following liver injury occurs only when there is a change (from normal) in the total lipid content of the organ.

Summary. Liver injury in rabbits resulting from the administration of arsenicals in the form of arsphenamine and neoarsphenamine cause no significant changes from normal in the amount or distribution of the liver lipids.

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Effect of Chlorination of City Water on Virus of Poliomyelitis.*

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Water was considered in early reports concerning the transmission of poliomyelitis. This method of spread seemed unlikely when later experimental evidence favored an air-borne infection entering the host through the olfactory tract. However, Kling,[1] observing European epidemics, reconsidered the question and additional evidence was accumulated incriminating water as a factor in the spread of the virus.

Poliomyelitis virus was found in human feces as early as 1912[2] and these observations have been amply confirmed. Unfortunately the technic of Sawyer[3] requiring a second monkey passage as an important criterion to verify the presence of the virus was ignored until 1938. In that year, Trask, Vignec, and Paul,[4] and Kramer, Hoskwith, and Grossman[5] improved the technic of virus isola-

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1 Kling, C., *Bull. Office internat. d'hyg. pub.*, 1928, **20**, 1779.

2 Kling, C., Petterson, A., and Wernstedt, W., *Communication Inst. méd. État*, Stockholm, 1912, **3**, 5.

3 Sawyer, W. A., *Am. J. Trop. Dis. and Prev. Med.*, 1915, **3**, 164.

4 Trask, J. D., Vignec, A. J., and Paul, J. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 147.

5 Kramer, S. D., Hoskwith, B., and Grossman, L. H., *J. Exp. Med.*, 1939, **69**, 49.

tion and included serial passage in monkeys. Using the new procedure Paul, Trask, and Gard[6] detected the virus in sewage in the Charleston and Detroit epidemics of 1939. In addition, Kramer, Gilliam and Molner[7] isolated the virus from stools of healthy contacts in a Detroit institutional outbreak. Others[8-12] during the past year have succeeded in isolating the virus and carrying it through a second animal passage.

The presence of the virus in human intestinal discharges led Levaditi, Kling and Lépine[13] to investigate the effect of chlorination. A concentration of 4 parts per million (ppm) destroyed the virus in a cloudy tap water emulsion of infected monkey cord in 24 hours; 0.40 ppm was equally effective with clarified preparations. The pH and temperature of the emulsions were not recorded. They concluded that chlorination by the usual methods was virucidal. This work is lacking in two essentials, namely, the minimum effective chlorine concentration, and the shortest effective contact period. The chlorine concentrations and contact periods they used were considerably in excess of those employed in this country. Because of lack of data on the virus-inactivating effects of chlorination as usually practiced, the problem was reinvestigated.

Fresh water was obtained for each experiment from the Ann Arbor Water Softening Plant where the water is treated by the ammonia-chlorine process in which chlorine is present as chloramines. The chlorine content of the water was determined by the ortho-tolidine test. Reducing substances in the water did not interfere with its accuracy. The MV virus was selected for these experiments. It had a minimal infective dose of approximately 0.001 g of spinal cord.

The suspensions to be tested were prepared by making a 10% emulsion of infected spinal cord in saline with subsequent centrifugation at 4,500 rpm (radius 10 cm). The supernatant contained a minimum amount of organic matter. This was desirable since

6 Paul, J. R., Trask, J. D., and Gard, S., *J. Bact.*, 1940, **39**, 63.

7 Kramer, S. D., Gilliam, A. G., and Molner, J. G., *Public Health Rep.*, 1939, **54**, 1914.

8 Lépine, P., and Sédallian, P., *Comp. rend.*, 1939, **208**, 129.

9 Toomey, J. A., *Arch. Ped.*, 1939, **56**, 693.

10 Howe, H. A., and Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 538.

11 Stimpert, F. D., personal communication.

12 Kempf, J. E., and Soule, M. H., unpublished.

13 Levaditi, C., Kling, C., and Lépine, P., *Bull. Acad. de méd.*, Paris, 1931, **105**, 190.

Mallman[14] and Fox[15] demonstrated a protective effect on bacteria of organic matter in the presence of chlorine.

