to initiate an infection despite the use of prophylactic penicillin.

The mechanisms of resistance to penicillin and tetracycline, their mode of action on bacteria, and their effects on mammalian hosts are fundamentally different. Nevertheless, situations analogous to those described in the present communication have been described; *i.e.*, apparent increases in virulence seem to be mediated through an antimicrobial when the infecting agent is resistant to that antimicrobial.

We have reported(1) that neither tetracycline-dependence nor enhancement of growth was found under a variety of *in vitro* conditions.

Other possibilities concerning the mechanism of action of tetracycline in this experiment include induced changes in the indigenous microflora of the animals and direct influences of tetracycline on host metabolism. Antimicrobial-induced(6) or environmentally-induced(7) alterations among the indigenous microflora of laboratory animals have resulted in increased susceptibility to staphylococcal infections. Although such changes were noted in the above experiments, the precise mechanisms whereby such changes alter susceptibility are not understood(6,7,8).

Tetracycline may exert profound effects on host metabolism(9,10) and may thereby increase susceptibility directly or through some impairment of the inflammatory reaction. Aside from the negative results discussed above, however, further work is needed to define precisely the infection-enhancing effect of tetracycline in this experimental model.

Summary. Administration of tetracycline markedly lowered the charge of tetracycline-resistant staphylococci required to initiate a standardized intracutaneous lesion in guinea pigs. The results were independent of the route of antimicrobial administration. Tetracycline administered before and during the course of an infection with tetracycline susceptible staphylococci appeared inconstantly to increase the infectious charge required to establish the lesion. The mechanisms underlying these observations are not understood.

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Received February 25, 1963. P.S.E.B.M., 1963, v113.

Abnormalities in Brain Esterases in Multiple Sclerosis.* (28415)

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It has been shown that lipolytic enzymes can cause demyelination both *in vitro* and *in vivo*(1-3). That "ali-esterase" may be concerned in myelin degradation during the later stages of Wallerian degeneration has been suggested(4). Since multiple sclerosis is a disease characterized by demyelination, an investigation of the esterases of nervous tissues from cases of this disorder might be justified on the basis that abnormalities, if demonstrable, could be correlated with the

* Supported by grants from Public Health Service, and from Nat. Multiple Sclerosis Soc.

degradation of myelin lipid which accompanies the disease.

An additional, and alternative, rationale for the study of esterases in multiple sclerosis is the possibility that these enzymes may be concerned in normal myelin anabolism. For example, the localization of non-specific cholinesterase to glia and Schwann cells(5) and the diminution in activity of this enzyme in the plaques of multiple sclerosis(6) has suggested that it may play a role in myelin maintenance.

Moved by these considerations we have used the "zymogram"(7) technique in the study of water-soluble non-specific esterases and cholinesterases of cerebral white matter from 13 male cases of multiple sclerosis. Starch-gel electrophoresis has been by the vertical method(8). Staining procedures for esterase activity have been described(9).

Generally the cadavers were refrigerated within one hour of death. In 11 cases one hemisphere was fixed in formalin for histologic study while the other was placed in the deep-freeze at −15°C until removal (usually within 2 weeks) for dissection and biochemical study. In 2 cases the hemispheres were sectioned coronally and facing slices were used, one for histologic and the other for biochemical examination. White matter, judged macroscopically to be uninvolved by the disease, and plaque material, usually periventricular, were carefully dissected from the still-frozen specimen as it began to thaw at room temperature. The conditions of homogenization, centrifugation, concentration and electrophoresis were as reported previously(9). The present report concerns results obtained by use of the substrates alpha-naphthyl acetate and alpha-naphthyl propionate for demonstration of non-specific carboxylic acid esterases and butyrylthiocholine iodide for demonstration of cholinesterases.

