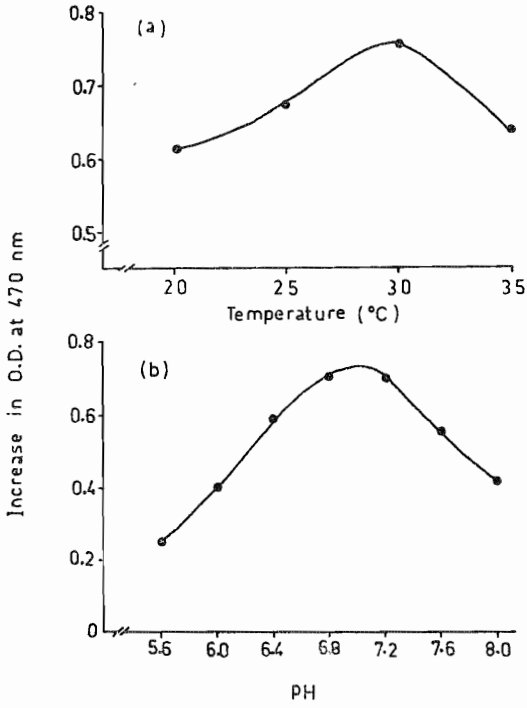

Fig. 2. Peroxidase estimation by guiacol method.
(a) Temperature optimum curve.
(b) pH optimum curve.

at 22°C in a reaction mixture of 0.1 M phosphate buffer, pH 7.0; 0.03% H_2O_2 and 0.01 M benzidine. Sections pretreated with 0.1% sodium azide solution for 20 min and in hot water (80°C) for 2 min were used as controls. Following incubation, the sections were transferred into Petri dishes, washed three times with cold water and left for 30 min at 5°C. This chilling stopped the reaction and removed air-bubbles from the sections. The sections were examined under the microscope as 1% glycerine mounts and representative ones were photographed within a few hours.

Results

Specific activity of the enzyme peroxidase in the regenerating and non-regenerating segments of the proximal and distal end of the root segments are presented in Fig. 3. Each value represents the average of three replicates. It was found that within

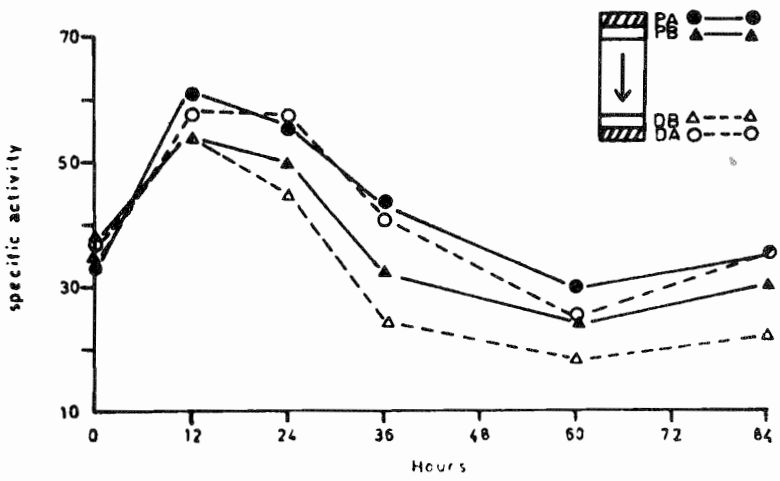

Fig. 3. Specific activity of peroxidase in root segments of *Taraxacum* during regeneration.
●——● PA, Regenerating proximal segment;
▲——▲ PB, Non-regenerating proximal segment;
o——o DA, Regenerating distal segment;
△- - - -△ DB, Non-regenerating distal segment.

12 hr of regeneration, peroxidase activity rose to a maximum level in both the regenerating and non-regenerating segments. This was followed by a gradual decline of peroxidase activity with the increase in regeneration time reaching either to the original value (zero hour) or even below. It was also observed that peroxidase level always remained at a lower level in non-regenerating segments as compared to the regenerating ones.

