was minimal transient arthritis in 1 of 10 rats with a 24-day interval and a significantly milder degree of arthritis in 8 of 10 rats when the interval was 35 days. Wax D adjuvant produced arthritis in 8 of 10 rats which had received LPS from *Salmonella typhimurium* 40 days earlier, but in most of these the disease was again very mild.

Substances inoculated in adjuvant which neither caused any apparent disease nor protected against later induction of wax D adjuvant arthritis were (i) LPS from *Escherichia coli* 0111 (Eichenberger) and *Brucella abortus*, (ii) waxes A, B, and C (Lederer) from the Canetti human strain and wax D (Lederer) from the Marmorek bovine strain of *Mycobacterium tuberculosis*, and (iii) fungi: *Coccidioides immitis* and *Trichophyton mentagrophytes*. There is some indication that LPS from *B. abortus* enhanced the severity of subsequently induced wax D arthritis.

Table 1 shows that lipopolysaccharides which were protective in adjuvant were not protective when inoculated in saline. Also, the arthritogenic character of wax D in adjuvant gave way to protection when it was inoculated in saline. These responses seem to imply that there exist three different degrees of stimulation of a common host reaction: (i) the clinically undetectable: *Salmonella typhosa* and *Staphylococcus* A LPS in saline; (ii) clinically protective: *Salmonella typhosa* and *Staphylococcus* A LPS in adjuvant, wax D in saline; and (iii) arthritogenic: wax D in adjuvant. The differences appear to be linked to the presence or absence of the synergistic action of the oily vehicle.

The factors which underlie the arthritogenic potential of wax D on the one hand and the protective action of lipopolysaccharides on the other are not as yet clear. It is possible that the basic mechanisms are qualitatively similar but quantitatively diverse, so that (i) the reticuloendothelial system may be blockaded by slow release of LPS from the depot site, rendering it unresponsive to the subsequent action of wax D, (ii) that this complex system, or others such as the lymphoidal elements, acquires a state of "resistance" that they cannot achieve if wax D is given initially, or (iii) other cellular elements, especially the periarticular, connective tissue components, are directly affected by LPS or wax D.

Whatever the exact nature of the protective action of lipopolysaccharides in the host may be, it seems apparent from our experiments on adjuvant arthritis in rats, that administration of some bacterial lipopolysaccharides before the disease process is initiated can repress, counteract, or blockade the heightened tissue reactivity induced by microbacterial adjuvant (*7*) and hence prevent arthritis from developing (*12*).

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18 January 1962

Tissue Cultures of a Cactus

Abstract. Tissue cultures have been established from stems of *Trichocereus spachianus* (Riccob.) for the purpose of studying alkaloid biosynthesis in cactus tissue. On a basal inorganic medium supplemented with glucose, coconut milk, and 2,4-dichlorophenoxyacetic acid, three distinct types of callus are initiated. One is greenish, compact, and slow-growing; another is firm and yellowish, with a moderate growth rate; the third is very friable and rapid-growing. The growth habit remains constant for a given clone in successive subcultures.

Several species of *Trichocereus*, a genus of South American columnar cacti, synthesize large amounts of candicine and related alkaloids (*1*). Although the occurrence of these compounds in cacti has been established, their biosynthesis has not been studied. This is easily understood, for the slow-growing *Trichocereus* species are not very suitable for laboratory experiments. It therefore seemed desirable to obtain a rapidly growing tissue that could be used to study this biosynthesis. This report describes a method for producing and maintaining callus tissue from stems of *T. spachianus* (Riccob.).

There have been few reports of successful *in vitro* culture of cactus tissue. Nitsch was able to grow *Opuntia* crown gall on medium containing tomato juice, but he did not describe cultures derived from normal tissue (*2*). King attempted to establish cultures from stems, leaves, roots, stamens, pistils, and ovules of several species (*3*). Ovules yielded the most rapidly growing and easily subcultured callus. The other organs (except for roots, which did not grow at all) showed a tendency to form callus, but permanent cultures rarely could be established from them.