In normal white matter, with alpha-naphthyl acetate as substrate, 11 bands of esterase activity are demonstrable consistently on the anodal side of the gel (Fig. 1). Three of these occur in a closely-approximated triplet and are, respectively, the 5th, 6th and 7th bands from the origin. A heavy doublet of activity constitutes bands 9 and 10. A faint band, numbered 8, is sometimes visible between bands 7 and 9. In some specimens additional minor zones of activity are apparent between bands 4 and 5 and 9 and 10. Brains affected by multiple sclerosis showed striking variations from this pattern. In plaque material from all but one case of this disease, bands 6 and 7 were absent or virtually so (Fig. 1). In the single exception, the staining intensity of bands 6 and 7 in plaque tissue was less than that of macroscopically normal white matter from the same specimen. In addition, in homogenates of plaques from 9 multiple sclerosis brains, the activity of band 9 was grossly evident to be much less than that of band 10 (Fig. 1). In contrast, in all normal brains examined, either activity in the 9-10 doublet appeared grossly to be equally divided between the 2 bands (Fig. 1) or band 9 contained the bulk of the activity. Of interest is the finding that macroscopically normal white matter showed an abnormality identical to that of the plaque in 11 of the 13 specimens.

When alpha-naphthyl propionate is the substrate, 3 to 4 dense bands are invariably demonstrable in homogenates of normal cerebral white matter (though not of gray matter) in positions between the 7th and 9th anodal bands of acetate preparations (Fig. 2). One of these is the faint 8th band alluded to in the description of the alpha-naphthyl acetate zymogram. The other bands developed with the propionate ester are absent from the usual acetate preparation, though they may be visualized after prolonged incubations. The 3-4 bands distinctive for the propionate zymogram of normal cerebral white matter were absent from homogenates of plaque tissue (Fig. 2) in all 5 cases where propionate was employed as substrate. In all but one of these 5 cases they were visualized in the macroscopically normal white matter.

The use of both alpha-naphthyl acetate and alpha-naphthyl propionate is associated with the development of 5 bands on the cathodal side of the gel. No abnormality in these enzymes has been demonstrable.

The patterns obtained with butyrylthio-

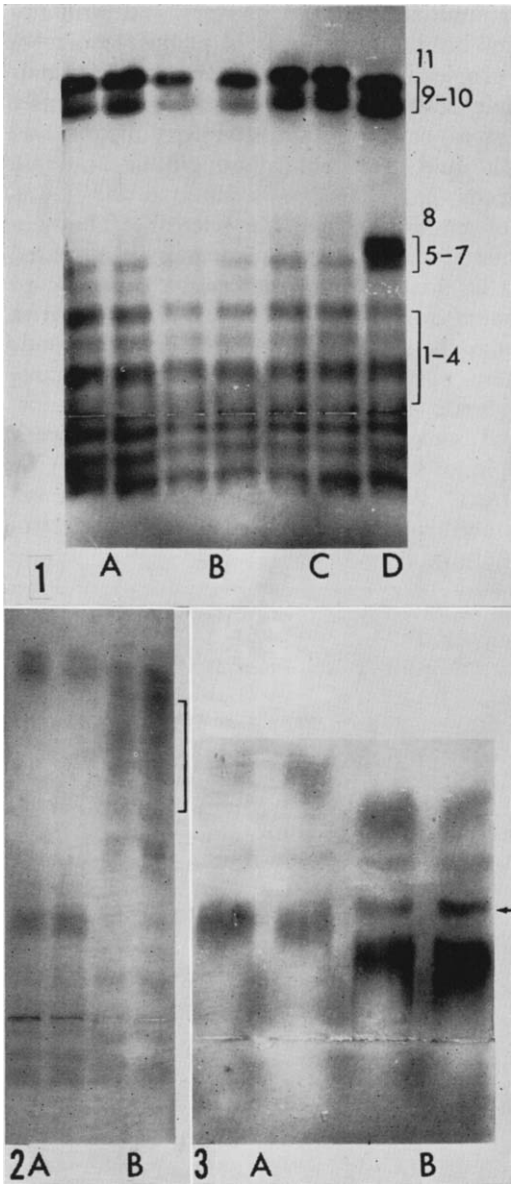

FIG. 1. The paired zymograms above A, B and C derive, respectively, as follows: macroscopically normal white matter of a multiple sclerosis brain; plaque tissue of the same case; macroscopically normal white matter of another multiple sclerosis brain. A single zymogram above D derives from white matter of a normal brain. Substrate is alpha-naphthyl acetate. Only the anodal bands are numbered. See text for description.

FIG. 2. Zymograms of multiple sclerosis plaque (A) and white matter of normal brain (B) with alpha-naphthyl propionate as substrate. Bands characteristic of propionate zymogram of normal white matter are included within bracket and are absent from the plaque.

