produced by others (*2, 11, 12*) over a longer period of time. The lesions were focal, vascular, and perivascular and were most marked in the brain stem, particularly in the pons, and in the cerebellum. In the cerebrum they were widespread but fewer and generally less intense; the spinal cord was least affected. There was a marked predilection for white matter with some tendency to periventricular clustering. Involvement of contiguous grey matter, as well as independent lesions in the latter, were common but far less intense and frequent. In two instances moderately severe lesions were present in the optic nerves.

The pathological changes were characteristically in the walls of and about capillaries, venules, and veins, but arterioles and small arteries were not spared. An initial mural and perivascular infiltration by polymorphonuclear leucocytes (and occasional eosinophiles) gave way to a lymphocytic infiltration and a marked multiplication and hypertrophy of local histiocytes apparently from the blood vessel walls. Giant cells were not encountered. Occasional blood vessels showed degeneration of their walls, fibrin impregnation, and rarely perivascular hemorrhages or fresh thrombosis. These last features seemed to be the result of an intensification of changes already in progress. Myelin degeneration began as myelin pallor about an affected vessel and often coalesced about a group of these. It went on in the longest surviving monkey (8 days) to complete breakdown in some lesions. There was a microglial proliferation with phagocyte formation and a mild astrocytosis in the perivascular lesions. The axones seemed well preserved and undiminished in numbers, although some loss of these structures could not be ruled out. It is possible that, had the monkeys survived for a longer period, degeneration of axones would have been encountered (cf. *2*).

No symptoms were observed in the fourth monkey injected with brain material nor were any abnormal signs noted in any of the animals which had received the emulsion of lung tissue.

The fact that adjuvants enhance the effect of brain tissue in producing this pathological process supports the hypothesis that an antigen-antibody reaction is involved in the formation of these lesions. This is also indicated on histological grounds by the abundant histiocytic response and is indirectly corroborated by the absence of lesions in other organs; its specificity for brain is evidenced by the failure to produce the same results with lung tissue.

Summary. Rapid production in the monkey of a pathological condition resembling acute disseminated encephalomyelitis, marked by demyelination, can be achieved by the use of adjuvants added to rabbit brain emulsions.

(Since this paper was submitted, I. M. Morgan has reported (*J. Bact.*, 1946, **51**, 53, abstract) obtaining similar results with monkey spinal cord plus adjuvants.)

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Plant Carbonic Anhydrase[1]

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This paper reports evidence for the existence of a substance in, or associated with, the green leaves of the common elderberry bush (*Sambucus canadensis*) which catalyzes the reaction, $H_2CO_3 \rightleftarrows H_2O + CO_2$. Its chemical properties are similar to, but not identical with, those of animal carbonic anhydrase. Neish (*7*) in 1938 discovered the presence of carbonic anhydrase-like activity in chloroplasts but did not attempt to isolate the catalyst or to describe it further. Stimulated by Neish's work, Mommaerts (*6*) searched for plant carbonic anhydrase but found no evidence for its existence. Similar negative results had been obtained earlier by Burr (*1*) and by Roughton (*8*). Proceeding from the assumption that this catalyst is absent, Burr argued that the first step in photosynthesis cannot be the hydration of carbon dioxide. His calculations indicated that the speed with which carbohydrate production proceeds is so great that if the reaction, $H_2O + CO_2 \rightarrow H_2CO_3$, is part of the process, a catalyst similar to carbonic anhydrase is required. Failing to find such a catalyst, he concluded that some other step must be the initial one in photosynthesis.

We have found such a catalyst in elderberry leaves but not in the leaves of some 15 other species growing in the vicinity of New York, including one of those tested by Neish (burdock). Perhaps the enzyme,

[1] In this work, which was supported by a grant from the John and Mary R. Markle Foundation, much assistance was received from Anne D. and Hans H. Zinsser, in collaboration with whom a more complete description of the enzyme will be published later. The authors wish to thank David E. Green and F. J. W. Roughton for many valuable suggestions.

though present, is difficult to demonstrate in most species. Even in the case of elderberry leaves the activity decreases rapidly after separation from the bush. Another possible explanation of its absence or irregular occurrence in other species is that the enzyme is actually not a functional part of the leaf but is associated with some pathological condition from which the elder is especially liable to suffer. We have, however, found activity in all the specimens tested including those picked from bushes apparently in excellent condition and from those obviously diseased. Bushes from five different counties in southern New York State were examined.