Effect of IAA application on the activity of peroxidase in regenerating root segments was also studied. The results presented in Fig. 4 indicate that after 12 hr of IAA application the level of peroxidase activity in the proximal segment (PA) remained unchanged whereas it increased to about 20% above the control in the distal segment (DA). At 36 & 60 hr, the enzyme remained at a higher level in the treated segments as compared to control. However at 84 hr stage, no significant difference was found among the treated and untreated segments.

Histochemical localization of peroxidase in the intact root revealed its abundance in the sieve tubes, some in the metaxylem and almost none in the phloem parenchy-

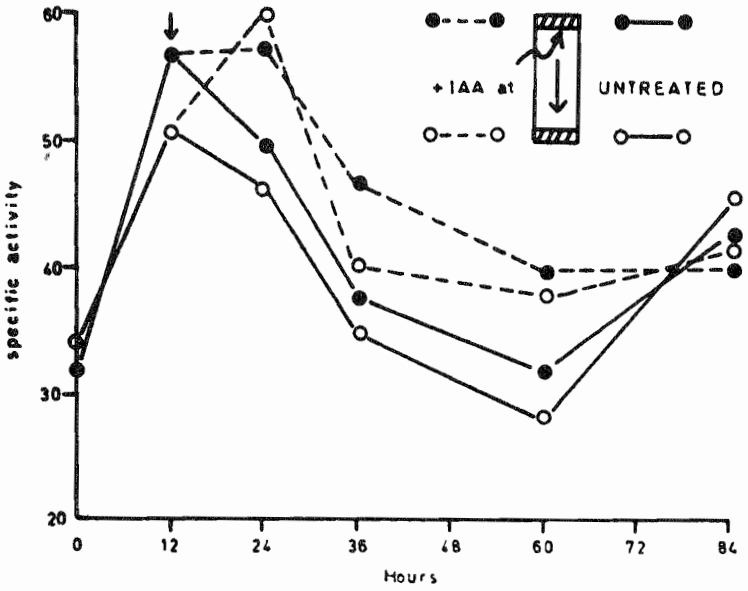

Fig. 4. Effect of IAA treatment on the activity of the enzyme peroxidase during regeneration of *Taraxacum* root segments. Arrow indicates the time and site of IAA (1000 ppm) application.
●———● Regenerating proximal segment (untreated);
o———o Non-regenerating distal segment (untreated);
● - - - - ● Regenerating proximal segment after the application of IAA.
o -- -- -- o Non-regenerating distal segment after the application of IAA.

ma (Plate I, Fig. 1). However, in regenerating roots the enzyme appeared in the phloem parenchyma as stained granules in the cytoplasm (Plate I, Fig. 2).

In roots just after excision (O hr), peroxidase was found only in the sieve elements and the xylem at both the proximal and distal ends of the cuttings (Plate I, Figs. 3 & 4). After 12 hr of regeneration, however, heavily stained cells were found at both the ends just below the cut surface and adjacent to the xylem (Plate II, Figs. 5 & 6). At 24, 36 and 60 hr, the intensity of the benzidine stain was comparatively less than at 12 hr stage (Plate II, Figs. 7 & 8; Plate III, Figs. 9-12).

At 15th day of regeneration, when shoots were well developed while roots were not, peroxidase was found to be present mainly in the cells adjacent to epidermis and

