In a final paper it will be shown that while the nervous system and other tissues of dogs dying from chronic phenobarbital poisoning contains no constantly occurring lesion typical of phenobarbital poisoning, phenobarbital produces a profound vascular disturbance in those tissues. In dogs a marked degree of tolerance to phenobarbital may be built up. After a period of pronounced irritability and sleeplessness, following withdrawal of the drug, clinical recovery takes place. The question of whether histological recovery parallels clinical recovery remains to be studied.

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### Inoculation of Mice with Poliomyelitis Virus.

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Nungester[1] demonstrated that when poliomyelitis virus was combined with mucin and injected intraperitoneally into 76 mice, death occurred in 22, muscular weakness in 13 and flaccid paralysis in 2 animals. The brains and cords of 12 of the 22 mice that died and 7 controls were examined by Weil.[2] In 2, the picture seen closely resembled the histopathology found in *M. rhesus* monkeys that died of poliomyelitis. These results were sufficiently important to encourage further work along the same line.

Commercial gastric mucin powder was sterilized dry by autoclaving, a 5% emulsion prepared and used as a diluent to make up a 2% suspension of potent poliomyelitis virus. All injections were made the same day that the mixture was prepared and each mouse received 1 cc. intraperitoneally. The results are tabulated in Table I. In controls, the diluent was either normal saline, normal cord in mucin, or mucin in saline. In one group, the diluent for the mucin and virus was convalescent poliomyelitis serum.

All mice of group I that died following the inoculation of virus and mucin showed a small amount of clear, straw-colored fluid in the peritoneal cavity. The upper part of the small intestine was swollen and yellow reddish brown in color. There was slight or

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1 Nungester, W. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1128.

2 Weil, W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1242.

TABLE I.

| Group | Injection Material | No. of Mice | Deaths | % of Deaths |
|---|---|---|---|---|
| I | 2% virus in 5% mucin | 57 | 23 | 40.+ |
| II | 2% " " normal saline | 30 | 1 | 3 |
| III | 2% normal cord in 5% mucin | 30 | 4 | 13 |
| IV | 5% mucin in normal saline | 15 | 0 | 0 |
| V | 2% virus, 5% mucin and convalescent serum | 30 | 5 | 17 |

moderate hyperemia of the lungs. A few had enlargement of the spleen and hyperemia of the liver. Autopsy examination of the mice of groups II and III showed their organs to be normal, while those of group V showed some swelling and redness of the small intestine, but it was distinctly less marked than that seen in the animals of group I.

The spinal cords, lungs and livers of all animals that died were examined microscopically. The lung and liver sections were stained with hematoxylin and eosin, and the cord by Nissl's stain.

*Cords.* Sections of the cords of 11 animals of group I showed some round cell infiltration and neuroglial increase throughout the gray matter. The capillaries were noticeable, but none of the sections showed perivascular cuffing. The anterior horn cells were absent in many specimens and where they were present, both nucleus and cytoplasm showed disintegrative changes. No hemorrhages were noted. In sections of the cords of 2 animals that died in group III, the findings were the same as in group I. One specimen was normal. Sections of 3 of the cords of those dying in group V showed round cell infiltration that was almost as severe as that of group I.

*Lungs.* Microscopic examination of the lungs of those dying in group I showed generalized capillary dilatation and engorgement, which encroached upon the alveolar spaces without any fixed tissue proliferation. The bronchial mucosa was normal and there was no exudate. Pneumonia was found in the lungs of 2 of the 4 animals that died in group III. A slight hyperemia was found in the lungs of 3 animals of group V.

*Liver.* The animals that died in group I had intense capillary dilatation and engorgement of the central veins with focal collections of round cells in the parenchyma and in the interlobular spaces, especially around the bile ducts. The cytoplasm of the liver cells showed diffuse granular degeneration, indistinct cell boundaries, and a shrinkage of the nuclei with occasional fragmentation. In a few sections, lobular degeneration had occurred and had gone on to necrosis. These reactions, however, were seen in sections of the

liver from animals of other groups, though comparatively less in extent.

Twelve animals were sacrificed to obtain normal sections. Four of the 12 supposedly normal liver sections had focal collections of round cells in the perilobular position, although they were comparatively less extensive than those seen in sections of those animals that died after injections of the mucin and virus mixture. Sections of other organs were negative.

Poliomyelitis virus in combination with gastric mucin caused death of less than half of the animals injected; the combination was 3 times more lethal than normal cord in mucin and 13 times more lethal than virus in saline.

Thirty animals were protected with human convalescent poliomyelitis serum. Three specimens of convalescent serum were tried, each sample being tested in 10 animals. Complete protection was had by the 20 animals protected with the first 2 specimens. The third specimen may not have contained neutralizing antibodies, since death occurred in 5 of the 10 mice protected with this serum. Unfortunately all the serum of the third specimen had been used and the point as to whether or not there were any poliomyelitis neutralizing antibodies present could not be settled.

Since only 40+% of the mucin and virus injected animals died, it is quite clear that mice cannot be used to test poliomyelitis virus where a 100% response is necessary. Our results might be changed by varying dosages to get a proper M.L.D. and further work would have been done along this line but for the fact that normal cord and mucin gave a comparable picture in 13% of the animals and for the fact that subsequent injections of another group of 20 animals with the same virus percentage from another lot of virus caused the death of only 4 animals. None of the controls of the latter group died.

Ten monkeys were injected intracerebrally with varying percentages and amounts of single and combined mouse cord suspensions, but the disease was not produced.

We confirm the observations of Nungester[1] that mucin added to poliomyelitis virus sometimes accentuated its potency when injected into the mouse. It is clear, however, that this animal cannot be used for experiments with poliomyelitis virus because of the inconstant results. The pathological sections examined by us were not distinctive enough to be considered typical. We were unable to infect monkeys with the cord emulsions of the mice that died.