fering with the ability of the virus to establish itself. It is known that thyroxin secretion is greater upon exposure to cold and that the metabolic rate is thereby increased. Hence, a series of investigations was begun on three groups of five-week-old mice inoculated intracerebrally with 0.05 ml. of a 3-per cent suspension of infected brain-cord suspension and held at room temperature (22° C.). One group was given a daily dose of 0.5 mg. of thiouracil by mouth, a second group received 0.5 mg. of thyroid extract by the same method of administration, and the third group served as controls. The amounts of thiouracil, a recognized basal metabolism depressant, and thyroid extract fed to the animals were arbitrarily employed after a limited series of experiments to determine toxic doses. There was no visible indication that the amounts used were toxic.

During the course of this experiment C. W. Turner, of the University of Missouri, suggested and supplied a thyroactive iodocasein prepared by the method of Reineke and Turner (*3*) and referred to by Koger and Turner (*2*) as thyroprotein. A second set of three groups of five-week-old mice was infected with virus. One group was given 0.5 mg. of thiouracil and another group, 0.5 mg. of thyroprotein daily by mouth. The third group served as controls.

The results obtained to date have been quite encouraging. Infected five-week-old mice treated with thiouracil have invariably shown symptoms of paralysis and have succumbed earlier than the controls, whereas those given thyroid extract or thyroprotein have undergone incubation periods much longer than those of the controls (Table 1). The lengthy incubation periods attained with thyroprotein treatment imply that it is much more effective than thyroid extract. However, this may be due to failure at the present time to employ these two agents in doses containing identical amounts of thyroactive substance.

TABLE 1

THE EFFECT OF THIOURACIL AND THYROACTIVE SUBSTANCES ON SUSCEPTIBILITY OF SWISS WHITE MICE TO POLIOMYELITIS VIRUS

Number of mice in each group	Type of treatment after inoculation	Incubation time (days) required to effect 50 per cent mortality in each group
20	0.5 mg. thiouracil daily	5
8	0.5 mg. thyroid extract daily	11
36	0.5 mg. thyroprotein daily	14
20	Nontreated controls	7

Attempts to measure the effect of thiouracil, thyroid extract and thyroprotein on metabolism of the mice, employing techniques adapted to small animals, have not proved highly successful, but there is an indication that the thyroactive substances increase the metabolic rate considerably. Hence, an earlier suggestion (*1*) that the marked decrease in the incidence of poliomyelitis with the onset of cooler weather may be due in part to a change in the metabolism of the host seems to have gained support.

The effect of altered metabolism on resistance to poliomyelitis virus needs careful study. Normally, four- and five-week-old mice tend to be the most susceptible to the virus. Experiments in this laboratory have shown that thyroactive substances create longer incubation periods in mice of these ages than in older animals. Possibly there is a critical metabolism range for virus growth that can be exceeded quite readily by use of thyroid stimulants in four- and five-week-old mice, whereas older animals may have their lower normal metabolic rate elevated to the critical range upon thyroid stimulation and thus become more susceptible than they normally are. When more is known about tissue metabolism and the safety with which thyroactive substances can be administered, it is possible that these agents may have prophylactic value for certain age groups during epidemic periods of poliomyelitis.

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The Failure of Poliomyelitis Virus to Grow in Certain Protozoa of Sewage[1]

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C. Kling, who is well known for his studies of poliomyelitis in Sweden during the past 35 years, has estimated from tests made in 1939 that the amount of poliomyelitis virus in the sewage from a section of Stockholm having a population of 100,000 could reasonably be accounted for only if 100 per cent of the people were excretors of the virus or if the virus grew in sewage. He has rejected the former possibility (*1*) and has suggested that the virus probably grows in some microorganism of sewage, the most likely one being, in his opinion, protozoa of the genus Bodo.

Unaware of Kling's observations, we were also led to speculate on the possibility that poliomyelitis virus might grow in some microorganism of sewage, and during the past several years we have investigated this question.