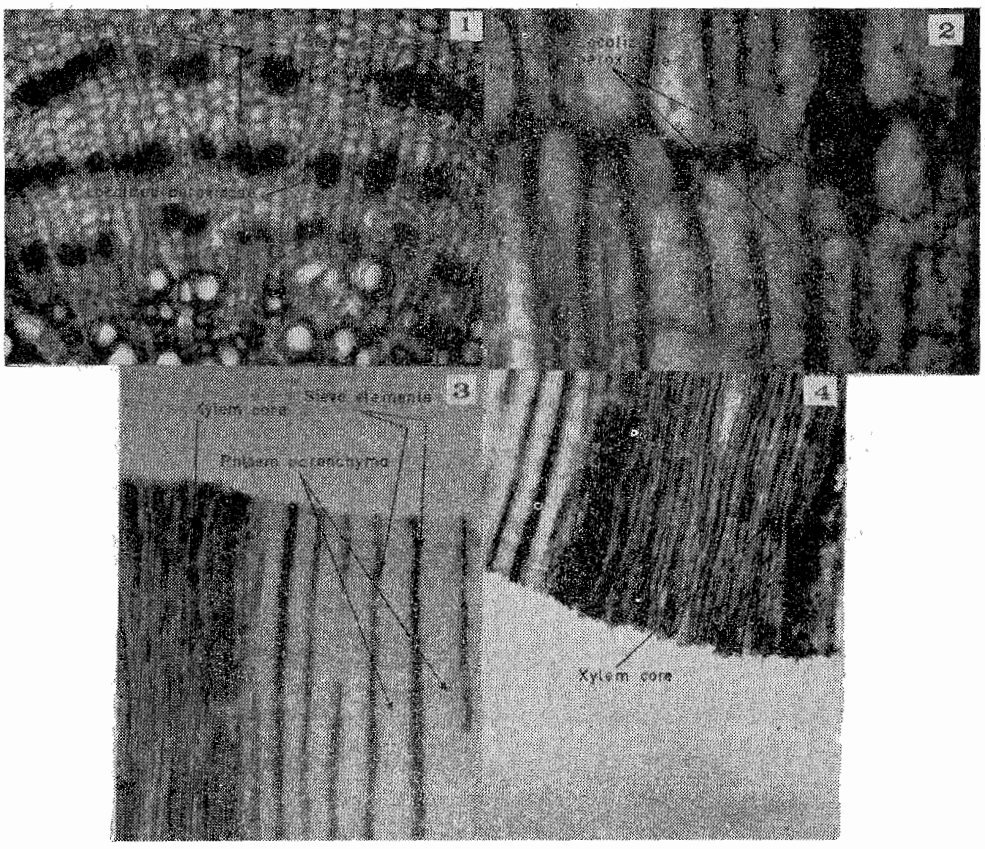

PLATE I.

Fig. 1. T.S. of *Taraxacum* root showing the localization of the enzyme peroxidase (Dark stained portions).

Fig. 2. L.S. of *Taraxacum* root showing the localization of the enzyme peroxidase in the phloem parenchyma.

Fig. 3. Proximal end of the root segment (L.S.) at the start of regeneration showing the presence of peroxidase in sieve elements and Xylem core.

Fig. 4. Distal end of the root segment (L.S.) at the start of regeneration.

xylem tissues (Plate IV, Figs. 13 & 14). At 21st day, the intensity of peroxidase increased once again in the proximal and distal ends of the cutting (Plate IV, Figs. 15 & 16). At this time the distal end, showed a clear line of peroxidase activity in cells beneath the cut surface, probably indicating that these are the most actively

