mum of 19 per cent of the ova cleaved when the number of spermatozoa was doubled (80,000) but suspended in 1 ml. of saline.

The advantage of a small volume of concentrated sperm suspension is clearly shown. It may be due to: (1) the detrimental effect of chemicals, ions, oxygen tension, etc. on spermatozoa when the proportion of chemical constituents in the medium to living tissue is excessive; (2) the fact that there might be beneficial chemical substances in the semen or in the spermatozoa which would be diluted too much in a large volume of solution with a consequent loss of fertilizing capacity; (3) the fact that the cervix can take up only a small amount of fluid and hence more sperms are taken up in a small volume of fluid. In any event, it is quite conclusive that the chance of fertilization is better when spermatozoa are suspended in a small volume of fluid.

Two implications arise from these findings: (1) In the diagnosis of male infertility, we have to take into consideration the volume of semen in relation to number of spermatozoa; that is, considering only the total number of spermatozoa in an ejaculate is not adequate for ascertaining the fertility of a male animal. It is the concentration of spermatozoa in semen that is more important. (2) In the practice of artificial insemination it is better to instill a small volume of fluid with a high concentration of sperms rather than a large volume of fluid with a low concentration of spermatozoa.

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Rapid Production of Acute Disseminated Encephalomyelitis in Rhesus Monkeys by Injection of Brain Tissue With Adjuvants[1]

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The production of multiple lesions of the central nervous system in monkeys by the repeated intramuscular injection of emulsions and extracts of rabbit brain has been reported by several investigators (*2, 11, 12*). The abnormal changes were marked by their wide dissemination, perivascular position, inflammation, proliferation of histiocytes, giant cell formation, and the associated demyelination. In all instances, large numbers of injections (30–100) and time intervals of from 3 to 13 months were required to induce the appearance of symptoms. Since this phenomenon may involve an immunological response to the injected brain material and the combination of the antibrain antibodies with the brain tissue of the animal to produce these pathological changes, it was thought that a more rapid effect might be obtained by the administration of brain tissue together with adjuvants. This procedure has been shown to result in an enhanced immune response with a variety of other substances (*1, 3–10*).

Two groups of four monkeys each were used. One group received an emulsion of 18 grams of rabbit brain in 20 ml. of saline, 20 ml. of "aquaphor," and 40 ml. of paraffin oil containing 95 mg. of dried, heat-killed tubercle bacilli (cf. *4*). The second group was given an emulsion of 27 grams of rabbit lung prepared in a similar manner. Both brain and lung materials contained phenol in a final concentration of 0.25 per cent and were heated to 60° C. for 45 minutes to destroy autolytic enzymes. Each monkey received three intramuscular injections of 1 ml. of material into the arm or leg at weekly intervals.

Three of the four monkeys that had received inoculations of brain tissue became ill from 25 to 33 days after the first inoculation or 9 to 19 days after the last injection. At first the animals were quieter than they had been before, sat hunched over, and were inadequately responsive to all stimuli. Shortly thereafter, focal signs of damage to the central nervous system appeared and grew rapidly worse. The localization, sequence of appearance, and speed of development of the signs varied in all three animals. Two showed marked trunk ataxia, and all showed some degree of weakness in one or more limbs. Rotation and retraction of the head and ptosis of the upper eyelids were noted in two animals, and a left internal strabismus and left facial weakness in one. Coarse muscular twitches were seen in all the limbs in one instance, and in another there was evidence of considerable reduction of vision.

The three affected monkeys became ill and were sacrificed by exsanguination on the day of the appearance of symptoms, and 2 and 8 days thereafter, respectively. In each instance it seemed that the animal might not survive for a longer period. Blood cultures proved sterile. Culture of cerebral tissue from one of the monkeys and intracerebral and intraperitoneal inoculation of a brain suspension into three mice and a rabbit were negative.

Post-mortem examination revealed lesions limited almost exclusively to the central nervous system. These resembled in all essential respects the changes

[1] Aided by grants from the William J. Matheson Commission.

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.