container had not often been opened. The other specimen was Armour's so-called insoluble pepsin which had been received from the manufacturers in July, 1909. Since its receipt it had been stored in its original container, a cork-stoppered bottle of amber glass, at the temperature of the laboratory. The bottle had been opened from time to time and about four-fifths of the contents used. At the time of sending the preparation, the manufacturer stated that it possessed a strength of 1–3000. The proteolytic strength was not verified at the time of the receipt of the specimen. The specimen used for comparison tests was Armour's "spongy granular, soluble" pepsin received from the manufacturer in May, 1919. The label claimed a strength of 1–3000 for the preparation.

The old laboratory specimen was a fine, cream-colored powder which was not noticeably hygroscopic. Its odor was not unpleasant, being similar to that of the specimen of recent purchase. Its taste was distinctly saline and somewhat bitter. The material was soluble in water, yielding a turbid solution which was strongly acid toward litmus paper. The ten-year old specimen was a greyish, somewhat lumpy powder. Its odor and taste were normal. The modern specimen was in the form of pale yellowish scales or granules.

Ash was determined by igniting a weighed quantity in a porcelain crucible, moistening the charred mass with a little ammonium nitrate solution, drying and again igniting. The very old specimen gave 48.8 percent of ash while the two more recent specimens gave, respectively, 4.14 percent and 3.01 percent of ash. As an average of several trials the proteolytic strength of the three specimens was found to be, respectively, 1–500, 1–2500 and 1–3000.

An examination was made to determine whether the literature contained reports of examinations of pepsin as old as the oldest specimen here studied, but no records were found. That a specimen nearly thirty-nine years old should retain any proteolytic activity is considered worthy of record.

LABORATORY OF THE AMERICAN MEDICAL ASSOCIATION.

THE LONGEVITY OF BACTERIA IN BOTTLED COMMERCIAL SPRING WATER.

BY MAUD MASON OBST.

The longevity of bacteria in natural waters after having been bottled for commerce does not seem to have been studied extensively. Many references are made in the literature to the longevity of significant individual bacteria in water under natural conditions. Sellards[1] states "Typhoid bacteria are neither harbored in lower animals, nor multiply in natural waters." Houston reports[2] "Outside the animal body the *B. coli* is usually known to be a decadent organism. At 20° C. it dies rapidly in both sea and tap water." Dunham[3] found that pure waters originally free from bacteria were contaminated mostly with chromogenic bacteria. Waters polluted with soil or vegetation contained *B. subtilis*, *B. my-*

[1] Sellards, "Water Bacteria," *Jour. Inf. Dis. Suppl.* No. 3, p. 41, 1907.

[2] A. C. Houston, "Significance of B. Coli in Water," *British Medical Journal*, No. 2699, p. 407, Sept. 1912.

[3] E. K. Dunham, "Value of Bacterial Examination from a Sanitary Point of View." *Jour. Amer. Chem. Soc.*, Vol. XIX, No. 8, p. 591, Aug. 1897.

coides, *B. figurans*, and organisms coming from the air. He also recorded the following data:

"One liter of distilled water inoculated with 1 Cc. of filtered suspension of *B. coli* contained an initial count of 42,971 per Cc. At the end of 24 hours the count was 14. One liter of distilled water enriched with 1 Cc. nutrient bouillon and inoculated as above, gave an initial count of 57,102 per Cc. at the end of 24 hours a count of 29,276. One liter of distilled water to which was added 1 Cc. hay infusion and *B. coli* as above had an initial count of 14,030 per Cc. and at the end of 24 hours a count of 439."

Streptococci and *B. coli*[4] introduced into tap water in the form of extract of feces died in a short time. Few of the streptococci survived more than two weeks, though the *B. coli* were still alive at the end of eleven weeks. The amount of organic matter introduced with the sewage, and the other kinds of organisms present were not stated.

Browne[5] studied the occurrence of organisms of the *B. coli* group in water into which he had introduced 1 gramme of fresh feces to 1 liter of water. *B. aerogenes*[6] is reported as being seldom found in stored waters and when present as indicating contamination from grain. When inoculated into water they decrease more rapidly than *B. coli*; 98 to 99 percent of both died off by tenth day.

