with uranyl acetate, then with phosphotungstate. In this way both the core and the surface structure of a single virus particle can be seen at the same time. Particles prepared in this manner are similar in appearance to the small fraction of coreless forms when they are stained and penetrated by phosphotungstate. There is no evidence of collapse. Such particles are usually as well preserved structurally as those treated with uranyl acetate only (Fig. 1, top).

Low-magnification electron micrographs were made of random fields of each preparation, and the particles were counted. The approximate concentration in the original vesicular fluid was calculated (*6*) although the precision of the original fluid volume measurement was poor. This volume could have varied by three-fold, therefore the dilution factor was by far the most variable element in the calculation. However, each of the five vesicular fluids contained sufficient numbers of particles to make quantitation possible. Concentrations ranged from 3×10^9/ml to 7×10^{10}/ml of vesicular fluid. The morphology of the particles was essentially the same in each preparation.

The approximate time required for preparing and examining three such specimens from time of receipt in the laboratory is 3 hours. The fine structure and staining characteristics of several viruses are sufficiently well known to make their tentative recognition possible in clinical specimens. It is apparent that the concentration of virus particles in some clinical materials is more than sufficient for direct electron microscopy. The methods employed in this study were sensitive enough to detect several particles per microscope field when the original vesicle fluid had been diluted 1:1000 prior to sedimentation. As preparative techniques become more refined and simplified, and thus more widely used, examining clinical materials for virus particles may become as practical as examining Gram-stained bacterial smears for the presumptive diagnosis of certain diseases (*9*).

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12 April 1962

Protection of Rats against Adjuvant Arthritis by Bacterial Lipopolysaccharides

Abstract. Lipopolysaccharides from both gram-negative and gram-positive bacteria in incomplete adjuvant caused rats to become resistant to mycobacterial (complete) adjuvant arthritis. Lipopolysaccharides which protected rats when they were administered in adjuvant did not protect when administered in saline. Mycobacterial wax D in saline was protective, while in adjuvant it produced arthritis. The degree of stimulation of a common tissue reaction may determine whether the result is protection or disease.

The varied physiological responses of animals to lipopolysaccharide (LPS) from gram-negative bacteria have been studied extensively (*1*). Stimulation of nonspecific immunity to bacterial and viral infections (*2*) and antiallergic effects (*3*) have been included. The nonspecific defense reaction has been credited both to the reticuloendothelial system (*4*) and to the properdin system (*5*). Recent research (*6*) indicates that inoculation of LPS into mice may cause "temporary elevation of a whole array of specific antibodies rather than the appearance of non-specific or cross reacting antibodies." Most previously reported inoculations have been given intraperitoneally or intravenously in an aqueous medium, rather than intracutaneously in a Freund-type (*7*) adjuvant. The dosages have been proportionately smaller than those we used.

Adjuvant arthritis was induced in young adult rats (Long-Evans, Wistar, Lobund) used in our experiments as described elsewhere (*7, 8*) by intracutaneous inoculation superscapularly with 0.5 ml of a water-in-oil emulsion. The inoculation contained wax D fraction (1 mg/ml) from virulent Canetti and Brevannes human strains of *Mycobacterium tuberculosis* (*9*). Although the mechanisms behind this interesting arthropathy have not been irrefutably established, some evidence indicates that they involve delayed hypersensitivity reactions (*10*).

To investigate further the factors influencing the pathogenesis of adjuvant arthritis, various lipopolysaccharides (*11*) were substituted for wax D in the adjuvant. These included LPS from gram-positive *Staphylococcus* A and from gram-negative *Salmonella typhosa* 0901, *S. typhimurium*, and *Escherichia coli* 055:B2. When technique and dosage were identical with those used for the inoculations of arthritogenic wax D adjuvant, no overt symptoms of disease developed. (Minimal joint swelling was noted with *Salmonella typhosa* LPS adjuvant in only two of four rats after a second inoculation. To date, this technique has not been investigated further.)

Subsequent injection of the same animals with wax D adjuvant showed that the animals were completely or partially refractory to adjuvant arthritis. The disease was considered to have been modified by previous injection of LPS if (i) no arthritis developed after challenge with wax D adjuvant, (ii) the disease occurred in a significantly smaller number of rats than in unprotected controls, (iii) its onset was markedly delayed, (iv) its symptoms were mild, or (v) recovery was more rapid than in controls.

With LPS from *S. typhosa*, only 9 of 36 rats (25 percent) developed arthritis as compared with 30 of 35 (85 percent) of the controls. Protection was much greater when the interval between the initial inoculation and the challenge was 8 days than when longer intervals (up to 60 days) were used. With LPS from *Staphyloccocus* A and intervals of 15 to 40 days, arthritis appeared in 14 of 31 rats (45 percent). When rats were protected with LPS from *Escherichia coli* there

Table 1. Effects of bacterial fractions, in incomplete adjuvant and in saline, on arthritis in rats. A second inoculation, consisting of wax D adjuvant, was given 24 days after the first. Evaluation: +, protection; 0, no protection.

First inoculation	Effects		Evaluation
	Arthritis/total	%	
In adjuvant:			
S. typhosa LPS	2/9	22	+
Staph. A. LPS	3/10	30	+
In saline:			
S. typhosa LPS	8/10	80	0
Staph. A. LPS	6/10	60	0
Wax D	2/10	20	+
Controls:			
Nothing	24/31	77	

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-