The cacti used in my work were 3- to 5-year-old *Trichocereus spachianus* plants (*4*). Tissue cylinders were removed aseptically with a No. 6 cork borer from the apical 5 to 7 cm of the stem and cut into disks 3.0 to 3.5 mm thick. Each disk was cut in half, and the pieces were planted on 50 ml of 0.7 percent agar medium in 125-ml erlenmeyer flasks. Heat-labile compounds were sterilized by passage through a Millipore filter. All other supplements were autoclaved with the basal medium.

The inorganic medium of Hildebrandt *et al.* (*5*) was routinely employed, although, in the presence of certain required organic supplements, callus formed equally abundantly on media with ⅕ or 5 times the salt concentrations listed, or on White's medium. Tests of sucrose and glucose in concentrations of 1 to 8 percent showed that 2 percent glucose was the best carbon source. Numerous combinations of the following supplements were tested: an auxin (indoleacetic acid, naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid [2,4-D]), 0.1 to 10.0 mg/l; kinetin, 0.2 to 2.0 mg/l; yeast extract, casein hydrolyzate, or a synthetic mixture of casein hydrolyzate amino acids, 10 to 1000 mg/l; a mixture of thiamine, pyridoxine, and nicotinic acid, each at 0.25 mg/l; filtered coconut milk, autoclaved coconut milk, or a filtered aqueous extract of *T. spachianus* or *T. lamprochlorus* stem, 2 to 20 percent by volume. Only autoclaved coconut milk and 2,4-D, in combination, stimulated vigorous callus formation on stem segments, although some cell enlargement or proliferation, or both, occurred on several other media. The optimum level of coconut milk was 10 percent, and the optimum level of 2,4-D was 2.5 mg/l. On this me-

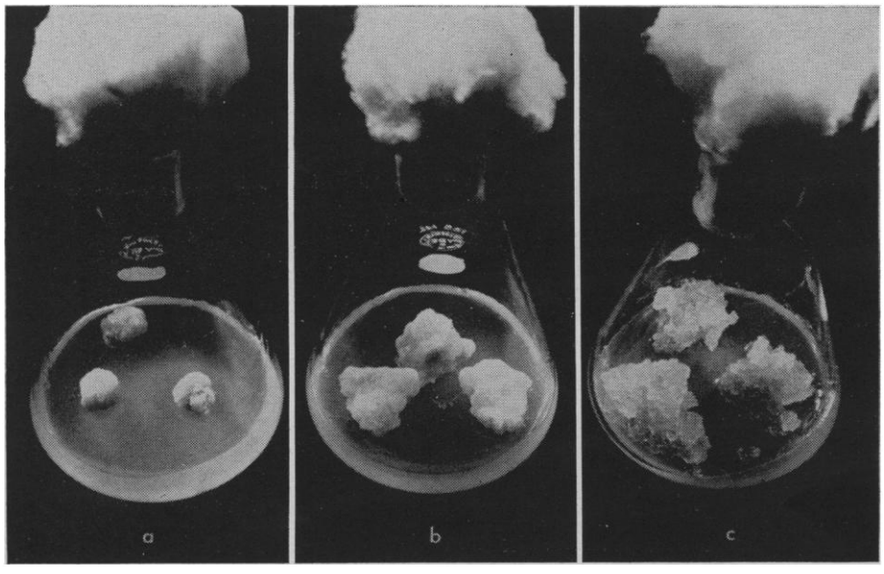

Fig. 1. Three types of callus from stem explants of *Trichocereus spachianus*: (*a*) compact, slow-growing form, (*b*) firm callus with a moderate growth rate, and (*c*) friable, rapidly growing tissue. The callus, in the 7th transfer, was planted on 21 May 1962 and photographed on 13 June.

dium about half the stem pieces produced callus abundantly enough for subculturing.

