TABLE 1

THE QUANTITATIVE STUDY OF THE ENCEPHALITOGENIC ACTIVITY OF PROTEOLIPIDE AND NEWBORN MOUSE BRAIN

Material	No. with signs			No. symptom-free but with CNS lesions	Reactors		B/A	Cumulative time and no. injections to onset			C×D/A
	A	B						C		D	
	No. of mice	Non-paralytic	Paralytic		Total no.	Percentage		No. mice	Days	No. injections	
Proteolipide from 150 brains*	20	0	20	0	20	100	1	6 8 9 10 11 13 20	6 9 14 15 16 17 20	1 2 2 3 3 3 3	3.0
Proteolipide from 25 brains*	21	6†	11	4	21	100	½	2 4 6 11 12 14 16 17	6 9 14 17 18 22 27 28	1 2 2 3 3 4 4 5	6.7
Whole, normal adult mouse brain (control)	20	13†	5	2	20	100	¼	4 5 6 7 9 11 12 14 18	5 8 16 19 23 26 28 33 37	1 2 3 3 4 4 5 5 6	11.1
Whole, normal newborn mouse brain	20	0	0	0	0	0	—	0	60	6	—

* These separate residues, after proteolipide extraction, of the 150 and of the 25 brains were inactive in 35 mice. The RD_{50}, dry weight, for whole brain = 0.40 mg/ml ($=10^{-2.75}$); for proteolipide, 0.013 mg/ml ($10^{-2.92}$).

† These showed neurological signs, such as paresis, tremors, weakness, excitability (*5*) but no extensive paralysis, as in Column B.

diluted 1:6, to equalize the weight of tissue used as control. The latter comprised 25 whole brains, or 10 g of tissue, secured from normal adult mice; 65 whole brains of 4-day old Swiss mice, yielding 10 g of tissue, were also included (Table 1). Each of these materials was mixed with the Freund-type adjuvant and homogenized in a Waring blendor for 2 min, as already described (*2, 3*). The methods, the use of H-line W-Swiss mice (*6*) as animals of choice for inoculation, and other details of procedure have been stated before (*3, 5*).

The tabulated results indicate clearly that proteolipide deriving from mouse brain induced encephalomyelitis in mice to a greater degree than did whole adult mouse brain. There is a correlation between the concentration of proteolipide and the degree of reaction noted in inoculated animals. Finally, newborn mouse brain from which no proteolipide is obtainable, treated in the same manner as adult brain, failed to bring about the disorder. It has already been shown that all the encephalitogenic power of mouse brain resides in the proteolipide fraction (*2*). The present results would therefore appear to confirm this finding.

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Fluorescence in Ultraviolet Light in the Study of Boron Deficiency in Celery

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A better understanding of boron deficiency in celery, *Apium graveolens* L. var. *dulce* Pers., has been attained by observing symptoms of brown checking and

cracked stem under ultraviolet light. The present report considers two fluorescent conditions associated with these symptoms.

Brown checking and cracked stem refer to two types of symptoms exhibited by a single physiological disease which occurs in most celery districts of California. Brown-checking symptoms appear on the adaxial side of the petiole along the median axis. On the main petiole, the affected area is commonly 0.4–0.9 cm wide and 3.6–11.0 cm long on the upper one

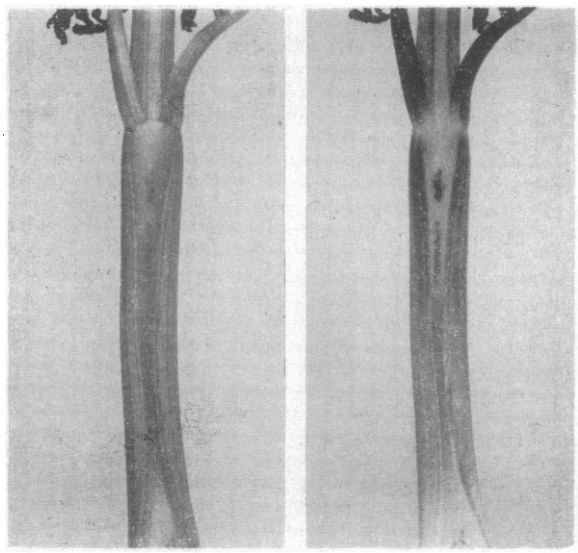

FIG. 1. Field-grown celery showing early boron-deficiency symptoms on the adaxial side of a petiole. Left figure photographed in visible light; right figure shows visible fluorescence induced by ultraviolet light. Note bright area which fluoresces blue around lesions.

