ing out of the seal. The shoulder on bearing A holds it in the top of the inner tube. Bearings A and B are drilled and turned down on the lathe as a single unit and cut with a hack saw.

For ordinary stirring, bearing C can be eliminated. Its purpose is to steady the end of the rotating tube, T, and to check mercury splashing. Use of this bearing is suggested when especially stable setups are desired, very long stirring periods are required, or larger setups are used.

The use of ball bearings, suggested by Hershberg (*1*), facilitates rapid stirring, but the nature of the materials involved in most chemical reactions makes it impractical to install a set of ordinary steel ball bearings inside the seal or flask. The ball bearings must therefore be mounted quite far from the free end of the stirrer shaft. Greater stability can be obtained by supporting the shaft nearer the stirrer end by a graphite bearing such as B.

The problem of splashed mercury finding its way into the reaction vessel is virtually excluded by the presence of bearing A. Bearing B decreases the refluxing of solvents within the seal and makes subsequent cleaning of the seal a much easier task. Other advantages of the graphite bearings are: ease of assembly of the seal, durability, self-lubrication, and chemical inertness of the materials involved.

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Tissue Potassium Determinations[1]

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A simple and relatively rapid method for determination of potassium concentrations of tissue has been developed in our laboratory.

From a series of ether-killed male albino rats of ca. 200-gm weight, brains and gastrocnemius muscles were removed and weighed. Tissues were then placed separately into 100 or 200 cc of distilled water and boiled under reflux condensers for 10 min. Solutions were stoppered and stored at 3° C for 36 hrs.

In the case of brain, sufficient agitation to fragment the tissues usually followed boiling, while in the case of muscle, a clear supernatant fluid was characteristically obtained.

At the end of 36 hrs aliquots taken from the supernatant liquid for potassium determination were analyzed by the flame photometer (Perkin-Elmer Model # 18); the boiled tissues, as a control of the method, were removed, dried at 105° C for 1–2 hrs, and then ashed in a muffle furnace at 550° C for 2 hrs. Tissue ashes were taken up in 0.1 N HCl, transferred through ash-free filter paper (S & S Blue Ribbon) to 50- or 100-cc volumetric flasks, and made up to volume with distilled water.

Analysis of the supernatant liquids of 23 brain and 17 muscle preparations yielded mean values of 3.42 ± 1.08 mg of K/gm of wet brain and 3.96 ± 0.12 mg of K/gm of wet muscle.

Application of Fisher's t test showed that there was no statistically significant difference between the standard errors of the mean potassium content values obtained by this method and the total potassium values determined by photometric analyses of a series of brain and muscle homogenates, P being 0.5 for brain and 0.4 for muscle.

Subsequent analysis of the tissue ash solutions described above, which were prepared at 2–4 times the concentrations of the solutions in which the tissues had been boiled, in a series of 20 experiments showed without exception that no more potassium remained in the boiled tissue than could be found in a corresponding volume of supernatant fluid. At the dilutions employed, this amount is negligible.

The method described above is recommended for total potassium analyses because, in addition to its simplicity and speed, it obviates the necessity of more arduous ashing or homogenizing techniques.

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Duration of Viability of Neurotropic Viruses in an Experimental Plumbing System Contaminated by Back-Siphonage[1]

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The possibility that poliomyelitis and other virus diseases may be spread by contaminated drinking water has been considered by many investigators. Various factors relating to the epidemiology of virus diseases have been studied. Laboratory experiments indicate that the poliomyelitis virus enters the body through the alimentary tract (*5*), and it has been shown that it is excreted in the stools of patients and persons not showing clinical evidence of the disease (*11, 14*). Furthermore, studies reveal that the poliomyelitis (*6*), St. Louis encephalitis, Japanese B (*3, 15*), and infectious hepatitis (*4*) viruses produce infection when given by mouth.

The poliomyelitis virus has been isolated from municipal sewage during epidemics in Europe and the United States (*10, 12, 13, 17*). The safety factor provided against bacteria through chlorination of water does not seem to apply to viruses. These studies and those carried

[1] These studies were supported by a grant from the American Society of Sanitary Engineering.

out by Kempf and Soule (*7, 8*) indicate that the amount of chlorine found in municipal drinking water is not enough to kill viruses of the neurotropic group.