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Attention was first directed to aquatic protozoa in general, and simple tests for the growth of poliomyelitis virus in the protozoa and other microorganisms occurring in lake water, pond water, river water, and sewage were carried on during the poliomyelitis season (August and September) in 1944. Samples of water were collected in 150-ml. amounts and kept in finger bowls or Erlenmeyer flasks at 70° F., the approximate temperature of the waters when collected. Protozoa were found in the usual abundance, with some increase of certain varieties during a one-week period of incubation. A pool of 12 strains[2] of poliomyelitis virus was prepared as a 10-per cent suspension of monkey spinal cord. Fifteen ml. of this pool was added to 135-ml. specimens of freshly collected water, giving an initial dilution of 1:100. Seven days later 15-ml. samples were transferred to dishes containing 135 ml. of freshly collected waters from the same sources. Serial weekly transfers involving 10-fold dilutions were carried out to a final dilution of 1:100,000. One week after the last transfer, specimens from each dish were treated with ether and injected into a total of eight monkeys. No evidence of poliomyelitis developed in the inoculated animals. Parallel tests with two strains of Theiler's virus (New Jersey I and GD VII) and with Armstrong's strain of mouse-adapted poliomyelitis virus also gave negative results.

In subsequent work, cultures of specific protozoa have been employed, and only those kinds of protozoa that occur commonly in sewage have been studied. Surveys of the protozoa of the Minneapolis and St. Paul sewerage system during the summer and fall of two successive years have shown clearly that the genus Bodo includes the protozoa most regularly present in all parts of the system tested and that one species or another of the genus is usually the most abundant protozoan present. Frequently the Bodos outnumber all other protozoa combined. Numerous other genera are represented, but only a relatively few occur with sufficient frequency or in sufficient numbers as conceivably to be of importance as hosts of the poliomyelitis virus.

In the past year six strains of Bodo were isolated from different samples of sewage and tested for their capacity to support the growth of poliomyelitis virus. At the same time one strain of Monas, one of Oikomonas, and three crude cultures containing significant populations of Tetrahymena, Uronema, Monas, and Pleuromonas, and other protozoa in smaller numbers, were similarly tested.

Six strains of poliomyelitis virus were used, including one strain capable of growing in rodents, "MEF" (*2*); one strain represented by infective human fecal material, kindly supplied by John R. Paul; one highly virulent laboratory strain; and three strains that had been passed only a few times through monkeys since original isolation from man.

The procedure employed was to cultivate the protozoa in flasks in 10 ml. of fluid wheat extract on the surface of 25 ml. of agar containing minerals and organic nutrients. Cultures were incubated in dim light at 70° F. Following each transfer, protozoan populations increased during a period of several days to a maximum of from 100,000 to 50,000,000 or more in each milliliter of culture. In our observations of raw sewage the total number of protozoa of all kinds has usually been from 20 to 100/ml.

Virus was added initially to the protozoan cultures as 0.25 ml. of a 10-per cent suspension of infected spinal cords, giving an initial dilution of 1:400. At intervals of 5 and 14 days (two experiments) subcultures were made by transferring 0.1 ml. to a freshly prepared flask. In this manner a 1:100 dilution of virus was accomplished with each transfer, so that after two subcultures the original virus was diluted to 1:4,000,000. In a control titration, none of the six strains of virus had infected monkeys in a dilution of 1:2,000,000. Samples of fluid taken from the third set of cultures (second subcultures) were treated with ether and by low-speed centrifugation to remove bacteria, and were tested for poliomyelitis virus by intracerebral injection of monkeys.

It was believed that no method of concentrating virus need be employed because the populations of protozoa in the cultures so greatly exceeded those in sewage that any growth of virus to the extent that would be significant in sewage should be easily detected.

Three monkeys were injected with material pooled as to protozoan culture in the first test (subcultured at 5-day intervals). In the subsequent tests, pools of all cultures of "MEF" virus, "Stool" virus, and "Virulent" virus were prepared separately and injected into different monkeys. Results were uniformly negative. Protozoan cultures to which the "MEF" "Stool," and "Virulent" viruses had been added were carried through one additional transfer in the 14-day series and were pooled and concentrated approximately 40-fold by centrifugation at 40,000 r.p.m. for one hour. Again three monkeys were injected with negative results.

Bacteria present in the protozoan cultures represent a complicating feature that would be difficult to avoid. However, bacteria are abundantly present in sewage, and we have found that the GD VII strain of Theiler's virus (which resembles poliomyelitis virus in stability)

[2] The MV, Hartford, and McKay strains plus 9 others, most of which had been passed only a few times in monkeys since originally isolated.

survives relatively well in sewage kept at 70° F., in spite of very considerable increases in bacteria during storage.