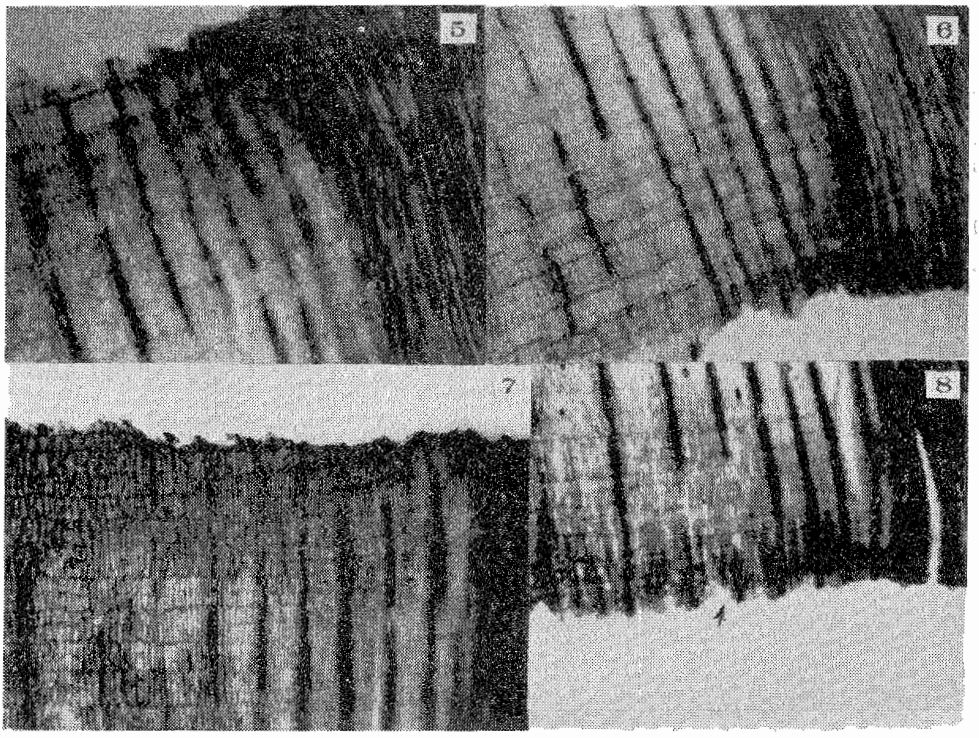

PLATE II. L.S. of *Taraxacum* root segments showing the localized peroxidase in the proximal end (5) and distal end (6) at 12 hr. The same at 24 hr. of regeneration proximal end (7) distal and (8).

dividing cells of the callus. The mature xylem nodule showed less activity of the enzyme, as observed by the intensity of the benzidine stain, compared to 15 day stage where the nodule was immature.

Discussion

High enzyme activity at 12 hr of regeneration is presumably the result of injury of the tissues. It is interesting to note that by this time the metabolic changes have also started (details to be published elsewhere). This might suggest that the initiation of the metabolic changes resulted due to increased enzyme activity. The decline of the enzyme activity, after 12 hr of regeneration, to the original level might suggest the lowering of the endogenous auxin. This was investigated by applying a high concentartion of IAA to the proximal end of the cutting 12 hr after the start of regeneration.

