TABLE III.
Toxicity Indexes of Iodine Alone and in Combination with an Oxidation-Reduction System.

Solution	Killing dilution			Toxicity index	
	Tissue (A)	*Staph. aureus* (B)	*Eberthella typhosa* (C)	*Staph. aureus* A/B	*Eberthella typhosa* A/C
Iodine alone	1:4,000	1:20,000	1:17,500	0.20	0.23
O-R solution alone	1:2,000	1:75	1:1,000	26.60	2.00
Iodine + 1,3000 o-r	1:3,750 I	1:80,000 I	1:60,000 I	0.05	0.06

TABLE IV.
Toxicity Indexes of Iodine in Combination with an Oxidation-Reduction System (O-R II).

Solution	Killing dilution			Toxicity index	
	Tissue (A)	*Staph. aureus* (B)	*Eberthella typhosa* (C)	*Staph. aureus* A/B	*Eberthella typhosa* A/C
Iodine + 1:3,000 o-r II	1:5,000	1:120,000	1:50,000	0.042	0.1

mixture of one mole each of manganous sulfate and ferric sulfate) killed *Staph. aureus* in a dilution of 1:80,000 and *E. typhosa* in a dilution of 1:60,000, both in the absence of organic matter. In the presence of organic matter the killing dilutions were 1:5000 and 1:4500 respectively.

A dilution of 1:2000 of the o-r solution killed embryonic chick heart tissue fragments in 10 minutes at 37°C. Iodine dissolved in a 1:3000 o-r solution killed the tissue fragments in a dilution of 1:3750 of iodine.

Toxicity indexes may be calculated by dividing the highest dilution of iodine required to kill the tissue by the highest dilution required to kill the test organism under the same conditions. The Toxicity Index of iodine + 1:3000 o-r solution was 0.05 for *Staph. aureus* and 0.06 for *E. typhosa*. The Toxicity Index of iodine alone was 0.2 for *Staph. aureus* and 0.23 for *E. typhosa*. Theoretically, an index less than one means that the germicide is more toxic to bacteria than to tissue; an index greater than one means that the germicide is more toxic to tissue than to bacteria. The smaller the index the more nearly perfect the germicide.

When iodine was dissolved in a 1:3000 o-r solution composed of manganous and ferric chlorides instead of the sulfates, the Toxicity Indexes were 0.042 for *Staph. aureus* and 0.1 for *E. typhosa*.

The importance of this observation is discussed.

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Attempts to Find Poliomyelitis Virus in Fish.*

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Each year since 1932 we have searched in vain for poliomyelitis virus in the gastrointestinal contents of fish caught in waters near epidemic areas with consistently negative results. There might have been several reasons for our failures. (1) The gastrointestinal contents used in our experiments

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could not always be rendered completely sterile and the pyogenic infection which usually followed intracerebral injections at that time may have overshadowed any effects from the virus that might have been present. (2) We might not have been fortunate enough to have obtained the fish from the place harboring the virus.

The first difficulty was solved when Kramer *et al.* showed that ether would kill organisms in the stool, but not appreciably affect the virus present. The 1941 epidemic of poliomyelitis in Cleveland began during the summer in the northeastern section of the city not far distant from Lake Erie. It also appeared about the same time in several isolated spots in the northeastern section of the state bordering the lake.

Fish were caught in waters infected by sewage drainage. Three such localities along Lake Erie were investigated in 1941, namely, polluted Brook D along the banks of which flies were collected and which carried the virus, and the mouths of two small rivers which emptied into Lake Erie.

The gastrointestinal tracts of several fish (mostly carp) were milked free of their contents, to which anesthetic ether was added to approximately 13% of the total volume. The ether was drawn off by vacuum. If the material was not sterile after standing, the procedure was repeated. The final sterile supernate was injected into 2 *M. mulatta* monkeys as follows: 40 cc intraperitoneally (I.P.) 5 times, 1 cc intranasally (I.N.) 13 times, 1 cc intracerebrally (I.C.) 2 times. One animal became furred. The other had weakness of one side, probably traumatic. The microscopic sections of its lumbar cord showed destruction of anterior horn cells, but no typical vascular reaction. Transfer attempts were negative. This and 2 subsequent experiments were negative.

Later the contents of the gastrointestinal tract obtained from at least 4 dozen fish, caught at the mouth of one of the rivers, were combined, centrifuged and the supernate treated with ether. The material was placed in a "Visking" sack and concentrated by fanning. On 10/3/41, monkey I was injected as follows: 40 cc I.P., 10 cc S.Q., 1.0 cc I.N., and 1.0 cc I.C. Seven days later, the animal had weakness in the legs; it was clumsy on the 13th day and on the 14th day it was sacrificed.