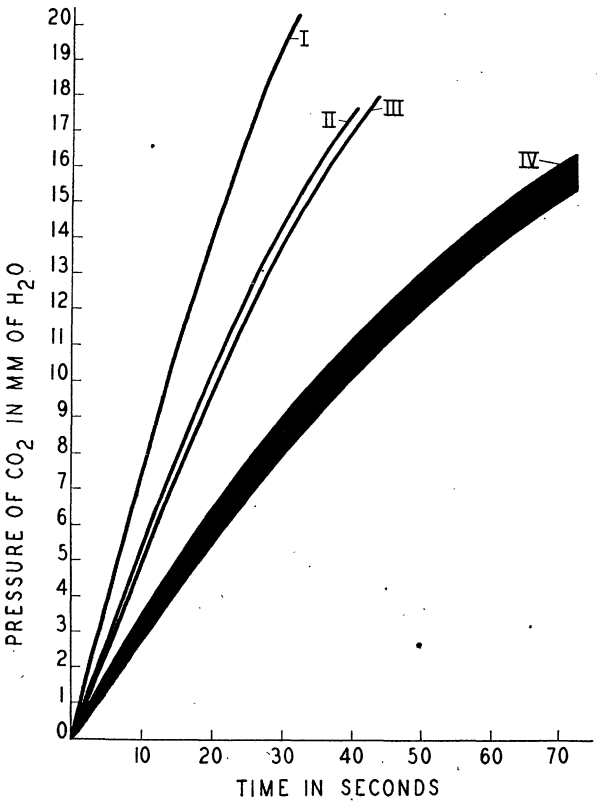

FIG. 1. Observations on the suspension. The curves indicate the rate of production of carbon dioxide under various circumstances when 2 cc. each of sodium bicarbonate solution and phosphate buffer of pH 6.8 are suddenly mixed at 15° C. (method of Meldrum and Roughton). For each curve a different substance is added to the substrate, as follows: I. 0.1 ml. of crude leaf suspension (curve identical to that obtained with added sulfanilamide); II. 0.1 ml. of 1 per cent laked human blood solution; III. 0.1 ml. of leaf suspension preserved 24 hours at 25° C. with 0.1 M stannous chloride; IV. Combined curve representing several curves which are so close together that separate identification is impossible. Included are three blank runs with no catalyst added and also observations with 0.1-ml. amounts of: (a) leaf suspension 24 hours old; (b) leaf suspension heated to 50° C. for 3 hours; (c) leaf suspension boiled 1 minute; (d) leaf suspension treated with 0.1 M $KMnO_4$; (e) suspensions of leaf substance of 15 different species of plants common to southern New York State.

Our original preparations were made by grinding a handful of leaves with sand and water for two minutes. The resultant green suspension was tested by the method of Meldrum and Roughton (5). Activity is expressed in units of E, an arbitrary velocity constant. In the test, 0.1 ml. of the green suspension is introduced into 4 ml. of substrate. This amount of crude suspension contains about 3 mg. of dried leaf; the resultant E is approximately the same as that obtained with 0.1 ml. of a 1-per cent solution of laked human blood containing approximately 0.2 mg. of solids. All the tests were made at 15° C.

The crude suspension is unstable. Activity is lost in 10 to 15 hours at 25° C. and in about 2 hours at 50° C. Boiling destroys all activity promptly. Activity is also destroyed by 0.001 M $KMnO_4$. Preservation of activity of the crude preparation can be achieved for several days by using 0.1 M $SnCl_2$ instead of water in making up the suspension. When the green particles, presumably chloroplasts, are separated by filtration or centrifugation, activity is entirely confined to the sediment, the clear supernatant being inactive (Fig. 1).

A solution, which is both clear and stable when stored at 10° C., was prepared by grinding the fresh leaves rendered brittle by contact with solid carbon dioxide and placing them in a cold, 25-per cent dextrose—0.1 M NaF solution. After a few hours all the leaf particles settled to the bottom, leaving a clear amber supernatant solution. The attempt to produce a stable dried powder failed. Leaves were ground in and desiccated by cold acetone. The resultant powder was highly active at first but deteriorated rapidly even when stored in the cold.

TABLE 1

INCREASE OF POTENCY WITH PURIFICATION

Specimen	Activity per unit of nitrogen expressed in arbitrary units				Average
	A	B	C	D	
Original solution of enzyme in 25% dextrose—0.1 M sodium fluoride	1.08	1.03	0.80	0.46	0.84
Sodium phosphate elution after adsorption on alumina-C-gamma gel	1.38	6.75	2.48	1.42	3.00
After both alumina gel treatment and precipitation with 50% saturated ammonium sulphate ...	8.35	7.75	4.42	2.91	5.86

The enzyme could be purified by ammonium sulphate fractionation between the limits of 40 and 60 per cent saturation. The activity ratio (ratio of enzyme units to protein concentration as determined spectroscopically by the absorption at 280 mμ) increased 3½-fold following ammonium sulphate precipitation and some 7-fold when ammonium sulphate fractionation was preceded by adsorption on, and elution from, alumina-C-gamma gel (Table 1). Since am-

monium sulphate itself accelerates the test reaction, blank determinations with ammonium sulphate added to the reagents are necessary.