In 1916 there were many samples of bottled water which had been stored for various lengths of time in the Bureau of Chemistry. Portions of the samples had been removed and bacteriological examinations made at the time of their receipt. The bottles, bearing identification numbers, had then been carefully sealed by the bacteriologist and stored without consideration of the conditions of light and temperature to which they might be exposed. In the summer and fall of 1916 a re-examination was made of all the water on which records of the previous examination were available. The standard methods prescribed by the American Public Health Association were followed in this work and confirmatory tests were made for *B. coli* whenever gas-producing organisms were found.

The results of the examinations of these samples are recorded as averages of the numbers obtained from all the individual bottles which connstituted one sample. (See Table 1.)

Sample No. 2 (Table 1) shows the uniformity of the counts obtained upon the individual bottles in the first examination. Each bottle contained a relatively high number of organisms, and this number is of the same magnitude for all of the bottles in this sample. Those samples which are bacterially clean generally vary much less than this, although occasionally one of four or six bottles may contain hundreds of bacteria when the remainder have less than 20 per Cc. Sample No. 23 (Table 1) was one of two out of 40 samples examined which showed an ap-

NOTE.—The writer is indebted to W. W. Skinner, in charge Water Laboratory, Bureau of Chemistry, for the use of the chemical data included in this paper and for many helpful suggestions.

[4] W. G. Savage and D. R. Wood., "Vitality and Viability of Streptococci in Water," *Jour. of Hygiene*, 16, No. 3, 227. 1917.

[5] W. W. Browne, "Predominance among the Members of the *B. coli* Group in Artificially Stored Water," *Jour. Inf. Dis.*, 17, No. 1 72–78, 1915.

[6] C. E. A. Winslow and B. Cohen, "Viability of *B. coli* and *B. aerogenes* Types in Water," *Jour. Inf. Dis.*, 23, No. 1, 82, 1918.

preciable variation in the counts at 25° C. In the routine work of the laboratory striking irregularities have been noted in the results obtained from imported samples which have naturally been bottled for some time before being examined.

TABLE I.—DETAILED RESULTS OF THE RE-EXAMINATION OF COMMERCIALLY BOTTLED WATERS AFTER PERIODS OF STORAGE.

					Bacteria per cubic centimeter developing on					
					Gelatin at 25° C.		Nutrient agar at 37° C.		Gas-producing bacteria present in Cc.	
Sample No.	Date of Examination First.	Second.	No. of days in storage.	Bottle No.	1st exam.	2nd exam.	1st exam.	2nd exam.	1st exam.	2nd exam.
2	2–19–13	11–17–16	1,357	1	3,700	0	900	0	1.0	*b*
				2	5,100	4	2,400	0	1.0	*b*
				4	28,200	3	11,500	3	0.1	*b*
				5	11,000	2	4,000	7	1.0	*b*
				6	68,000	31	12,000	20	0.001	10.0
Averages..............................					23,200	8	6,160	6	++	*a*
23	3–17–15	11–8–16	607	1	9,300	0	500	9	1.0	*b*
				2	37,000	60	270	9	0.1	*b*
				4	370	23	250	40	1.0	*b*
				6	290	170	220	720	1.0	*b*
Averages..............................					11,690	63.2	310	194.5	+	o

"Experimental work † has shown that when certain types of mold infection are present in the water or in the cork, mold development may follow. In certain cases the cork becomes deeply infected with mold hyphae which fruit constantly. The spores drop into the water as they ripen or are washed into the water whenever the container is handled. Once in the water these forms germinate and produce little, submerged, colorless, cottony tufts of mycelium, which often remain sterile and in some cases finally die. In other cases, the mold spores float, develop into pin point colonies on the surface of the water, which produce considerable numbers of spores. Any living colony when the bottle is thoroughly shaken may provide a liberal seeding for cultures made from the sample. It is clear that, aside from the substance of the cork, the very small amounts of inorganic and organic matter present is quite carefully handled; waters and their containers can be utilized by certain species of molds."