The stem cultures required dim light during at least part of the day, in order to initiate callus. The laboratory bench offered the optimum available conditions of temperature (about 20°C) and light (40 to 100 ft-ca during the day). No callus formed on stem explants kept in a dark growth chamber at 26°C, or in a growth chamber with a 12-hour light period (500 ft-ca) at 19°C followed by a 12-hour dark period at 10°C.

Most of the initial stem pieces first formed red pigments and then turned black. Callus formed most abundantly on explants that maintained their green color for a week or longer, although even in these cultures the original stem tissue ultimately turned black. The callus itself was white, and if a mass of new cells free from the darkened stem tissue could be obtained, it usually grew and maintained its light color in subsequent transfers. It seemed likely that polyphenoloxidases were responsible for the darkening of the stem explants (*1*), and since the extent of callus initiation was inversely correlated with the rate of pigment synthesis, attempts were made to retard the action of the postulated enzymes. Polyphenoloxidase was implicated in the darkening by the observation that feeding 40 mg of tyrosine per liter so greatly accelerated pigment synthesis that the cultures were completely black 24 hours after planting. Omission of copper from the medium had no effect on pigment formation, nor did addition of glutathione, cysteine, or ascorbate. (The reagent grade salts used were not treated to remove traces of copper, however.) Certain materials other than tyrosine also hastened the darkening of the stem tissue. These included casein hydrolyzate and amino acid mixtures, with and without tyrosine; indoleacetic acid and naphthaleneacetic acid; glutamine; and high levels of sugars, especially sucrose. Attempts to prevent darkening by lowering the oxygen tension around the tissue failed. In one experiment the stem pieces were covered, after planting, by a thin layer of 1 percent agar; in another, a layer of light mineral oil was added. No growth was observed in either set of cultures, and the tissue became black even more rapidly than it did when exposed to air.

Although the cactus plants themselves grow most rapidly in the spring and summer, no seasonal effect on the growth of stem explants in vitro was noted. In some cultures, callus growth was initiated over the entire surface of the explant. In others, small spherical outgrowths appeared which might have originated from the division of a single cell and its progeny. During the first subculture period the callus established one of three growth habits (Fig. 1). The cultures illustrated were 23 days old and in the 7th transfer. Figure 1*a* shows a type of callus which grows in the form of an inverted bowl, doubling in weight during the usual 3- to 4-week growth period. The outer shell is firm and greenish; the center is filled with brown, dead cells and incorporated agar. The firm, pale yellow callus in Fig. 1*b* grows more rapidly; the callus pieces illustrated are 5 to 6 times their original size. The third type of callus (Fig. 1*c*) is very friable and easily dissociates into single cells and small clumps of cells. After a 3-week growth period the pieces weigh 7 to 8 times as much as they did when planted. Knobs of bright pink or red cells are frequently formed, but because the pigmented areas grow more slowly than the clear ones, the cultures in general remain white or grayish.

It was of interest to determine more exactly the growth requirements of these distinct morphological callus types. Of the media tested, however, only that containing coconut milk and 2,4-D could support continued growth; nor were the growth habits altered by manipulation of the auxin and coconut milk levels, as might have been predicted from other reports (*6*). Like the stem explants, all types of callus turned dark and failed to grow when kept in darkness or in light of 500 ft-ca intensity.

Future experiments will be designed to determine whether the various stem tissues give rise to different types of callus, or whether the distance of the stem explant from the apex of the intact cactus influences the subsequent form of growth. Studies of the exact nutrient requirements of the callus are planned, as well as a study of alkaloid synthesis in the tissue. The results of a preliminary experiment suggest that the callus has retained the enzymes involved in the methylation reactions leading to candicine synthesis (*7*).

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7. I thank Dr. S. H. Mudd for helpful discussions and for reading the manuscript.

17 July 1962