I produced paralysis in the test monkeys in all cases but one. This latter monkey had the non-paralytic form of the disease as evidenced by a negative clinical course coupled with poliomyelitic lesions in its spinal cord.

Comment. The attempt will not be made to determine the significance of finding virus in the blood of 4 of 10 paralyzed monkeys. There is no reason to suspect that this represents an extra-neural pathway for the primary distribution of virus in the body; it may well be the direct result of the overflow or of the breakdown of virus-laden nerve cells. The distribution of lesions[9] and of virus[10] only in certain regions of the central nervous system both in the experimental and human disease would lead one to favor the view that virus in the blood does not play a predominant role in determining the location of virus within the central nervous system, once the latter has gained access to this tissue. In these experiments the presence of *detectable* amounts of virus in the blood stream was neither a prerequisite nor a predisposing factor for the appearance of *detectable* amounts of virus in the colon contents during the acute disease.

All of the positive results were found in monkeys inoculated with one strain (Hartford) of poliomyelitis virus. Three tests on two other strains were negative. This finding may recall the fact that certain strains, usually in their early passages, are more infectious by the cutaneous route than are others.[11,12]

Summary. Of 10 monkeys, paralyzed after being inoculated with poliomyelitis virus of recent human origin, three rhesus and one capuchin monkey were found to have the virus in the blood stream. All of the positive tests in the blood were due to a single strain. The colon contents of three of these latter monkeys were examined for virus and it was found in only one instance. Of 4 monkeys in whose blood virus could not be detected, one was found to have virus in its colon contents.

[9] Howe, H. A., and Bodian, D., *Neural Mechanisms in Poliomyelitis*, New York, 1942.

[10] Sabin, A. B., and Ward, R., *J. Exp. Med.*, 1941, **73**, 771.

[11] Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

[12] Trask, J. D., and Paul, J. R., *Science*, 1938, **87**, 44.

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Effect of Aspergillic Acid on Equine Encephalomyelitis Virus.*

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During the current outbreak of equine encephalomyelitis among horses in the Southwest, a few human cases occurred.[1] This prompted study on treatment of the disease, since no effective therapeutic agent is available. Although antiserum may be effective in the treatment of horses when administered during the early stages of the disease, such therapy is of little value in man because the central nervous system lesions are usually too far advanced at the time when the diagnosis of encephalitis is made. A study of the effect of aspergillic acid on a neurotropic virus seemed of interest because, to our knowledge, no reports have appeared in the literature concerning the effect of this substance on viruses, despite its efficacy on a wide variety of bacteria.[2] Also, there are indications that this drug diffuses rapidly into the central

* This work was aided by a grant from Eli Lilly and Company, Indianapolis, Indiana.

[1] Sulkin, S. E., and Ashworth, C. T., to be published.

[2] Jones, H., Rake, G., and Hamre, D. M., *J. Bact.*, 1944, **45**, 461.

TABLE I.
In vitro Effect of Aspergillic Acid on Equine Encephalomyelitis Virus (Western Type).

Experiment No.	Procedure*	Dil. of virus suspension	Results§
1	Virus and aspergillic acid†	10^{-7}	2,2,2,2
	" alone (control)		2,2,2,2
2	" and aspergillic acid	10^{-8}	3,S,S,S
	" alone (control)		2,2,3,S
3	" and aspergillic acid	$10^{-8.5}$‡	3,3,3,4,5,S,S,S,S,S
	" alone (control)		2,3,3,3,3,5,S,S,S,S

* Virus and drug shaken at room temperature for 3 hours; mixture injected intracerebrally into 3-week-old mice (inoculum 0.03 ml).
† 1 mg aspergillic acid per ml virus suspension.
‡ Intracerebral LD_{50} titer.
§ Each figure represents one mouse and the day of death. S = mouse survived.

nervous system when injected intraperitoneally into mice.[3]

The following experiments are concerned primarily with the *in vitro* effect of aspergillic acid* on the virus of equine encephalomyelitis. The Western strain of equine encephalomyelitis virus obtained from Dr. W. McD. Hammon in 1943 was used. The virus has been maintained by intracerebral injection in mice. Before the experiments were started several passages were made in mice to insure a high virulence. The cerebral titer was determined by the 50% end point of death of 3- to 4-week-old mice.