"The cork, on the other hand, has shown itself to be one of the chief vehicles of mold contamination in bottled waters as in other bottled products. Examination of the stoppers from bottles long in storage shows clearly that the substance of the cork is regularly attacked by certain fungi, which, together with their products, contaminate the water.

"It would appear that this condition of the corks is a considerable factor in old and musty flavors in water, since diffusible by-products seem to be very quickly produced by many molds. Mold spores are occasionally found in considerable numbers in fresh water. These occasions are few and can usually be closely correlated with inspection data. As a rule in actual inspection practice, numerous mold colonies in cultures from commercial bottled waters are indicative of rather long storage."

As shown in Table 2, there were only two samples in which the counts actually increased during the periods of storage. In many samples *B. coli* survived for a long time. In several instances, where no molds were noted at the time of the first examination there were molds present when finally examined.

† NOTE.—The paragraphs upon molds in water were prepared by Dr. Charles Thom.

TABLE II.—RESULTS OF THE RE-EXAMINATION OF COMMERCIALLY BOTTLED WATERS AFTER STORAGE FOR VARYING LENGTHS OF TIME.
(Expressed in the Average for the Number of Bottles Examined.)

Sample No.	Date of Examination. First.	Date of Examination. Second.	No. of days in storage.	No. of bottles examined.	Bacteria per cubic centimeter developing on: Gelatine 20° C. Total. 1st exam.	Gelatine 20° C. Total. 2nd exam.	Gelatine 20° C. Liquefiers. 1st exam.	Gelatine 20° C. Liquefiers. 2nd exam.	Nutrient Agar. 1st exam.	Nutrient Agar. 2nd exam.	Gas-producing bacteria present in Cc. 1st exam.	Gas-producing bacteria present in Cc. 2nd. exam.	Remarks.
1	10–8–12	11–6–16	1,490	4	5	Molds	....	..	4	Molds	+	0	Plates sterile except for molds
2	2–19–13	11–17–16	1,363	5	23,200	8	....	..	6,150	6	++	...	
3	12–30–13	11–17–16	1,053	3	66,000	360	....	..	16,900	12	++	(a)	
4	12–30–13	11–17–16	1,053	4	3,120	225	....	..	220	11	+	0	
5	2–6–14	11–6–16	1,004	6	5,390	5	....	..	200	1	++	0	
6	2–11–14	11–17–16	1,010	5	110	1	....	..	70	1	0	0	
7	4–27–14	11–10–16	928	4	970	Molds	....	..	50	Molds	0	0	Plates sterile except for molds
8	5–29–14	11–10–16	896	3	124,500	Molds	55	0	300	Molds	0	0	Plates sterile except for molds
9	5–29–14	11–17–16	903	8	6	Molds	....	..	2	Molds	0	0	Plates sterile except for molds
10	4–28–14	11–10–16	927	6	600	Molds	31	0	8	Molds	0	0	Plates sterile except for molds
11	5–11–14	11–10–16	914	3	115	Molds	....	..	1	Molds	0	0	Plates sterile except for molds
12	6–2–14	11–10–16	892	3	1	315	....	..	2	165	0	0	
13	6–20–14	11–17–16	881	6	7,850	225	1,330	0	1,030	23	++	2+	
14	6–23–14	11–17–16	878	6	7,850	490	....	..	990	225	++	0	
15	10–10–14	11–8–16	759	5	21,500	970	350	0	3,100	515	++	+	
16	11–14–14	11–16–16	733	5	340	2,100	13	0	900	920	++	0	
17	10–31–14	11–8–16	739	6	178,000	9	....	..	190,000	7	0	0	2 bottles showed molds
18	12–7–14	11–6–16	689	3	4	(b)	4	..	7	(b)	0	0	
19	1–23–15	11–16–16	662	6	1,570	125	125	0	2,500	9	++	0	
20	2–24–15	11–16–16	630	5	48,000	14	190	0	4,150	115	++	(a)	
21	3–23–15	11–16–16	605	6	12,000	105	2,150	0	16,500	28	+	0	
22	3–17–15	11–8–16	602	2	32,000	34	400	0	22,000	50	++	(1.0)	
23	3–17–15	11–8–16	602	4	11,700	63	....	..	310	195	++	0	
24	5–3–15	11–14–16	562	6	3	0	....	..	1	0	0	0	
25	5–4–15	11–6–16	551	4	25,000	265	645	0	3,450	96	++	(1.0)	
26	5–25–15	11–10–16	534	4	53	14	....	..	290	6	0	0	
27	5–22–15	11–6–16	533	5	3,500	0	14	0	138	0	+	0	
28	6–14–15	11–6–16	544	6	8,900	13	500	0	26,500	47	+	(a)	
29	6–23–15	11–10–16	539	8	9,500	64	410	0	580	38	++	+	
30	7–16–15	11–14–16	487	3	800	4	26	0	325	4	0	0	
31	7–17–15	11–14–16	486	5	555	18	175	0	54	18	0	0	
32	7–19–15	11–16–16	486	2	6,500	85	100	0	32,500	122	+	+	
33	7–27–15	11–14–16	476	9	.....	....	....	..	60,000	240	++	++	
34	7–31–15	11–10–16	468	11	4,600	225	265	0	5,500	190	+	0	
35	8–9–15	11–14–16	463	9	.....	....	....	..	32,000	200	++	0	
36	2–28–16	7–24–16	147	8	0	0	0	0	1	0	0	0	
37	2–29–16	7–24–16	146	6	2,900	58	0	0	6,000	41	+	0	
38	3–14–16	7–24–16	132	11	9,700	81	10,000	0	18,000	60	0	0	
39	3–16–16	7–24–16	130	8	43,000	31,000	0	28	11,000	2,200	++	+	
40	4–5–16	7–24–16	110	6	2,600	38,000	100	0	138	60,000	0	0	(C) Many micrococci