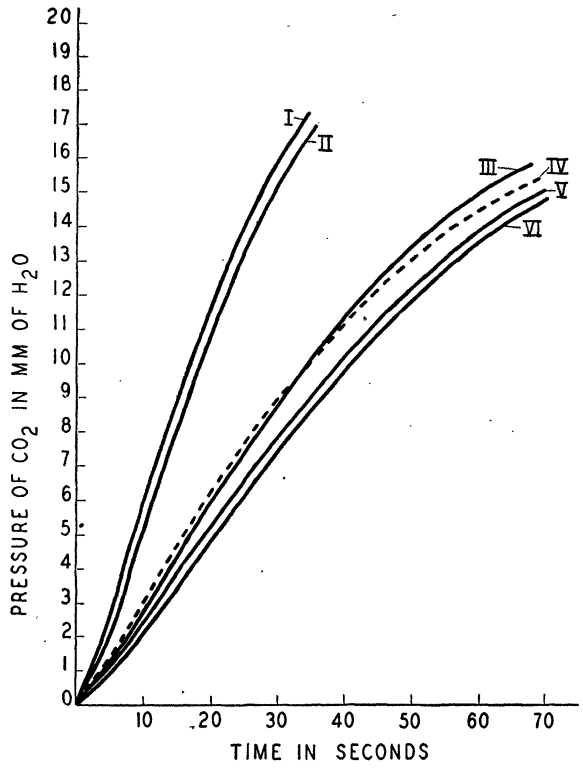

FIG. 2. Observations on plant enzyme solution: I. 0.1 ml. of dextrose-fluoride-enzyme solution after dialysis for 8 hours; II. 0.1 ml. of dextrose-fluoride-enzyme solution after storage for 4 weeks at 10° C.; III. 0.1 ml. of the same solution in 0.001 M KI; IV. 0.1 ml. of same solution as in II in 0.001 M $CuSO_4$; V. Same as II except heated at 80° for 15 minutes; VI. Blank. Curves I and II also are virtually identical to those obtained when the same solutions are used with sulfanilamide and sodium cyanide added.

The behavior of the active principle in the above-described procedures indicates that it is a protein. This opinion is supported by its lability to heat and by its failure to pass through a dialyzing membrane. A solution of the enzyme in sodium phosphate was dialyzed against 30 per cent ethanol for 8 hours. At the end of this time activity was unimpaired, but the concentration of phosphate had decreased to 2.5 per cent of the starting value.

The plant enzyme shows certain similarities to animal carbonic anhydrase but is different in other respects. Both are sharply inhibited by low concentrations (0.01–0.001 M) of H_2S, $KMnO_4$, $CuSO_4$, and I_2. On the other hand, neither sulfanilamide nor cyanide appears to inhibit the plant enzyme, though both inhibit that obtained from animals (*4*) (Fig. 2).

Both the animal and the plant enzymes are soluble and stable in 30 per cent ethanol but are inactivated at concentrations of this alcohol above 40 per cent. In purified solution the plant enzyme is less heat labile than in the crude suspension; in fact, it is destroyed at the same temperature as the animal enzyme. Both are inactivated by 5 minutes at 80° C., at which temperature a precipitate forms. At 70° C. there is only slight inactivation even after half an hour.

Zinc, known to be a constituent of animal carbonic anhydrase (*3*), was found to be present in our solutions by the method of Hibbard (*2*). However, it cannot be stated whether this is associated with the active principle or with some other protein. Dialysis for 48 hours does not remove the zinc.

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News and Notes

About People

Irving Hill Blake has been appointed chairman of the Department of Zoology, University of Nebraska, to succeed David D. Whitney, who will remain on the staff as a professor of zoology.

L. W. Chubb, director of the Westinghouse Research Laboratories, is the recipient of the John Fritz medal and certificate, awarded annually for notable scientific or industrial achievement. The award is made by representatives of four national engineering societies: the American Society of Civil Engineers, American Institute of Mining and Metallurgical Engineers, American Society of Mechanical Engineers, and American Institute of Electrical Engineers.

Capt. Guy Wheeler Clark, USN, was appointed superintendent of the U. S. Naval Observatory, Washington, D. C., on 1 September, to succeed Capt. R. S. Wentworth, who has retired.

Howard F. Hunt has been appointed acting assistant professor of psychology at Stanford University, where he is connected with the general training program in