FIG. 3. Zymogram of plaque (A) and macroscopically normal white matter (B) from a multiple sclerosis brain. Note overall loss of activity in A and absence of the band marked by arrow. Substrate is butyrylthiocholine iodide.

choline iodide are highly variable. A maximum of 4 bands is visible. All migrate anodally. One band is resistant to inhibition by eserine (10^{-5}M). When grossly normal white matter of a multiple sclerosis brain was compared with plaque tissue from the same case, a generalized diminution of activity in the plaque was apparent and one or more bands were absent (Fig. 3).

The Figures show that the enzymes of the plaque and macroscopically normal white matter of multiple sclerosis tissue exhibit an electrophoretic mobility which, under identical conditions of electrophoresis, differs from that of enzymes present in homogenates of white matter from normal brain.

The histologic data have been analyzed. A positive correlation has not been found between the histologic character of the plaques (cellularity, presence or absence of axis cylinder destruction, etc.) and the type of esterase abnormality occurring in a particular specimen. This negative finding is not surprising since macroscopically normal white matter, where little or no histologic abnormality is evident (as concluded from microscopic examination of fixed tissue from facing blocks), may show changes in esterase population identical to those of the plaque.

The possible specificity of these findings has now been examined in the light of data obtained over a period of more than 2 years from 36 normal specimens subjected to identical laboratory procedures. The normal brains have been obtained from patients dying of a variety of acute and chronic ailments without a history of neurologic disability other than the report, in a few instances, of obtundity or mild confusion 24-48 hours before death. In 6 of the 36 normal specimens activity in the 6th and 7th bands of alpha-naphthyl acetate and propionate preparations has been diminished or absent. Findings to date suggest that this variation from the usual pattern bears a positive correlation with senility.

Absence of or diminution in activity of

the 6th and 7th anodally-migrating bands of the naphthyl acetate zymogram are, then, not specific for multiple sclerosis. However, relative diminution in activity of band 9 of the 9-10 doublet and the loss from plaque tissue of those enzyme activities against alpha-naphthyl propionate which are characteristic of normal white matter, are, to date, specific findings and seem of special interest. Because of the variations that occur in normal specimens, the significance of observed changes in cholinesterase zymograms of multiple sclerosis brain is difficult to evaluate.

No increase in esterase activity of multiple sclerosis tissue has been detected by densitometric analysis of zymograms or by quantitative assays of tissue homogenates(10). Experiments employing long-chain compounds (laurate and myristate esters of alpha- and beta-naphthol) have not disclosed the presence of a lipase activity(10). Presumably, if the abnormalities found in the esterase population of multiple sclerosis tissue have basic importance in the genesis of the disease they are related, in some way, to a defect in the normal metabolism of myelin.

Summary. Vertical starch-gel electrophoresis has been used for preparation of esterase zymograms of postmortem specimens of human brain. The specimens derived from 36 cases without a history of significant neurologic disability and 13 cases of multiple sclerosis. Certain esterases hydrolyzing alpha-naphthyl propionate were consistently demonstrable in, and characteristic of, zymograms of white matter from normal specimens. These were absent from zymograms of multiple sclerosis plaques. Alpha-naphthyl acetate zymograms of plaques and macroscopically-normal white matter from multiple sclerosis specimens showed frequently loss of, or diminution in activity of, the 6th, 7th and 9th anodally-migrating esterase bands, but alteration in bands 6 and 7 was not specific for multiple sclerosis. The cholinesterase activity of plaque tissue appeared to be diminished in comparison with that of macroscopically-uninvolved white matter from the same case, and one or more bands were absent in the plaque. The electrophoretic mobility of esterases of the plaque, and sometimes of grossly uninvolved multiple sclerosis white matter, appeared to be greater than that of normal specimens examined under identical conditions of electrophoresis.

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Received March 7, 1963. P.S.E.B.M., 1963, v113.

Myocardial Catecholamine Contents in Vitamin E and Sulfur-Deficient Chickens.* (28416)

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Changes in myocardial function have been reported in chickens made dystrophic through Vit. E deficient diets(1). The basis for this cardiac dysfunction is not clear. Since alterations in catecholamine content of the heart have been implicated in the pathogenesis of

* Supported by grants from U.S.P.H.S. and from John A. Hartford Foundation.