It would be expected that the number of days which a specific water remained in storage would have a marked effect upon the numbers of bacteria present. This is proved to be true by a comparison of samples numbered 2, 13, 14, 15 and 23. These are all samples shipped from one spring in interstate commerce. A proportional difference is shown in the counts obtained after incubation at 37° C., the decrease being greatest on the water which has been stored the longest. After nearly two years' storage (samples 13 and 14) this water still showed an undesirable number of bacteria for a bottled water, and an excessive number of gas-producing organisms.

TABLE III.—PRELIMINARY EXPERIMENT.

Water "C."—Inoculated with *B. coli* on 1–10–16.

Date examined.	No. of days stored.	Total counts on bottle numbers (per Cc.) A.	B.	C.	D.
1–10–16	0	2,000	100,000	600	400
1–12–16	2	2,300	110,000	540	370
1–14–16	4	1,900	90,000	390	300
1–19–16	9	1,800	70,000	400	310
1–28–16	18	1,600	76,000	350	290

Water "C."—Inoculated with *B. coli* on 1–14–16.

Date examined.	No. of days stored.	Total counts on bottle numbers (per Cc). A.	B.	C.	D.
1–14–16	0	1,300,000	3,000,000	2,100,000	1,600,000
1–17–16	3	1,400,000	3,000,000	2,000,000	1,200,000
1–28–16	14	1,200,000	2,700,000	1,700,000	1,300,000

Water "B."—Inoculated with *B. coli* on 1–10–16.

Date examined.	No. of days stored.	Total counts of bottle numbers (per Cc.) A.	B.	C.	D.
1–10–16	0	4,000	10,000	7,000	6,000
1–12–16	2	4,000	10,000	5,900	5,400
1–14–16	4	2,700	7,000	5,000	5,100
1–19–16	9	2,600	5,800	5,100	5,000
1–28–16	18	2,100	5,000	4,700	4,300

The samples (Table 2) which gave high averages of bacterial counts after storage for several months were shown by chemical analysis* to be relatively high in total solids, including a variety of elements in calculable quantities. Aside from waters with relatively high total solids, these examinations showed no multiplication of bacteria during periods of storage but ordinarily a marked reduction. This indicates that the number of bacteria reported as present when commercial samples, and especially imported ones, are received in the laboratory are always less, rather than more than the number present in the water at the time of bottling.

