

probably from others, stand as a memorial to his vision and perseverance, for he worked under the growing burden of impaired health. In spite of this handicap he accomplished much and encouraged others to carry on the work he could not finish. He

was a leader in the field of desert ecology, for his understanding of desert life and desert problems was founded on long experience, keen observation, and an analytical mind. He became almost a part of the desert he studied and knew so well.



## Technical Papers

### The Question of Extraneural Growth *in vivo* of Poliomyelitis Virus

Harold K. Faber

Department of Pediatrics,  
Stanford University School of Medicine,  
San Francisco, California

The cultivation of poliomyelitis virus in cell suspensions of various tissues (1-3) (skin, muscle, intestine, kidney, testis, etc.) has been interpreted as invalidating the assumption of obligate neurocytotropism of this virus in the intact animal, an assumption which Syverton and his associates explicitly state is "no longer tenable." There is a serious fallacy in inferring from *in vitro* growth of a virus in particular tissues that the same tissues are capable of supporting growth *in vivo*. It has yet to be shown in the living animal that cells of skin, muscle, kidney, or testis, in contrast to those of the nervous system, either support growth of poliomyelitis virus or display specific lesions, or, indeed, lesions of any kind, during the early stages of poliomyelitis. Certainly, dermatitis, enteritis, nephritis, and orchitis are not features of the clinical picture of the disease. Enders himself, in whose laboratory the first successful cultures of the virus were made on extraneural tissues, has made no such claim. On the contrary, he has stated (4), in discussing the factors influencing multiplication of viruses and rickettsiae in tissue cultures, that:

The results of many studies with different viruses, however, have made it clear that the degree of pathogenicity exhibited by an agent for the intact animal is frequently not correlated with its capacity to increase in cultures prepared from the tissues of such an animal; . . . [and] Extracellular inhibitory mechanisms present in the living body may be eliminated in cultures, thus permitting multiplication.

There is some reason to believe that these remarks may well apply to the case of poliomyelitis virus. The method used, with various modifications, by Enders and others, in the cultivation of poliomyelitis virus, is that of Maitland and Maitland (5), using one of Hanks' salt mixtures and Simms' ox blood serum ultrafiltrate. Preliminary washing of the tissue appears to be important, both in the original preparation and in subcultures. The salt solutions depart widely from normal mammalian interstitial fluid in

respect to electrolyte composition. The importance of electrolytes, at certain critical concentrations, in promoting the attachment of virus to host cell has recently been noted by Puck and his associates (6). The part played by ox blood serum ultrafiltrate in virus cultivation also appears to be critical. Simms (7), who introduced this material for tissue culture, found that normal tissue and serum contain several factors that affect cell growth and metabolism, one of which is inhibitory, one (A) stimulative, one (B) causative of fat granule production, one (C) degenerative, and one (D) causing cohesion of cells. The ultrafiltrate contains only A, removes B, C, and D from cells and counterbalances the inhibitory factor.

The presence of poliomyelitis virus in the intestine in the disease has been offered by Evans and Green (8), and more recently by Syverton and his associates, as evidence in support of extraneural growth of the virus, presumably on the cells of the oral and intestinal mucous membranes. The usual lack of signs of inflammation in these membranes early in the disease is suggestive contrary evidence. An alternative explanation, based on the characteristic neurotropism and axonal conduction of the virus, has been demonstrated by us in recent experiments (9), which showed that the virus is excreted into pharynx and gut as early as 3 days after neural exposures in which primary exposure of the pharyngeal or intestinal surfaces was rigorously excluded. At this time virus was demonstrable in the regional ganglia (10). In other experiments heavy exposures of the gastrointestinal tract, in which the oropharyngeal surfaces did not participate, were not followed, after the immediate postexposure period, by continuing excretion of virus such as might have been expected if the mucosal epithelium had become infected. In a single instance, excretion of virus began later, at the time when paralytic symptoms appeared, an indication of a neural source. In recent experiments, as yet unpublished, we found that nontraumatic oropharyngeal application of the virus was followed by the appearance at 2 days of specific lesions and at 3 days of recoverable virus from regional peripheral ganglia, whereas no evidence of infection of the CNS had appeared then nor for several days later. The experiments indicate an almost immediate entry and centripetal passage of virus through the superficial nerve fibers to the ganglia, without any lag such as

might be anticipated if primary implantation in the epithelial cells were the first step of the infective process. The findings are also inexplicable on the basis of early viremia.

Proof of the negative is always difficult, but it is a good general rule to give preference to explanations for which there is sound positive proof. In the case of poliomyelitis, neurocytotropism and axonal conduction of the virus *in vivo*, which have been proved beyond reasonable question (11, 12), provide an adequate explanation of the essential characteristics of the disease, whereas the theory of extraneural affinities, although revived periodically for several decades, remains without factual proof and only serves to becloud the pathogenesis of the disease.

