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UNPUBLISHED PRELIMINARY DATA

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Semi-annual Status Report covering the period 1 October 1963 through 31 March 1964 on the research program entitled

GROWTH OF A PLANT TISSUE CULTURE IN THE GRAVITY-FREE STATE

Following the experiments reported by Skoog and Miller (1957) it was proposed to grow as regenerative outgrowths from tobacco stem segments the following categories of organs or tissues:

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1. Normal shoots (stems with their attached leaves)
2. Roots.
3. Callus.

Since these organs or tissues constitute the normal repertoire of the vegetative growth process in a normal plant under the attraction of gravity, the experiments are considered important as a means of ascertaining the effect of the gravity-free state upon the processes of regeneration and normal growth.

The results of Skoog and Miller (1957) have been checked by continuing experiments; as these authors indicated, the various manifestations of growth (production of the organs or tissues) generally occurs near the end of a period of time approximately 40 days after the start of the experiments.

The main problem of the present researches has been the acceleration of the growth responses; instead of obtaining these responses at the end of 40 days, it is essential to obtain them at the end of 20 days.

By utilizing an improved mineral supply in the culture medium for tobacco (Murashige and Skoog, 1962) it has been possible to obtain the various required growth responses from stem segments. The accompanying photographs show the tobacco stem segments with stem plus leaves, apical callus, stem plus leaves and basal roots, roots alone, numerous buds without basal roots.

All of these pictured results have been achieved in light at ca. 10 foot candles. It has not been possible to achieve these results in total darkness. However, since this apparent light requirement is of such small magnitude, it should not be too difficult to modify the experimental set-up in the satellite to accommodate it.

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Many supplementary experiments have been carried out on the callus tissue, since it was the main point of interest by the writer in entering this research program for the biosatellite. One of the most remarkable results achieved in this program has been from the growth of the stem pieces in callus-producing culture medium rotated constantly on a klinostat. The last three photographs show a representative result of such growth. Generally, the growth of the callus tissue on the klinostate is from 2 to 3 times as great (fresh weight) as that on the same medium not rotated. Other important differences are that the klinostat callus is compact, while that from stationary medium is loose, friable; the klinostat callus has such a high concentration of chlorophyll in the plastids that the whole appears light green, while that from stationary callus has so little chlorophyll that it is almost white (cf. figs. 2 and 5).

These differences between the klinostat-grown and stationary-grown callus are being investigated by light- and electron microscopy, and by determinations of dry weight, and contents of protein, carbohydrate and fat.

Brain (1939) suggested that the rotation of a shoot system during growth on a klinostat brings about a constatn redistribution of the auxin and causes a profound modification of the external and internal growth patterns. No studies have yet been made to determine what growth factors are being redistributed by the constant reorientation of the callus by the klinostat, but the responses so far noted indicate others of more fundamental aspect than auxin.

References

Brain, E. D. 1939. Studies in the effects of prolonged rotation of plants on a horizontal klinostat. II. Anatomical structure. New Phytol. 38: 240-256.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Skoog, F. and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. In: The Biological Action of Growth Substances. Symposia of the Society for Experimental Biology, No. XI, pp. 118-131.