A commercially bottled water which had been condemned as unfit for drinking was stored in the bottles to ascertain the practicability of attempting to purify polluted waters in this way. It was held from December 4, 1914, to April 17, 1915. During this time there was no marked decrease in the total counts of bacteria. The *B. coli* in those bottles held at room temperature did not decrease. It is probable that the temperature of the room was much less than it would have been in

* NOTE.—Analyses were furnished by the Water Laboratory of the Bureau of Chemistry.

summer, and if the water had been stored during the warmer months, the change in the bacterial content might have been slightly more marked. The bottles of this water which were stored at 36° F. showed no change in the numbers of bacteria. A decrease in the bacterial count was observed in a few bottles which stood in the direct sunlight for four weeks. There appeared to be no difference in the effect of using brown glass or colorless glass bottles. *B. coli* were present in 0.1 Cc. quantities in several bottles at the time of the last examination.

TABLE IV.
Water "A."

Inoculated with	Date examined.	No. of days stored.	Average number of organisms per Cc.[1] from bottles stored at 20° C.	Room temperature.
B. dysenteriae	4–27–16	0	6,550,000	1,350,000
	4–29–16	2	4,150,000	465,090
	5– 2–16	5	(1 bottle) 20	(3 bottles) 83.3
	5–15–16	18	0	0
B. typhosus	4–14–16	0	1,592,500	1,383,000
	4–18–16	4	Less than 1,000	3,428
	4–21–16	7	(1 bottle) 340	(2 bottles) 520
		Others less than 1 per Cc.		
B. coli	4–27–16	0	99,750,000	16,700,000
	4–29–16	2	47,600,000	99,213,333
	5– 4–16	7	7,825,000	1,405,222
	6–11–16	45	Less than 10	Less than 100
	6–21–16	55	......	......

No bottle contained gas-producing organisms in 10 Cc. quantities.

Chemical Constituents (Hypothetical Combinations).

	Mg. per liter.
Sodium chloride	6.6
Sodium sulphate	0.5
Magnesium sulphate	11.7
Magnesium bicarbonate	36.9
Calcium bicarbonate	283.2
Silica	12.4
Total	351.3
Ammonia, free	.012
Ammonia, albuminoid	.060

Mixed flora introduced by adding sewage to bottled waters has been referred to in the literature cited above, although little work has apparently been done with known mixtures. No reference was found regarding the action of pure cultures of specific organisms in spring waters containing known chemical constituents. Water possessing the following characteristics were, therefore, used in experiments with pure cultures of organisms:

(A) High in mineral salts, and low in organic matter.
(B) High in both mineral salts and organic matter.
(C) Medium high in both mineral salts and organic matter.
(D) Very low in both groups of constituents.

[1] All samples were incubated on nutrient agar 2 d. at 37° C.

The waters were collected directly from the springs either by the bacteriologist or by a Food and Drug Inspector under special instructions. They were sent to the laboratory in Washington, in sealed, 5-gallon carboys. There the water was transferred to colorless 1-liter, glass-stoppered bottles, which had been previously carefully washed, rinsed with distilled water and then with the water with which they were to be filled. The water was sterilized in these small bottles under pressure. The water high in carbonates was sterilized in bottles filled very full, and with the stoppers firmly tied in place to prevent the precipitation of the carbonates.

TABLE V.
Water "B."

Inoculated with	Date examined.	No. of days stored.	Average number of organisms per Cc.[1] from bottles stored at 20° C.	Room temperature.
B. typhosus	6–23–16	0	26,375,000	5,037,000
	6–26–16	3	12,050,000	3,412,500
	6–30–16	7	3,900,000	423,750
	7– 7–16	14	162,750	21,183
	8–31–16	69	866	0
B. coli	6–15–16	0	39,750,000	23,400,000
	6–17–16	2	31,250,000	16,350,000
	6–21–16	6	15,000,000	4,210,000
	8–31–16	77	966,666	449,100
	11–3–16	141	343,333	17,755

Chemical Constituents (Hypothetical Combinations).