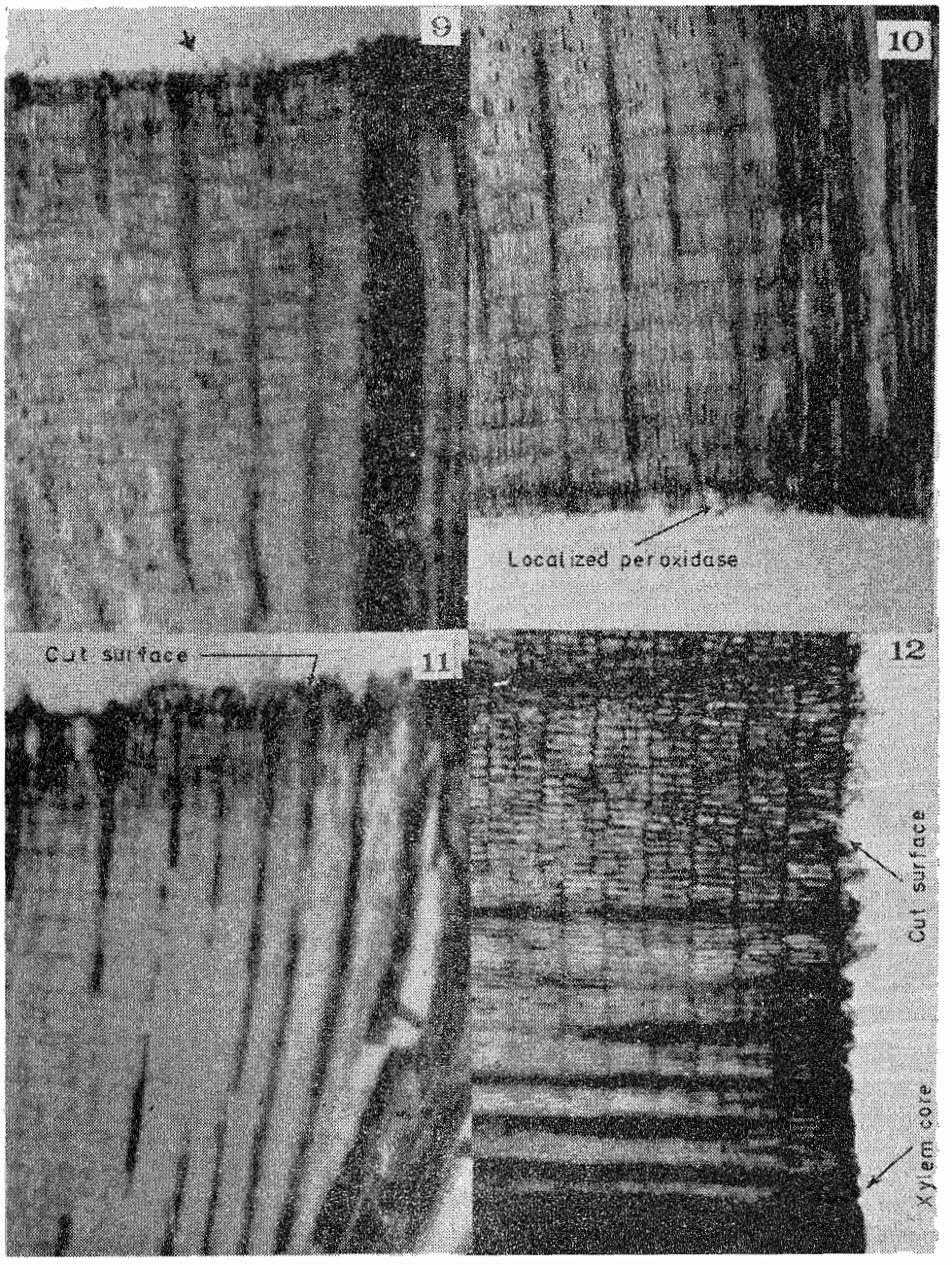

PLATE-III. L.S. of *Taraxacum* root segment showing the localized peroxidase in the proximal end (9) and distal end (10) at 36 hr. The same at 60 hr. of regeneration proximal end (11) distal end (12).