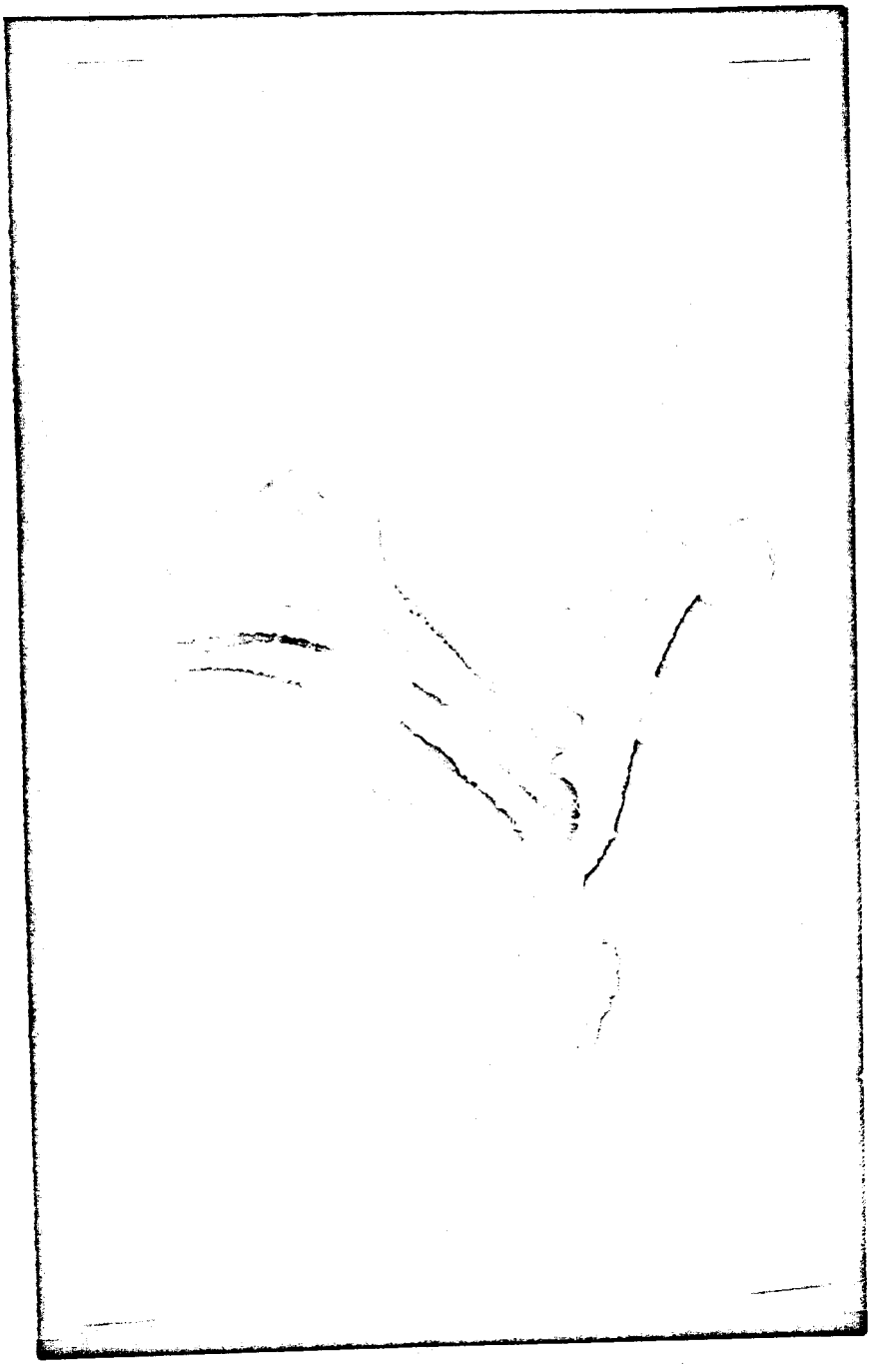

Figure 1. Tobacco stem segment with a single large leafy shoot twenty days after inoculation on Murashige, 20 g./l. sucrose and 0.2 mg./l. kinetin. The callus is of limited amount and quickly forms one or more fast-growing buds. 1X.