In 1939 Kling (*9*) reported the isolation of a polio-like virus from well water in Sweden. The well was located on the premises where a child was afflicted with poliomyelitis. Aside from this report, however, we have been unable to find evidence of virus isolation from water.

Although the presence of virus has not been established in drinking water, the possibility that virus diseases may be transmitted by contaminated water should not be disregarded. The studies of Cronkright and Miller (*1*),

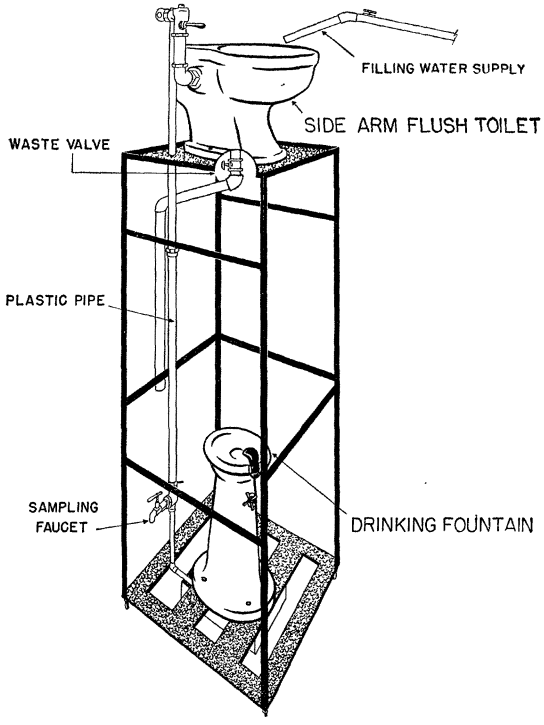

FIG. 1

Stockton (*16*), and Dawson and Kalinske (*2*) show that water supplies may be contaminated with sewage by many means of cross-connections and back-siphonage. Several factors may be responsible for the failure to isolate virus from water. Water in a plumbing system is continually changing, and by such a process the virus may be washed away or diluted so much in a relatively short time that its isolation could not be made. In addition, the methods of demonstrating the presence of virus are difficult, and in most cases the studies are made too late in the course of the epidemic.

Our studies were undertaken to determine if a plumbing system could be contaminated experimentally with neurotropic viruses by back-siphonage and to determine how long these viruses remained infectious when suspended in chlorinated drinking water and retained in the plumbing system.

The studies were made with the Western equine encephalomyelitis, St. Louis encephalitis, poliomyelitis (Lansing strain), and lymphocytic choriomeningitis viruses. The source of the virus in each case was infected mouse brains. Municipal drinking water containing about 0.1 ppm of chlorine was used for suspending the viruses. In each experiment the virus was diluted 1–5,000. A plumbing system representing that found in a two-story residence building was constructed for the study. The toilet bowl was of a flush valve type with a side-arm inlet. It was placed on a stand 9′ above the floor, and a faucet and a drinking fountain were connected to it at floor level. All the parts used were of standard make. Fig. 1 shows the plumbing system.

The experiments were performed by filling the toilet bowl with water to the lower edge of the top rim. The descending pipe was also filled with water, and its upper end was closed. When the temperature of the water in the bowl attained that of the room, the virus was added to make a 1–5,000 dilution. The experiments were carried out at room temperature which ranged from 24 to 28° C. The siphon was started by opening either the faucet or the drinking fountain. This represented a simple form of back-siphonage, and with it, it was possible to draw the virus suspensions out of the bowl only to the level of the side outlet pipe.

Thirty minutes after the virus suspensions were drawn into the plumbing system by back-siphonage, 100-cc samples were obtained from both the faucet and the drinking fountain and tested for the presence of virus. Thereafter, samples were drawn from the faucet at 24-hr intervals to determine how long the viruses remained infectious when held in the plumbing system.

Since it was not practical to sterilize the entire plumbing system before the addition of the viruses, it was necessary to free each sample of bacterial contamination. Those containing the Western equine encephalomyelitis, lymphocytic choriomeningitis, and St. Louis encephalitis viruses were filtered through and N Berkefeld filter. The samples of poliomyelitis virus were treated with ether. Immediately after treatment the samples were cultured in thioglycollate media and then inoculated subdurally in Albino Swiss mice. The remainder of each sample was stored in dry ice, and, if bacterial contamination was found in any, the treatment was repeated and another set of animals was inoculated. In each experiment the presence of virus was established in the original virus suspensions and in the samples taken from the plumbing system.