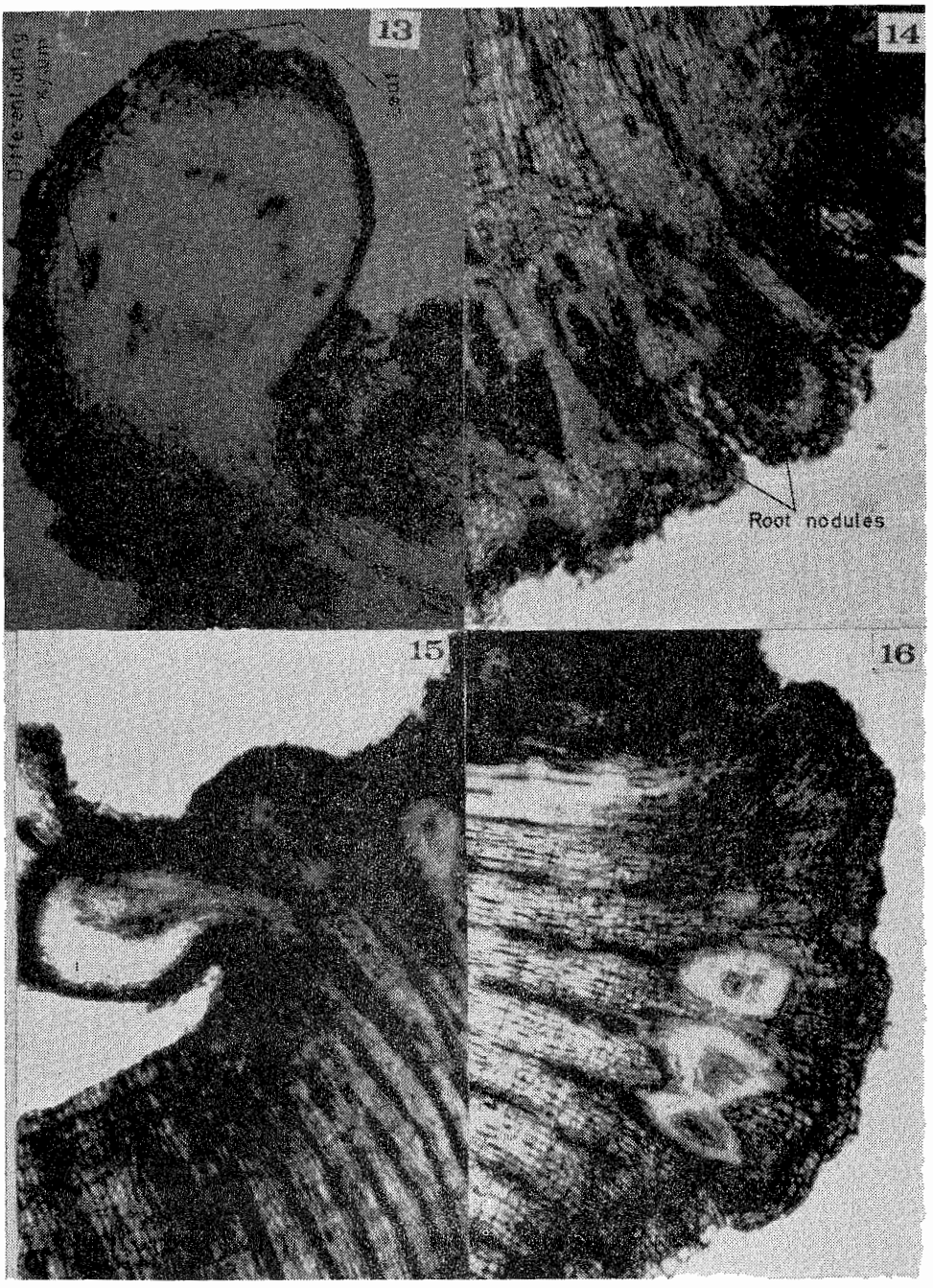

PLATE-IV. L.S. of *Taraxacum* root showing the localized peroxidase in the proximal end (13) and distal end (14) at 15th day. The same at 21st day of regeneration, provisonal end (15) distal end (16).

Satchuthananthavale (1966) found that when the proximal end of the *Taraxacum* root segments were treated with 100 ppm IAA, roots were formed instead of shoots. This concentration of IAA was chosen since it clearly led to an exceptionally marked morphogenetic response. The ratio of the enzyme activity in the proximal to the distal end slice of the treated roots was not substantially different to that of the un-treated roots even though the proximal end had been in contact with IAA solution (Fig. 4.). This suggests that the decline in the activity of the enzyme peroxidase after 12 hr of regeneration is not due to the deficiency of the endogenous auxin.

The presence of the enzyme peroxidase at later stages of regeneration (15 and 21 days) appears to be associated with the onset of the meristematic activity which led to the formation of the callus. Surrey (1956) and Van Fleet (1947, 52) also found peroxidase to develop at an early stage in the origin of new meristems in several plant parts. Van Fleet (1959) observed that the development of peroxidase activity in advance of or accompanying cell division declined after the division phase was over. He attributed the decline of the enzyme to the increase of phenols, napthols and phenolase.

It may be concluded that there exists a relationship between the peroxidase activity of the regenerating tissues and the formation of new tissues.

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