#### References

1. ENDERS, J. F., WELLER, T. H., and ROBBINS, F. C. *Science*, **109**, 85 (1949).
2. WELLER, T. H., ROBBINS, F. C., and ENDERS, J. F. *Proc. Soc. Exptl. Biol. Med.*, **72**, 153 (1949).
3. SYVERTON, J. T., SCHERER, W. F., and BUTORAC, G. *Ibid.*, **77**, 23 (1951).
4. ENDERS, J. F. In T. M. Rivers (Ed.), *Viral and Rickettsial Infections of Man*. Philadelphia: Lippincott, 121 (1948).
5. MAITLAND, H. B., and MAITLAND, M. C. *Lancet*, **2**, 596 (1928).
6. PUCK, T. T., GAREN, A., and CLINE, J. J. *Exptl. Med.*, **93**, 65 (1951).
7. SIMMS, H. S. *Science*, **83**, 418 (1936).
8. EVANS, C. A., and GREEN, R. G. *J. Am. Med. Assoc.*, **134**, 1154 (1947).
9. FABER, H. K., et al. *J. Exptl. Med.*, **92**, 571 (1950).
10. *Ibid.*, **88**, 65 (1948).
11. FAIRBROTHER, R. W., and HURST, E. W. *J. Path. Bact.*, **33**, 17 (1930).
12. HOWE, H. A., and BODIAN, D. *Neural Mechanisms in Poliomyelitis*. New York: Commonwealth Fund (1942).

Manuscript received July 17, 1951.

## *Fissidens pauperculus* Howe and *Orthodontium gracile* Bruch & Schimper: Mosses Associated with the Coast Redwood Forest

Leo Francis Koch<sup>1</sup>

Bakersfield Junior College,  
Bakersfield, California

The geographical distribution of mosses appears to correspond closely with that of conifers in California. This hypothesis was first formulated by the writer (1) as a result of comparing the known distributional patterns of mosses with the units of various systems of classifying vegetational units pertaining to California. At that time a distribution pattern among mosses identical with the coast redwood forest was unknown, although known stations for *Fissidens pauperculus* and *Orthodontium gracile* were recognized as restricted to redwood areas (2-4).

With these facts in mind, the writer initiated a study of the bryophytes of the coast redwood forest, supported by a postdoctoral fellowship for the aca-

<sup>1</sup> Postdoctoral fellow, Horace H. Rackham School of Graduate Studies, and collaborator in phytogeography, Botanical Gardens, University of Michigan, Ann Arbor, 1950-51. Now instructor of life sciences, Bakersfield.

demic year 1950-51 from the Horace H. Rackham School of Graduate Studies at the University of Michigan.

The known range of the coast redwood is from Curry County, Ore., southward in the coastal counties of California to Monterey County, covering an area roughly 450 miles long and averaging 25 miles wide (5).

*F. pauperculus* was originally discovered in Marin County near Mill Valley in 1893 (6) and was not rediscovered until 1947 (3), when it was found near Larkspur and on Mount Tamalpais in Marin County, and also in the Big Basin Redwood State Park in Santa Cruz County (4). It has been listed as one of 16 mosses endemic to California and Baja California (7).

*O. gracile* was also first found in California by Howe, who found it on a redwood stump near Eureka, Humboldt County, in 1896 (8). In the same year Howe also found it in Mendocino and Sonoma Counties (2). In 1906, H. B. Humphrey collected it on King Mountain in San Mateo County. The writer found the moss in the Big Basin Redwood State Park in Santa Cruz County in 1947 (4). *O. gracile* has an interesting history in Europe (9), where isolated stations in Great Britain and France are known.

In September 1950, the author<sup>2</sup> visited the Chetco River Redwood Park in Curry County, Ore., and discovered both *F. pauperculus* and *O. gracile* there. This northernmost remnant of redwood forest is in grave danger of losing its vegetational character because of indiscriminate lumbering in the immediate vicinity.

The coast redwood forest has its farthest inland station on Howell Mountain in Napa County, where it grows in stream canyons. *F. pauperculus* was found there in Wildcat Canyon on the property of W. L. Wright, but *O. gracile* was not seen. If the latter species no longer grows on Howell Mountain, the thorough disturbance of the area by owners and Boy Scouts is no doubt a contributing factor.

Recently the Big Sur Redwood State Park in Monterey County was visited, and collections of both mosses were made in the park. Both species have been found to be abundant in the Armstrong Redwood State Park and in Russian Gulch in Sonoma County. Undoubtedly they will also be found in the remaining unexplored areas in which the coast redwood predominates.

The remarkable parallel distribution of *F. pauperculus* and *O. gracile* with the coast redwood appears too exact to have been the result of chance. Both ecological and historical factors are probably involved. The writer has previously (1) postulated a closer correspondence between the distribution of bryophytes and conifers than between either of these groups of plants and flowering plants. It was suggested that the common antiquity of bryophytes and conifers, when compared to flowering plants, may

<sup>2</sup> Funds for traveling expenses from the Botanical Gardens of the University of Michigan are gratefully acknowledged.