The presence of the virus was established by inoculating from 6 to 12 mice with each sample. Each mouse was given 0.03 cc of the suspension by the subdural route. Albino Swiss mice, each weighing about 12 gm, were used. After recovery of the virus from the plumbing system from the first sample taken, its identity was established by the neutralization test with specific serum. Three experiments were performed with each virus.

The results in Table 1 show that in each experiment

the virus was recovered from the experimental plumbing system which had been contaminated with it by back-siphonage. Although the viruses were suspended in chlorinated municipal drinking water, they survived in

TABLE 1

CONTAMINATION OF AN EXPERIMENTAL PLUMBING SYSTEM WITH NEUROTROPIC VIRUSES BY BACK-SIPHONAGE

Type of specimen	No. of experiments	Viruses employed				Ident. of virus
		L.C.M.	Polio (Lansing strain)	W.E.E.	St. Louis	
Original	3	15/15*	12/18	18/18	18/18	Pos.
From faucet	3	32/36	28/36	35/36	36/36	Pos.
From fountain	3	34/36	31/36	36/36	32/34	Pos.

* Mice dead/mice used.

the plumbing system for several days in each set of experiments. Survival time for the different viruses in Experiments 1, 2, and 3, respectively, was as follows: lymphocytic choriomeningitis—7, 3, and 4 days; poliomyelitis (Lansing strain)—4, 1, and 2 days; Western equine encephalomyelitis—5, 2, and 4 days; and St. Louis encephalitis—4, 3, and 2 days.

These experiments show that a plumbing system may be contaminated with the neurotropic viruses studied by a simple form of back-siphonage. They also show that, when these viruses were suspended in chlorinated drinking water and retained in the plumbing system at room temperature, they remained infectious for several days.

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Role of Sulfhydryl Compounds in Pigmentation

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In recent studies (*8*) it was found that aqueous extracts of human epidermis inhibit the oxidation of *l*-tyrosine and *l*-dihydroxyphenylalanine (dopa), thus preventing the formation of melanin. Since this effect is counteracted by iodoacetamide, it has been assumed that the inhibition is due to the presence of sulfhydryl compounds in the extracts.

Further evidence in support of this assumption is submitted in this communication. The inhibitory principle is water soluble, dialyzable, and heat stable and is counteracted by *p*-chloromercuribenzoic acid and by cupric ions. Direct evidence is supplied by *in vitro* experiments demonstrating the relationship between the inhibitory activity of epidermal extracts and their sulfhydryl content. It is found that the degree of inhibition of melanin formation varies directly as the logarithm of the molar concentration of –SH.

A similar relationship is demonstrated *in vivo* following ultraviolet irradiation of the skin. In these experiments an increase in melanin formation is found to be preceded by a decrease in the –SH content of the skin. Immediately following exposure of the shaved skin of rabbits to large doses of ultraviolet light there is a decrease of from 24% to 83% in the concentration of –SH in the skin. This suggests that pigment-producing stimuli act by eliminating the –SH inhibition, allowing the enzymatic oxidation of pigment precursors to occur.

A clue as to the possible mechanism of this elimination is provided by another defense reaction of the tegument to ultraviolet irradiation and to other injurious stimuli, namely, an increased production of keratin, manifested as a thickening of the horny layer in man (*7*) and as excessive hair growth in animals (*6*). The production of keratin is characterized by the formation of disulfide (–S–S–) bridges from sulfhydryl groups of native proteins. Possibly the same biochemical process, the oxidation of sulfhydryl groups to disulfides, is responsible for both intensified keratinization and pigmentation.

Additional information concerning the role of sulfhydryl compounds in melanin formation is provided by two observations: first, that a copper-containing enzyme, tyrosinase, is apparently essential for the formation of melanin in mammalian tissues (*2, 4, 5*), as it is in plants and lower animal forms; and second, that sulfhydryl-containing epidermal extracts neutralize the effect of both tyrosinase and cupric ions in pigment production. Copper is known to be an essential dietary factor in the maintenance of the color of fur (*3, 10*). Moreover, our analyses have confirmed and extended earlier data (*1, 9*) which show that in most cases the black and gray hair of rabbits, guinea pigs, and rats contains significantly

[1] American Cancer Society Fellow, 1948–49.