It is concluded that in our experiments the naturally occurring microorganisms in water from a pond, a lake, and a river failed to yield significant increase in several strains of poliomyelitis virus, and that six strains of Bodo, two of Monas, and one each of Pleuromonas, Oikomonas, Tetrahymena, and Uronema, derived from sewage, failed to support the growth of poliomyelitis virus to an extent that would be significant with reference to the finding of the virus in sewage.

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Influence of Anesthesia on Experimental Western Equine Encephalomyelitis[1]

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Past attempts at therapy in neurotropic virus diseases have been largely unsuccessful. The results have been essentially negative because once the symptoms of a virus disease have become evident, the virus is already well established within the host cell. Therapeutic agents are therefore unable to gain access to the virus, with the result that treatment is of little value. Nevertheless, there have been suggestive positive results with both serotherapy and chemotherapy in this group of diseases. Zichis and Shaughnessy (*14*) reported successful treatment of experimental western equine encephalomyelitis infections using hyperimmune rabbit serum, but their observations have not been fully confirmed (*8*). Wherever positive results have been obtained, early administration of potent antiserum has been found to be essential. Successful chemotherapy has been limited to the so-called lymphogranuloma venereum-psittacosis group (*10*), which, however, some workers place intermediate between true filtrable viruses and rickettsiae. Generally, in true virus diseases, chemotherapy has been ineffective (*5*).

The ideal therapeutic agent for use in the treatment of neurotropic virus diseases would bring about a reversible change in the metabolism of the host cell sufficient in degree and duration to destroy the virus without causing permanent injury to the host cell, and must have predilection for cells of the central nervous system. General anesthetics seem to fall within this category. Our interest in the use of anesthetics in neurotropic virus diseases was further prompted by a number of reports on the influence of anesthesia on the course of several other diseases affecting the central nervous system. Bronfenbrenner and Weiss (*1*) noted that anesthetics, alone and in combination with specific antitoxin, decreased mortality in experimental botulism. Similarly, avertin (or tribromethanol) has been used to alleviate muscular spasms in tetanus (*4, 7*).

The *in vitro* effect of ether on viruses has been observed by several workers. When used in relatively high concentrations, this anesthetic is an effective bactericidal agent and can be used *in vitro* to destroy bacterial contaminants in tissues infected with poliomyelitis (*6*), rabies (*12*), and measles (*9*) without affecting the virus. The method, however, is not applicable in removing contaminants from tissues infected with St. Louis encephalitis or equine encephalomyelitis viruses (*2, 3*).

In the present studies a strain of western equine encephalomyelitis virus, obtained from W. McD. Hammon, was used. The virus was maintained by intracerebral injection of Swiss mice. A 20-per cent suspension of infected mouse brain tissue was prepared using 10 per cent sheep serum-broth as diluent. The supernate, removed after centrifugation, was dispensed in 0.2-ml. amounts in sterile glass ampules which were then sealed, frozen quickly, and stored in the deep-freeze cabinet. Under these conditions the titer of virus remains constant for several months. The quantity of virus present in the stock suspension was determined by intracerebral inoculation of three- to four-week-old mice, and the 50-per cent end point (LD_{50}) was calculated according to the method of Reed and Muench (*11*).

In the experiment to be described each three- to four-week-old Swiss mouse was inoculated intracerebrally under light ether anesthesia with 0.03 ml. of a $10^{-8.5}$ dilution of stock virus suspension (approximately $3 \times LD_{50}$). In order to avoid errors due to sequence of inoculation, all inoculated mice were placed in one large cage and then separated at random into three groups. Group I served as controls and received no subsequent treatment. Group II received three four-hour periods of diethyl ether anesthesia during the first 36 hours after intracerebral inoculation, while Group III received two four-hour periods of ether anesthesia beginning at the time the animals first showed symptoms of encephalitis. The open method was used for the administration of the anesthetic. All animals were observed for central nervous system symptoms and death from encephalitis during

[1] The study of which this is a preliminary report was aided by a grant from the National Foundation for Infantile Paralysis, Inc.