

activity of these two fractions, as revealed by the response to antibody formation they elicit. In these experiments, two groups of large rabbits, of between 4 and 5 kg body weight, were given successive, intravenous injections of native and regenerated horse serum albumin, respectively, over a period of not less than four weeks. Antibody titer of the rabbit sera was determined by the optimal proportion method of Culbertson.<sup>6</sup> In several cases, antibody content was also determined by the method of Heidelberger and Kendall,<sup>7</sup> as well as by quantitative comparison of the electrophoretic patterns of normal and immune sera.<sup>8</sup>

Although individual variations in the response to antibody formation are apt to be great, nevertheless, with eleven animals that have been tested, the antibody titer of the sera of rabbits immunized with the regenerated serum albumin was, on the average, only 10 per cent. of that obtained by immunization with the native protein. In a typical pair of experiments, 1 cc of an anti-native protein rabbit serum was found to combine optimally with 1.23 mg of the homologous antigen whereas 1 cc of an anti-regenerated protein rabbit serum combined optimally with only 0.13 mg antigen.

While the regenerated protein is thus seen to possess a greatly reduced capacity to incite antibody formation, the immunological specificity of the native protein was not impaired by the regeneration process. This is borne out by the finding that native and regenerated protein not only exhibited strong cross reactions when tested with the corresponding antisera, but, in all but two cases, were actually immunologically equivalent. Immunological equivalence is defined, according to Marrack, as: "Two antigens A and B will

be said to be 'equivalent' when the antiserum to A reacts in the same titer with both A and B, and vice versa."<sup>9</sup>

Preliminary measurements indicated the regenerated horse serum albumin to be completely non-antigenic in sheep, even when administered in fairly large amounts.

The present findings suggest that native and regenerated serum albumin differ from each other in antigenic activity but not in antigenic specificity, and that the antigenic activity of the native protein resides primarily in the very structural features that are altered or destroyed during the regeneration process.

This work is being extended in various directions so as to include the action of several types of denaturing agents on the albumin fraction of certain normal sera, and on the globulin fractions of normal and immune sera. A full account of the present findings will be published elsewhere.

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# QUANTITATIVE INTERNEURONAL RELATIONSHIPS IN THE HUMAN SPINAL CORD<sup>1</sup>

BODIAN<sup>2</sup> has recently reviewed the problems in the field of synaptology, and to his excellent summary the reader is referred for introductory notes and bibliography. Among the problems requiring attention is that of ascertaining on a quantitative basis the interrelationships of the various cell groups in the human spinal cord. The determination of these values

TABLE 1  
INTERNEURONAL RELATIONSHIPS IN THE HUMAN SPINAL CORD

Source of Adjacent Terminals (Number and per cent. of total for region)						
Cell column	Number of adjacent boutons	Dorsal root	Substantia gelatinosa	Sensory and internuncial	Subcortical nuclei	Cerebral cortex
Sensory and internuncial (5,440,000 cells) . . . . .	676,740,000	20 per cent. (135,348,000)	14 per cent. (94,743,600)	50 per cent. (338,370,000)	10 per cent. (67,674,000)	6 per cent. (40,604,000)
Nucleus dorsalis (188,600 cells) . . . . .	65,632,800	65 per cent. (42,661,320)	10 per cent. (6,563,280)	15 per cent. (9,844,920)	10 per cent. (6,563,280)	0 per cent.
Intermediolateral (1,023,000 cells) . . . . .	124,806,000	0 per cent.	50 per cent. (62,403,000)	40 per cent. (49,922,400)	10 per cent. (12,480,600)	0 per cent.
Somatic efferent (1,456,000 cells) . . . . .	925,503,000	0 per cent.	5 per cent. (46,275,150)	65 per cent. (601,576,950)	20 per cent. (185,100,600)	10 per cent. (92,550,300)
Number and per cent. of total boutons contributed to cord cells from each source . . . . .	1,792,681,800	178,009,320 (9.5 per cent.)	209,985,030 (11.8 per cent.)	999,714,270 (55.8 per cent.)	271,818,480 (15.2 per cent.)	133,154,700 (7.5 per cent.)

<sup>6</sup> J. T. Culbertson, *Jour. Immunol.*, 23: 439, 1932.

<sup>7</sup> M. Heidelberger and F. E. Kendall, *Jour. Exp. Med.*, 61: 559, 563, 1935.

<sup>8</sup> A. Tiselius and E. A. Kabat, *Jour. Exp. Med.*, 69: 119, 1939.

<sup>9</sup> J. R. Marrack, "The Chemistry of Antigens and Antibodies." London, 1938.

<sup>1</sup> Read before the American Association of Anatomists, New York, N. Y., April 2, 1942.