	Mg. per liter.
Sodium nitrate	5621.0
Sodium chloride	2604.0
Magnesium chloride	1349.0
Magnesium sulphate	17517.0
Calcium bicarbonate	1408.0
Ferric oxide alumina	8.0
Silica	22.0
Total	29126.0

In preparing the cultures of bacteria for inoculation, transfers were made in standard nutrient bouillon for three successive days and from the last 24-hour culture in this medium streaks were made upon several nutrient agar slants. These were incubated at 37° C. for 36 hours. Then the surface growth was removed by adding a few cubic centimeters of sterile distilled water and loosening the growth beneath this water with a heavy platinum loop. If any agar was taken up in this way with the growth, it was easily discernible, and the tube was discarded. The liquid containing the bacteria from all the tubes was combined in a sterile flask, thoroughly shaken with glass shot, and finally transferred to the sterile spring water. The water was at once tested for contaminations and if the bottle was found to contain any but the organism with which it had then been inoculated, it was discarded. This method of inoculation may have introduced very small quantities of food material, and many clumps of bacteria. The clumps,

[1] All samples were incubated on nutrient agar 2 days at 37° C.

however, were broken apart as much as possible by shaking the bottles vigorously. Whenever samples were to be removed the bottles were inverted and shaken 25 times through an excursion of 1 foot. In order to reduce errors further, a number of bottles were used in each experiment and the results upon which the discussion is based are the averages of the counts of bacteria obtained from four or more bottles.

TABLE VI.

Water "C."

Inoculated with	Date examined.	No. of days stored.	Average number of organisms per Cc.[1] from bottles stored at 20° C.	Room temperature.
B. dysenteriae	4–14–16	0	7,750,000	3,532,000
	4–15–16	1	6,092,500	3,940,000
	4–18–16	4	516,666	6,250
	4–21–16	7	0	0
B. typhosus	4–14–16	0	5,250,000	1,772,000
	4–18–16	4	5,425,000	1,960,000
	4–21–16	7	20,300	66,300
	6–11–16	58	0	0
B. coli	2– 8–16	0	8,050,000	1,474,000
	2– 9–16	1	8,550,000	1,676,000
	2–11–16	3	8,025,000	1,922,000
	3– 8–16	29	6,975,000	753,000
	4–16–16	76	......	88,000
	4–18–16	78	1,612,500	......
	6–11–16	132	......	12,650
	6–15–16	136	902,500	......
	8–31–16	213	480,000	208.3
	11–2–16	276	21,533	17.5

Chemical Constituents (Hypothetical Combinations).

	Mg. per liter.
Magnesium chloride	9.4
Magnesium bicarbonate	70.3
Calcium sulphate	1317.9
Calcium bicarbonate	139.6
Total	1537.2
Ammonia, free	.016
Ammonia, albuminoids	.120

Four bottles of each set were stored in an electrically regulated 20° C. incubator, and ten bottles at room temperature in the dark. Counts and identifications were made each time of examination to prove that the original organism of inoculation was still present in condition to grow on culture media.

A preliminary experiment was carried out to determine the best degree of inoculation. Two waters, both fairly rich in organic matter were given heavy and light inoculations with *B. coli*. The results are recorded in Table 3. No noticeable difference was found. It was decided to give all of the water a fairly heavy inoculation hoping it might thus be made uniform.

[1] The samples were incubated on nutrient agar 2 days at 37° C.

TABLE VII.
Water "D."