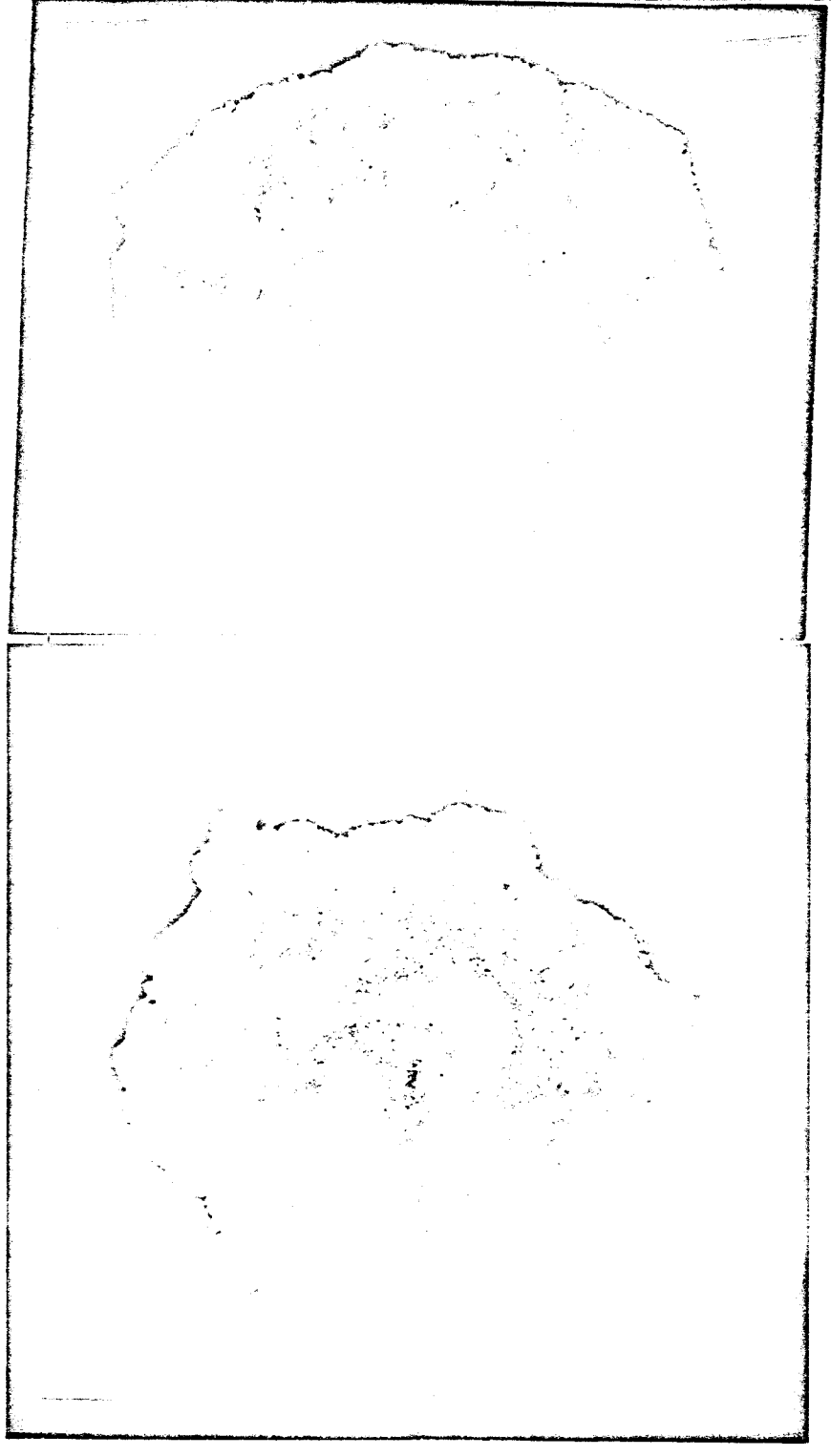

Figure 2. Callus of tobacco stem with large terminal callus twenty days after inoculation on Murashige, 20 g./l. sucrose, 0.2 mg./l. kinetin and 2 mg./l. indoleacetic acid. Top is the lateral view, and bottom is view from above. Note the friable nature, and the almost white color of this callus. 1X.

