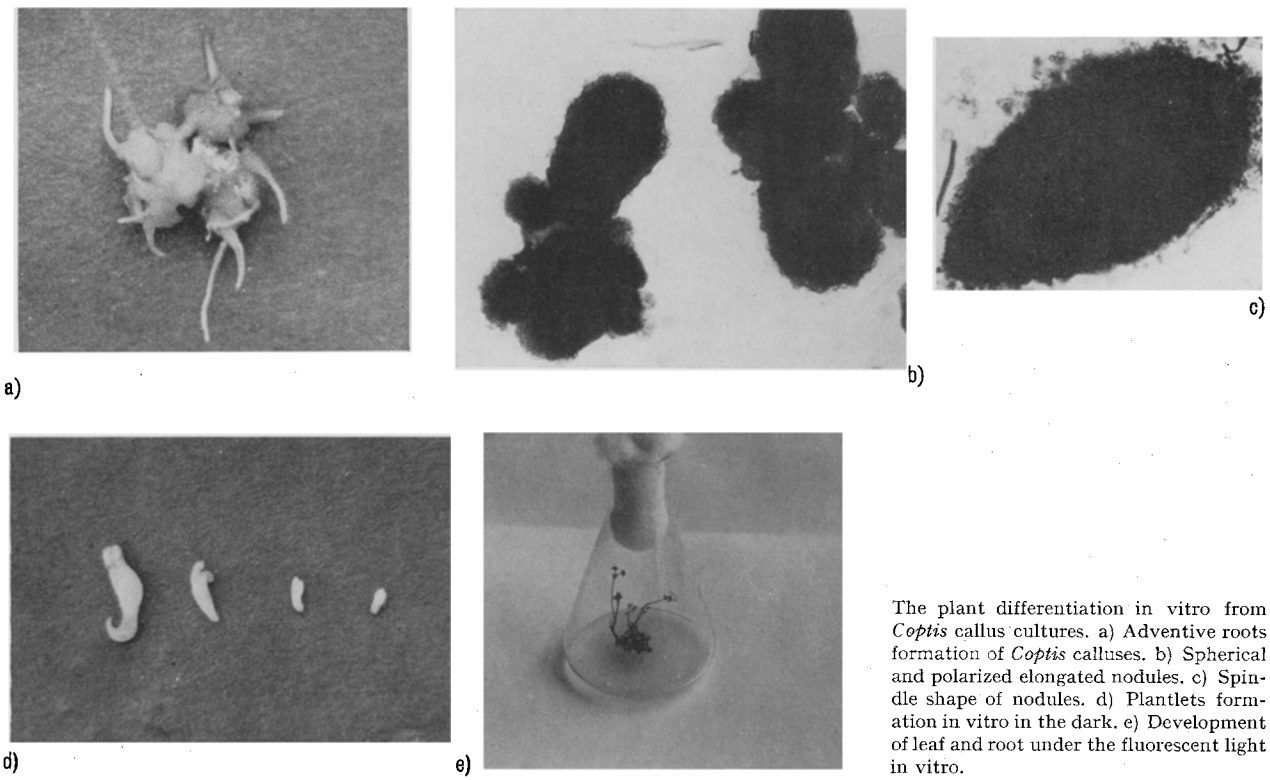
The Differentiation of *Coptis* Plants in vitro from Callus Cultures[1]

The berberine-bearing rhizome of *Coptis japonica* Makino var. *dissecta* (Yatabe) Nakai (Seriba-woren in Japanese) belonging to Ranunculaceae is widely used, together with berberine hydrochloride, for an all round tonic and stomachic in Japan. Since the rhizome of *Coptis* plant grows very slowly, it must be of pharmaceutical significance to investigate callus cultures of this plant for berberine production. This report describes a successful isolation of *Coptis* callus with the berberine synthesizing activity retained and also the differentiation of whole plants from the callus cultures.

Petiole section (5 mm) of *Coptis* plant, surface-sterilized for 10 min in 10% hypochlorite solution, was aseptically cultured on MURASHIGE and SKOOG's agar medium[2] (minus glycine) containing 1 mg/l 2,4-dichlorophenoxyacetic acid and 0.1 mg/l kinetin as growth regulators in the dark at 26 °C. Friable yellow callus was isolated from the cut end of sections in August, 1968, and subcultured in the dark every 6 weeks on medium of the same composition as described above.

Adventive yellow roots (Figure 1a) and occasionally numerous pale yellow nodules (0.5–3 mm in diameter) were observed when the calluses were cultured for more than 6 weeks. The nodules initiated in the friable callus as spherical masses of tissue, about 40–80 μm in diameter, under microscopical observation and increased in size (Figure 1b). In some cases, they showed polarized elongation to the spindle shape (Figure 1c). Some of them developed into root-bearing nodules which showed no signs of a shoot apex, or into nodules bearing both shoot and root (Figure 1d). The latter was transferred to the MURASHIGE and SKOOG's medium containing none of the growth regulators and cultured under fluorescent lamp (40 W) for about 1 month at 25–30 °C. From the nodules, green leaves expanded and yellow roots elongated (Figure 1e). The differentiated plantlets were transferred to soil in pots. They grew vigorously to about 10 cm in height.

In methanol extract of *Coptis* callus cultured for 6 weeks, at least 7 Dragendorff's reagent- and iodoplatinate reagent-positive spots (alkaloids A-G) were separated by thin-layer chromatography [Silica gel G, $CHCl_3$-CH_3OH (3:1)]. Among them hydrochloride of alkaloid B was isolated as yellow needles, m.p. 192–193 °C (decomp.), and identical with authentic samples of berberine hydrochloride by UV-, IR- and mass spectra[3].



The plant differentiation in vitro from *Coptis* callus cultures. a) Adventive roots formation of *Coptis* calluses. b) Spherical and polarized elongated nodules. c) Spindle shape of nodules. d) Plantlets formation in vitro in the dark. e) Development of leaf and root under the fluorescent light in vitro.

Zusammenfassung. Kalluskulturen aus Petiolastücken von *Coptis japonica* (Ranunculaceae) synthetisieren das Alkaloid Berberin. Unter geeigneten Bedingungen werden auf den Kallusstücken ganze Pflanzen herangebildet.

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[1] Part XIII in the series 'Studies on Plant Tissue Cultures'. Part XII, see reference [3].

[2] T. MURASHIGE and F. SKOOG, Physiologia Pl. *15*, 473 (1962).

[3] T. FURUYA, K. SYŌNO and A. IKUTA, Phytochemistry, in press.

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