Inoculated with	Date examined.	No of days stored.	Average number of organisms per Cc.[1] from bottles stored at 20° C.	Room temperature.
B. dysenteriae	2–19–16	0	3,450,000	739,000
	2–21–16	2	330,000	184,500
	2–23–16	4	900	64
	4– 8–16	49	All bottles sterile	
B. typhosus	2– 1–16	0	207,500	270,100
	2– 3–16	2	292,500	200,333
	2– 5–16	4	120,250	126,200
	2–14–16	14	10,000	82,750
	2–16–16	16	Contaminated	63,100
	3– 8–16	37	......	630
	4– 8–16	67	......	0
B. coli	1–29–16	0	1,665,000	2,380,000
	1–31–16	2	1,270,000	1,228,000
	2– 2–16	4	647,500	616,400
	2–14–16	16	95,000	718,888[2]

Chemical Constituents (Hypothetical Combinations).

	Mg. per liter.
Potassium chloride	0.80
Sodium nitrate	2.43
Sodium chloride	1.35
Sodium sulphate	2.89
Sodium bicarbonate	1.46
Magnesium bicarbonate	10.63
Calcium bicarbonate	21.37
Ferrous bicarbonate	1.78
Silica bicarbonate	35.07
Total	77.78
Ammonia, albuminoid	0.005
Nitrogen as nitrates	0.400

DISCUSSION OF RESULTS.

Three waters were inoculated with *B. dysenteriae* from a culture furnished by the Hygienic Laboratory of the U. S. Public Health Service. It was inoculated in numbers varying from 120,000 to 16,000,000 per Cc. These organisms decreased rapidly during the first two days of storage, and in most cases by the end of 5 days there were less than 1 per Cc. remaining alive. In the water described in the tabulation as "C," the *B. dysenteriae* remained alive in appreciable numbers after four days' storage at 20° C., but at the end of seven days they were nearly all dead.

B. typhosus did not apparently multiply in any of the waters. Counts on this organism showed, in waters "D" and "C," averages which increased between the first and second examinations from 207,500 to 292,500 and from 5,250,000 to 5,425,000 per Cc. There was nothing, however, to indicate that these increases were due to contamination, or to multiplication. It is evident that they may have been due to the breaking apart of clumps of bacteria which were not separated at the

[1] The samples were incubated on nutrient agar for 2 days at 37° C.

[2] Several bottles became seriously contaminated before the next examination. At that time (3–8–16) nearly all of the bottles contained millions of bacteria but no *B. coli*.

time of inoculation. After the second examination the *B. typhosus* decreased rapidly in numbers. In water "D" the numbers were greatly decreased at the end of 14 days' storage at both temperatures. In water "C" they were reduced from an average of 5,250,000 to an average of 20,300 within 30 days, and 28 days later there were practically none. In waters "C" and "D" this organism decreased more rapidly at 20° C. than at room temperature. In water "B" large numbers survived at 14 days' storage and some remained alive at 20° C. for 68 days. Water "A" seemed to possess a mild germicidal action. *B. typhosus* survived in it less than four days, except in three of the fourteen bottles which showed an average of 500 organisms per Cc. after 7 days. This water showed the same effect upon *B. coli;* they were all dead at the end of 45 days, or before June 11th. It is noteworthy that no bottle of this water showed any form of contamination, although 3 bottles were left unstoppered in the laboratory for six hours in an effort to contaminate them. Repeated examinations of routine samples of this water seldom showed many bacteria. This water contains a moderate amount of mineral matter comprised mostly of magnesium and calcium bicarbonate (see chemical data) and very little organic matter.

The average counts of the *B. coli* show only water "C" to have given upon the second examinations an increase over the numbers present at the time of the first examination. In this instance this increase is at 20° C. from 8,050,000 to 8,550,000 and at room temperature from 1,474,000 to 1,676,000. The same water, examined two days later showed a decrease at 20° C. and a slight increase at room temperature. It may be possible that if the increase was not due entirely to the shaking apart of the clumps, it may have been aided by a single subdivision of part of the bacteria after inoculation. Possibly it was the completion of subdivisions which had been started before the removal of the bacteria from the agar. There were, however, not sufficient increase to warrant the conclusion that either *B. coli* or *B. typhosus* multiplied in the bottled spring water.