<sup>2</sup> David Bodian, *Physiol. Rev.*, 22: 146-169, 1942.

is attempted in the present study through observations on central bouton changes incident to known lesions in the central and peripheral nervous systems. The data on which the present computations are based have been presented in previous reports.<sup>3,4,5</sup> These data include: (1) determinations of cell size, number and surface area in the various main nuclear groups; (2) quantitative estimates of the number of boutons adjacent to individual cells, to cell groups and in the entire cord; (3) establishment of criteria of bouton alteration under pathological influence which include alterations in number (both increase and decrease) and alteration in morphology; (4) establishment of circumstances dictating the variable changes including direction of influence, effect of age, autolysis and technique, the part played by the duration of the pathological process, the fundamental nature of the pathology, and the bearing cell size may have on the effect; and (5) the establishment of patterns of bouton change peculiar to specific lesions or cord conditions. With this information at hand it is possible to relate alterations in specific locations to known lesions and deduce certain interrelationships which are presented in Table 1.

The restrictions imposed by dependence on chance lesions in human material have necessitated limiting these observations to only the main nuclear groups and their connections. Work is in progress as material comes in which suggests more explicit sources for the groups included in the sensory and internuncial cell columns and also in the somatic efferent columns. It should be pointed out that the quantitative data from control groups at all ages varies plus or minus 5 per cent. necessitating caution in interpreting 0 per cent. values which have been established through comparison with control averages. These values mean only that no change in the synapses at the designated locations is detectable with the technique used.

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#### GROWTH STIMULATION OF PEAS BY TETRACHLORO-PARA-BENZOQUINONE, A FUNGICIDAL SEED PROTECTANT<sup>1</sup>

THE first strictly organic, non-metallic compound to show much promise as a plant protectant against fungous diseases was tetrachloro-para-benzoquinone. In field tests made on pea seed during the past three

years, it has very effectively prevented seed decay by soil-inhabiting fungi and has usually induced better yields than metallic compounds of equal fungicidal potency (cuprous oxide, hydroxymercurichlorophenol and ethyl mercury chloride). In tests made under conditions where there was no seed decay, it was the only treatment that increased the yields. The plants from pea seed treated with tetrachloro-para-benzoquinone yielded 9 to 22 per cent. more than untreated controls.

The treatment apparently stimulated the peas in many field plots since the plants were brighter green in color, made more terminal growth and frequently had stronger stems. Such field observations can not be accepted as proof of stimulation because a fungicide such as tetrachloro-para-benzoquinone might promote growth by preventing any one of several diseases of the seeds and roots. As a matter of fact, it has been found that both it and the metallic fungicides frequently increase the yield per plant by preventing post-emergence seed decay, a deleterious but frequently overlooked aspect of the seed disease problem.

In order to prove that tetrachloro-para-benzoquinone was stimulating the plants, it was considered necessary to test its effect on seed in the absence of disease organisms. Disease-free seed were treated with it and other fungicides and then sown alongside untreated seed in steamed soil in the greenhouse. Plants of the varieties Surprise, Wisconsin Early Sweet, Thos. Laxton, Alderman and Perfection have consistently produced 5 to 20 per cent. more dry matter in a 3 to 4-week growing period when grown from seed treated with it than from untreated seed. The other fungicides did not increase the growth of the plants. The seeds and roots of the controls were as healthy as those in the various treatments.

Typical results were obtained in a test with the variety Surprise, using 5 replications of 100 seed each. After 23 days, the average yield of dry plant tissue was: from untreated seed, 9.6 gm; red cuprous oxide, 9.9 gm; hydroxymercurichlorophenol, 9.7 gm; tetrachloro-para-benzoquinone, 10.9 gm; and yellow cuprous oxide, 9.3 gm. A difference of 0.8 gm was significant at the 5 per cent. point in an analysis of variance. The differences in yield were due to differences in growth rates, since there was almost identical emergence (97 to 98 per cent.) from the various treated lots. On the other hand, when seed from the same lots were planted in soil infested with *Pythium ultimum*, all four fungicides increased the emergence by 23 to 25 per cent. and consequently improved the yields. Tetrachloro-para-benzoquinone gave the largest yield increase under these conditions.

These data obtained under controlled conditions provide a suitable explanation for the results ob-

<sup>3</sup> Jeff Minckler, *Anat. Rec.*, 77: 9-25, 1940.

<sup>4</sup> *Ibid.*, *Arch. Neurol. Psych.*, 45: 44-55, 1941.

<sup>5</sup> *Ibid.*, *Am. Jour. Path.* (in press).

<sup>1</sup> Approved by the director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 513, June 1, 1942.