In the first experiment a 10^{-7} dilution of infected mouse brain from a moribund animal was prepared, and aspergillic acid was added as the sodium salt in the arbitrary amount of 1 mg per ml of virus suspension. The virus and drug mixture, together with virus suspension alone, which served as control, were shaken at room temperature for 3 hours. The virus and drug mixture, together with virus suspension, was then injected intracerebrally into 3-week-old Swiss mice (inoculum 0.03 ml). A titration of the virus suspension was carried out simultaneously; the intracerebral LD_{50} was found to be approximately $10^{-8.5}$/0.03 ml. The aspergillic acid appeared to have no effect whatever on the virus suspension used, since all animals inoculated with the virus-drug mixture died within 2 days, as did the controls receiving virus alone (Table I). All animals showed typical symptoms of encephalitis. Since the concentration of virus used in this *in vitro* experiment seemed too severe an inoculum, the number of lethal doses was reduced. In the second experiment, the drug was added to a 10^{-8} dilution of mouse brain and the procedure was repeated as above. Three out of 4 animals receiving virus-drug mixture survived the observation period of 15 days, while only one of the animals receiving virus alone survived. These results prompted us to undertake an experiment using a larger number of animals. In this experiment (No. 3) the drug was added to a $10^{-8.5}$ dilution of virus (LD_{50}), and the procedure was repeated as above. The results of this experiment indicate that the differences obtained in experiment 2 were probably due to chance.

In conclusion, then, these experiments indicate that a contact for 3 hours at room temperature with aspergillic acid had no effect on the infectivity of the equine encephalomyelitis virus.

Although it seemed improbable that favorable therapeutic results could be expected from the use of aspergillic acid, one such experiment was conducted. Twenty mice were infected by intracerebral inoculation of 0.03 ml of 10^{-7} suspension of Western type of equine encephalomyelitis virus. This dose of virus was

[3] White, E. C., and Hill, J. H., *J. Bact.*, 1944, **45**, 433.

* Obtained from the Squibb Institute for Medical Research, New Brunswick, N. J., through the courtesy of Miss Clara M. McKee.

used because in man the virus is usually present in high concentration before the diagnosis is made and therapy instituted. Ten mice received aspergillic acid in the amount of 50 mg per kilogram intraperitoneally immediately following the inoculation of the virus and twice daily thereafter. All mice died within 3 days, the treatment thus being completely ineffective.

Conclusion. Aspergillic acid in high concentration had no effect *in vitro* on the infectivity of the Western type of equine encephalomyelitis virus. There was also no effect on the course of the infection in mice.

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Relation of the Milk Influence to the Carcinogenic Induction of Mammary Cancer in Mice.*

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The administration of carcinogens to mice of inbred strains may result in the appearance of epitheliomas, sarcomas or other tumors to which the animals show no spontaneous susceptibility. In certain strains in which lung tumors, hepatomas, leukemia, or mammary cancer appear spontaneously carcinogens have accelerated their onset.[1,2,3,4] This suggests that the synthetic carcinogen might, in these instances, potentiate the spontaneous mechanism of carcinogenesis.

Three known influences are of etiologic significance in the development of mammary cancer of the "genetic" type which occurs in high incidence in certain strains of mice.[5] These factors are (1) a genetic susceptibility, (2) an hormonal influence, and (3) a milk influence, which is a substance contained in the breast milk of high mammary cancer strains.

The relationship of the carcinogen-induced to the spontaneous neoplasm can be studied in the case of mammary cancer by using the dba strain, in which mammary cancer appears spontaneously and can also be induced by the administration of one of the carcinogenic hydrocarbons, methylcholanthrene.[6] The present experiments were designed to test the role of the milk influence in governing the response of the mammary tissue to carcinogens. Previous experiments with these mice demonstrated that the mammary gland stimulation coincidental with forced breeding was an important factor in conditioning the neoplastic response to methylcholanthrene.[7]

Strain dba males† were crossed with C3H females possessing the milk influence (Z stock) and with C3H females lacking the milk influence (ZB stock). The F_1 hybrid animals so obtained were observed as either virgin or as forced-bred females during treatment with methylcholanthrene. Breeding females of the genetic constitution (ZB♀ × dba♂)♀ × dba♂ were also skin-painted with methylcholanthrene. Of the factors necessary for the development of spontaneous mammary cancer,

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1 Andervont, H. B., *Pub. Health Rep.*, 1939, **54**, 152.

2 Strong, L. C., Smith, G. M., and Gardner, W. U., *Yale J. Biol. and Med.*, 1938, **10**, 335.

3 Mider, G. B., and Morton, J. J., *Am. J. Cancer*, 1939, **37**, 355.

4 Engelbreth-Holm, J., *Cancer Research*, 1941, **1**, 109.

5 Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1590.

6 Engelbreth-Holm, J., and Lefèvre, H., *Cancer Research*, 1941, **1**, 102.

† Sublines 12 and 212.

7 Kirschbaum, A., Lawrason, F. D., Kaplan, H. S., and Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 141.