half to two thirds of the petiole. The lesions, involving the necrosis of superficial parenchyma and collenchyma cells, are brown, and in advanced stages of the disease they usually have conspicuous transverse cracks.

In the major celery varieties grown in California the brown-checking symptoms are predominant. Of 298 plants with these symptoms collected from five celery districts in California, 48.3% also exhibited cracked stem in varying degrees. Plants with both types of symptoms are more seriously affected than those with brown checking alone. Cracked stem appears on the abaxial side of the petiole. The primary symptoms are numerous isolated cracks across the ribs or small tears on the ribs; in advanced stages the affected tissue becomes brown in color. Both types of symptoms also occur on the upper rachis and secondary petioles.

Cracked stem in celery is attributed to boron deficiency by Pruvis and Ruprecht (*1*), Maier (*2*), and Reed (*3*). Studies now in progress by other members of the Department of Vegetable Crops and by the Extension Service suggest that boron deficiency in the plant is a factor causing brown checking. Symptoms on both the inside and outside of the petiole are reported by Bardin and McCormick (*4*) and Lachance *et al.* (*5*). The latter also describe a condition of heart atrophy as the most severe symptom of cracked stem.

In the phase of the work reported here, macroscopic examinations were made under ultraviolet light on affected plants from commercial fields, and on plants grown in nutrient solution at deficient levels of boron (0.0–0.05 ppm) in the greenhouse at Davis. Plants were also grown in nutrient solution at normal and toxic levels of boron. An A-H4, 100-w mercury lamp with Corning filter 5860 (color spec. 7–37) provided peak ultraviolet radiation at the 365 mμ line of the mercury arc spectrum. Several Utah varieties of celery were observed in this study, of which the principal one was Utah 10-B.

Blue fluorescent characteristics of the disorder. Under ultraviolet light normal young, growing petioles appear dull reddish green. In contrast, the early brown-checking symptoms on the inside of the petiole exhibit a bright, light-blue fluorescence around the affected tissue, extending about 3 mm beyond the margin of the lesions (Fig. 1). In effect, the fluorescence resembles a blue halo around the lesion. This blue fluorescence also extends within the tissue of the petiole to a depth of about 3 mm. The blue color, most intense near the necrotic cells, diminishes in intensity with distance from them. Petioles with symptoms on the abaxial side develop a blue fluorescence in the living tissue adjacent to the cracks; a blue fluorescence is associated also with some mechanical injuries to the petiole.

Although early brown-checking symptoms commonly have a characteristic location on young, growing leaves, they are often quite inconspicuous or not apparent when viewed under visible light. The vivid fluorescence associated with them, however, makes possible the detection of incipient stages of the disorder.

The development of a blue fluorescence in relation to a variety of diseases and injuries in plants may be more significant than has heretofore been realized. It is reported in several species of the Solanaceae, especially in the potato (*6–11*), and also in citrus fruits (*12, 13*). I have also observed intense, light-blue fluorescent halos around the necrotic lesions of "internal cork" in sweet potato. Best (*14*) and Andreae (*11*) working on tobacco and potato, respectively, have identified a blue fluorescing material as scopoletin. The possible metabolic role of this coumarin compound in plants has been discussed recently by Best (*15*), Andreae and Andreae (*16*), and Goodwin and Kavanagh (*17*). Bottini (*18*) identified several blue-fluorescing compounds in injured citrus fruits, including two derivatives of coumarin in the lemon and the bergamot orange. The relationship of the coumarins to necrosis and wounding should be explored further.