In experiment 1, 2.0 cc of the supernatant were added to 100.0 cc of chlorinated water and the same amount to a distilled water control. The initial chlorine content at the time of adding the virus was 0.58 ppm; this dropped to 0.10 ppm in an hour at which time 2.0 cc of each solution were injected intracranially into monkeys. Both animals developed quadriplegia, the control in 6, the other in 8 days.

Experiments 2 and 3 (Tables I and II): One part of the virus suspension was diluted with 165 parts of chlorinated water; a distilled water control was also prepared. Five minutes later the chlorine content, temperature and pH were determined. At stated intervals, monkeys were inoculated as in Experiment 1. The temperature readings were made at the time of each monky injection.

Attention should be directed to the persistence of a chlorine

TABLE I.
Effect of Chlorination on MV Virus in 1:1650 Dilution.†

Monkey No.	Chlorine concentration, ppm: Original Conc.	5 min after exposure to virus	At time of inoculation	Contact period, hr	Neurological signs	Time of onset, days
3	(Control)				Quadriplegia*	12
4	0.55	0.55	0.50	1½	"	35
5	0.55	0.55	0.35	4	Negative	
6	0.55	0.55	0.20	10	"	
7	0.55	0.55	0.05	24	"	

*Histopathological picture was compatible with that of acute poliomyelitis.
†Temperature 21-24°C; pH 8.5.

TABLE II.
Effect of Chlorination on MV Virus in 1:1650 Dilution.†

Monkey No.	Chlorine concentration, ppm: Original Conc.	5 min after exposure to virus	At time of inoculation	Contact period, hr	Neurological signs	Time of onset, days
8	(Control)				Quadriplegia*	10
9	0.80	0.55	0.40	1	"	14
10	0.80	0.55	0.40	2½	XI nerve paralysis* Leg paralysis Arm paresis	10
11	0.80	0.55	0.25	5	Negative	

*Histopathology was compatible with that of acute poliomyelitis.
†Temperature 21-23° C; pH 8.3.

[14] Mallman, W. L., *Mich. Eng. Exp. Sta., Bull. No.* 59, 1934.
[15] Fox, L. A., *Military Surgeon*, 1936, **78**, 329.

content of 0.50 ppm for 1½ hours in Experiment 2, indicating a negligible chlorine demand by the organic matter. In Experiment 3, apparently there was more organic material present because the residual chlorine dropped from 0.80 ppm to 0.50 in 5 minutes and to 0.40 ppm in 1 hour. The contact period required for inactivation of the virus was approximately the same in both instances. As an additional control 0.45 ppm was adequate to kill *B. coli* in a concentration of 24,000 organisms per cc in ½ hour.

In municipal practice, a residual chlorine content of 0.10 to 0.20 ppm for ½ to 2 hours is considered adequate for the production of a safe water. The results in this paper indicate that a higher concentration and a longer contact period are necessary to inactivate the virus of poliomyelitis. The possibility that drinking water, adequately chlorinated according to accepted standards, may be a factor in the epidemiology of poliomyelitis must be recognized as a result of these findings. As a corollary, attention is directed to the shortcoming of this method for the protection of swimming pool water since carriers may discharge the virus from the intestinal tract or the naso-pharynx and the chlorine content of swimming pools is apt to drop significantly during the peak bathing loads. The need for more sensitive methods for detecting the poliomyelitis virus in water should be emphasized. Even persistently negative results would not necessarily assure the absence of the virus from water, because organisms such as *B. typhosus* are seldom found by direct bacteriological methods.

Whether the aluminum hydroxide sedimentation process previous to chlorination would produce virus-free water cannot be answered in this paper. Experiments are being continued to determine whether the chlorine concentrations usually used in swimming pools are sufficient to inactivate the virus.

Summary. Chlorine in a concentration of 0.5 ppm, which is an amount in excess of that usually employed in municipal practice, did not inactivate the virus of poliomyelitis in 1½ hours.