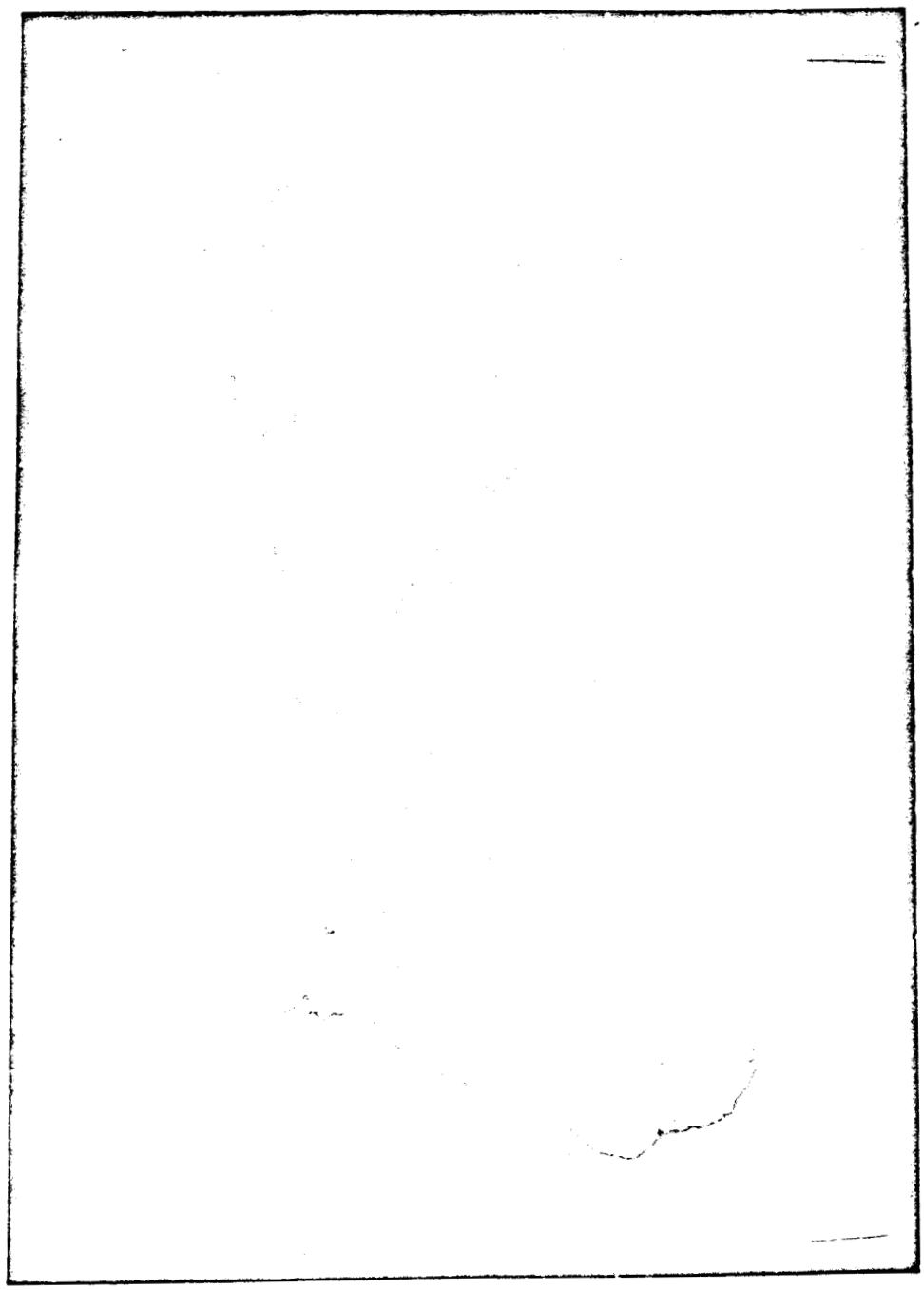

Figure 3. Tobacco stem segment with a conspicuous callus and a large upright leafy shoot twenty days after inoculation on Murashige, 20 g./l sucrose, 0.2 mg./l. kinetin, 2 mg./l. IAA, and KH_2PO_4 at 400 mg./l. A small adventitious root is seen at the base of the shoot. The phosphate and/or the auxin greatly stimulates growth in length of the shoot as compared with figure 1.

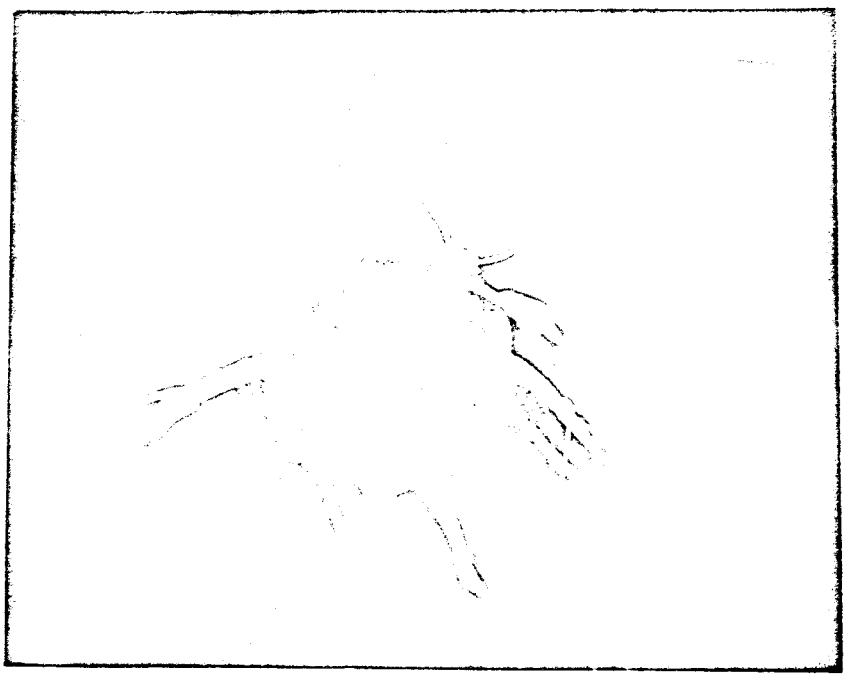

Figure 4. Adventitious roots from tip of stem segment of tobacco inoculated 20 days previously on Murashige, 20 g./l. sucrose, 0.02 mg./l. kinetin, and 2 mg./l. IAA. The relatively high concentration of kinetin with respect to the one of IAA has brought forth a prolific rooting response, with no formation of buds. IX.