The two waters "A" and "D" which were low in organic matter did not support the pure cultures of bacteria as long as waters "B" and "C." Water "D," containing very small quantities of either organic or inorganic compounds apparently had little effect upon the bacteria but harbored them till they died. Water "A" seemed to contain some element slightly germicidal. Waters "B" and "C" did not induce any increase in numbers of bacteria but did sustain them for a comparatively long period.

In some cases when the bottled water became contaminated with bacteria from the air the *B. coli* remained alive longer than in the uncontaminated water. Other proof of this is shown in the re-examination of stored commercially bottled water. The air contaminations consisted of two forms of micrococci, one forming a small, white pin-point colony, and the other a yellow colony on nutrient agar. The former multiplied with great rapidity in the bottled waters.

CONCLUSIONS.

1. Water can be stored in bottles so that contamination will not enter.

2. A re-examination of a stored bottled water within 30 days may, or may not give the same total count as the first examination, but it is improbable that the *B. coli* will ever be found to have increased.

3. Pollution can be detected in a bottled water even after three years of storage. Such water may not be safe to use for drinking purposes.

4. The presence of certain salts seems to aid the longevity of bacteria in commercial waters, while the presence of other salts seems to have the opposite effect.

5. The presence of molds in large numbers in a bottled water suggests storage.

6. *B. coli*, in symbiosis with water bacteria, may live in bottled spring waters for several years. It is not safe to assert that *B. typhosus* and others of these groups will not survive long periods of storage under symbiotic conditions.

7. *B. coli, B. dysenteriae* and *B. typhosus* in pure culture did not multiply when inoculated into sterilized bottled spring waters.

8. *B. typhosus* was obtained alive from spring water "B" after inoculation and two months' storage.

9. *B. dysenteriae* remained alive from four to five days in pure culture in spring waters, "A," "C," and "D."

10. From the results obtained with water "A" it is indicated that certain chemicals in natural spring waters may inhibit the existence of bacteria.

11. A steady decrease in the numbers of the inoculated bacteria was evident in waters "B," "C," and "D." This decrease was more rapid in water "D," which was low in both organic and inorganic matter than it was in waters "B" and "C," which contained, respectively, large and medium quantities of organic and inorganic matter.

SOME SUGGESTIONS FOR NATIONAL FORMULARY REVISION.*

BY WILBUR L. SCOVILLE.

Comments on National Formulary preparations since the advent of the Fourth Edition have been very meagre. This is probably due more to war conditions, the diverting of attention, and the restrictions placed upon materials, as well as upon time and men for experimenting than to a special satisfaction with the National Formulary. Thus we have come to the time for appointment of a new revision committee, and our pharmaceutical literature offers but few suggestions for improvement. The following may be of help in getting work started, and are offered with this in mind:

Compound Elixir of Glycerophosphates precipitates on standing. Glycerin does not help this, and the amount of glycerin in the preparation might be reduced without detriment in this respect, though not without detriment to the taste. Probably more acid is needed.

Emulsions.—Nearly all commercial emulsions are made to contain tragacanth as well as acacia, in order to preserve homogeneity in appearance. Those pharmacists who make their own emulsions probably make some weeks' supply at a time, and this factor is of advantage to them. A small amount of tragacanth prevents the formation of layers in the emulsion for a considerable time, and in some instances adds to palatability.

Solution of Aluminum Subacetate is directed to be adjusted to a definite specific gravity. Such adjustments are difficult to make and not in accord with the usual methods. Adjustment to a definite volume, with a descriptive clause would be desirable.

Solution of Ferric Hypophosphite precipitates on standing. Glycerin again does not help. Probably more Sodium Citrate is needed.

Compound Solution of Phosphates also precipitates quite badly. Probably more acid is needed in this.

Liquid Petroxolin.—Complaints have been made that this does not always make a clear preparation. Experiments on the use of potassium or sodium hydroxide are desirable to learn whether more certain results are likely to follow than when stronger ammonia water is used. The present formula is probably satisfactory when the materials are standard, but it is not always practicable to get

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