Yellow fluorescent characteristics of the disorder. A second fluorescent phenomenon associated with brown checking and cracked stem in celery is the dis-

play of a bright-yellow or gold color on the surface of adaxial and abaxial lesions (Fig. 2). The fluorescence is not general over the lesion, but occurs in small scattered spots or flecks on the surface. In some cases the flecks are so numerous as to give the lesion an over-all yellow appearance. This fluorescence is absent in some lesions, and in others it is weakly developed because the spots of fluorescing material are small and scattered. The material is a dry exudate, which may develop wherever the surface tissue is broken. The nature of this material is unknown. Under the unfiltered light of a mercury lamp it appears colorless, whereas under an incandescent lamp it is light tan in color. Of 298 affected plants collected from five celery districts, 87.9% showed some degree of yellow fluorescence. Usually, plants with severe symptoms display a striking fluorescence, whereas plants with moderate or weak symptoms may show little or none. In individual plants, the fluorescence is best developed on mature leaves having lesions, is less well developed on younger affected leaves, and does not occur at all on leaves with incipient lesions. In plants grown in nutrient solutions deficient in boron, lesions on both the inside and outside of the petiole often develop a high degree of yellow fluorescence very much like that occurring in field-grown plants.

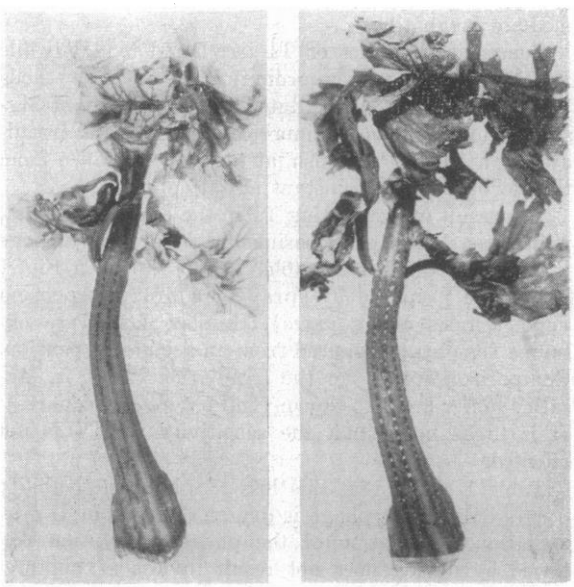

FIG. 2. Celery grown in boron-deficient (0.03 ppm) nutrient solution showing advanced symptoms on the abaxial side of the petiole. Left figure photographed in visible light; right figure shows visible fluorescence induced by ultraviolet light. Note bright spots which fluoresce yellow on cracks along ribs of petiole.

Plants grown at toxic levels of boron (10.0 and 33.0 ppm) develop superficial brown necrotic areas on the ribs and on the adaxial side of the petioles. These lesions, which do not initially involve any of the collenchyma tissue, appear as russeted or beaded vertical streaks. Transverse cracks may also develop on either side of the petiole in association with the affected tissue. The surfaces of these lesions, in contrast to those of plants grown in deficient boron, do not show any distinctive macroscopically observable fluorescence under ultraviolet light.

To determine whether yellow fluorescence is exhibited by other diseases and injuries in celery, plants from most of the celery districts were examined. Growth cracks and miscellaneous injuries occasionally showed weak displays of yellow fluorescence; diseases including aster yellows, blackheart, late blight (*Septoria*), pink rot (*Sclerotinia*), and western celery mosaic had little or none. In no instance where such fluorescence occurred could the symptoms be misinterpreted as being those of brown checking or cracked stem.

Yellow fluorescence has been observed over a period of two growing seasons in a wide range of celery material from both the field and that grown in boron-deficient nutrient solutions. Although there is considerable variation in the display of fluorescence by plants with brown checking and cracked stem, it is frequently prominently developed in celery thus affected. The yellow fluorescence may be considered as an added feature in the array of symptoms exhibited by plants with this disorder. Its significance in relation to boron deficiency in celery is not as yet understood.

The parallel development of similar fluorescent conditions in plants grown in the field and in nutrient solution supports other phases of this study which implicate boron deficiency in the plant as a factor causing brown checking and cracked stem. These fluorescent conditions also serve as an aid in the study and diagnosis of the disorder.

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