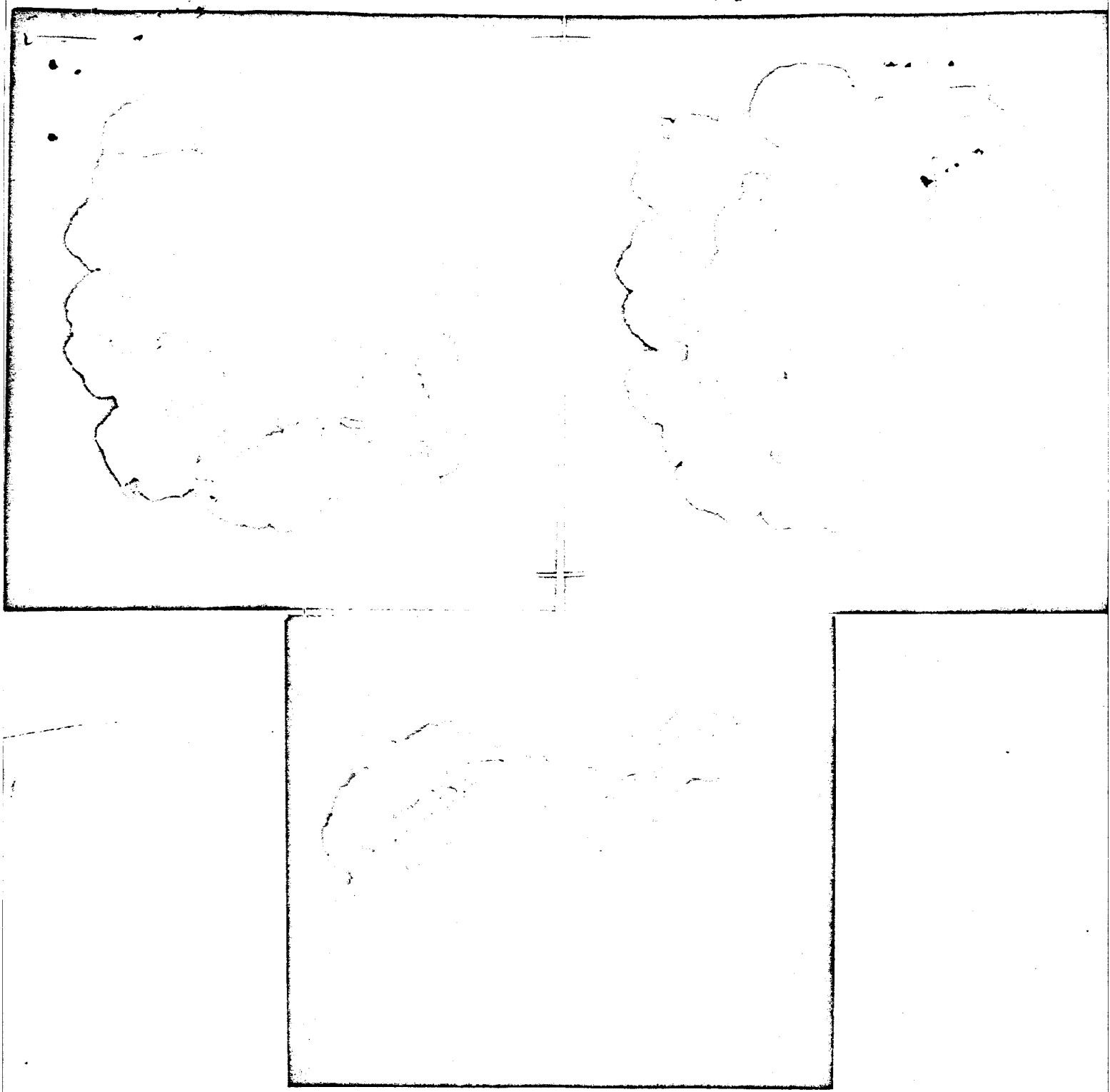

Figure 5. Calli grown from tobacco stem pieces inoculated 20 days previously on Murashige, 20 g./l. sucrose, 150 ml./l coconut milk, 0.5 mg./l. 2,4-D. Upper left is top view, showing original diameter of stem inoculum, upper right is bottom view that was in contact with agar medium, and lower photo is lateral view of the callus. This callus is of firm consistency, and light green in color; compare with fig. 2. 1X.