The second monkey was injected on 10/20/41 with a suspension made from the cord obtained from the first animal. The doses were: 1 cc I.C., 1.0 cc I.N, and 30 cc I.P. Two days later, a similar set of injections was given. The animal had a tremor and became slow and deliberate in its movements; on the 5th day, it had weakness in both legs. It was sacrificed on the 14th day. On 11/22/41, 1 cc of a 20% unpurified suspension of its lumbar cord was injected I.C. into another animal (monkey III). Five days later the muscles of the arms and left leg were week and on the 7th day the left leg was dangling. On the 7th and 15th days, it was given another injection I.C. with no added results. The animal was sacrificed on the 25th day. Sub-transfer of its cord into another animal (monkey IV) was negative.

On 11/26/41, a large batch of fish was caught in harbor F. The guts were prepared in the usual way and injected into a monkey (V) on 12/1/41. It received 30 cc I.P., 30 cc S.Q., and 2 cc I.N. On the 11th day, the animal had weakness of the right leg, but it did not seem typical and it recovered completely. It was reinjected with the same dosage of material on 12/5/41, observed for 44 days and sacrificed. The microscopic sections of the cord were negative.

Although paresis was found in monkeys I and II and paralysis in monkey IV, there was no evidence that these conditions were due to poliomyelitis virus.

Subsequent attempts to obtain virus from fish caught in (a) Lake Superior, (b) Lake Michigan, and (c) on the Canadian side of Lake Erie were failures.

Our next experiments were directed toward determining whether fish possess natural neutralizing antibodies against poliomyelitis virus. The scavenger carp was selected for this work. Blood was obtained and neutralization tests made in all instances by adding the questionable neutralizing antiserum, as was, to 10% poliomyelitis virus of known potency. The virus used in all experiments was Flexner M.V. strain. *M. mulatta* monkeys were used

as the test animal. Cotton rats and Flexner M.V. strain adapted to this species were used in the final experiment only. In 6 experiments, no neutralizing antibodies were demonstrated in the blood specimens of 6 carp, indicating possibly that carp do not develop antibodies because either they are never exposed or are naturally resistant to poliomyelitis virus.

We next tried to infect carp artificially. There were difficulties in obtaining live fish, or at least partly frozen fish that would survive. The iced fish that arrived all winter could not be easily revived. It was not until the spring of 1942 that we obtained carp for our purpose. There was some difficulty in keeping them alive and every one of several batches died, even though our tank was large and kept filled with water running constantly. Our city water is heavily chlorinated in the springtime. We were advised to paraffin the sides of the copper tank, to fill it with water and to allow it to remain there 24 hours, and then to start the current going slowly. Eight fish, weighing about 3 pounds each, were placed in the tank prepared in this manner. They all lived. Two of the fish were sacrificed. Their stools were tested for virus and their bloods for neutralizing antibodies. The results were negative.

The 6 remaining fish were fed virus in various ways—in balls of bread, whole pieces of cord, etc. Finally, the virus was merely ground up and poured into the tank, after which the water became milky white in color. The water drain was plugged for a few days and the running water stopped so that the virus material would not be dispersed. Two months later 2 fish were killed. Tests were made for neutralizing antibodies and a search was made for virus in the gastrointestinal tracts. Again, the results were negative.

The fish left were tested 2 months later and again neutralizing antibodies were not found in the blood serum. Three months passed and one fish was sacrificed. There was neither evidence of virus in the gastrointestinal tract nor evidence of neutralization antibodies in the blood. One fish was accidentally killed.

The 2 remaining carp were given 1 cc of 10% virus suspension intraabdominally every 10 days for 10 doses, starting on 11/25/42. The fish were bled on 5/26/43 and on 11/27/43. Neutralization tests of the blood serums were made in monkeys with negative results. Since antibodies may appear over one year later in some species under certain circumstances,[1] the fish were again bled on 6/11/44, about a year and a half after immunization had been started. Once more the results were negative. Cotton rats were used in this experiment (10 controls injected with a mixture of virus and saline, and 10 with a mixture of virus and immune serum). All of the animals had paralysis and died, these experiments also being negative. It is most unusual that the virus antigen did not produce serum antibodies in the fish when it does so easily in other species. It raises most interesting speculations as to why they are immune.

Thus, we were not able to demonstrate the presence of virus in the carp. Nor could we with the methods and the amounts used immunize these fish to the point where they would show neutralizing antibodies; neither could we demonstrate live virus in their stool contents.

[1] Toomey, John A., *Am. J. Dis. Child.*, 1937, **53**, 1492; 1938, **55**, 1261.