ferred to steam sterilizer, and heated on four successive days during ten to fifteen minutes. Sterilized test tubes, also plugged with cotton-wool, may be partly filled and sterilized in the same way. Thus prepared, the medium may be preserved in the dark for months.

For the actual tests, at least four dishes are required for each sample. and these together with all other glass apparatus should be sterilized as usual in the air oven, several pipettes divided into $\frac{1}{10}$ cc. will be needed, also glass flasks of 100 cc. capacity for diluting the water.

The dishes having been placed on a level table and the nutrient melted at about 40° C, about 10 cc. is carefully introduced into each dish, removing the cover only so far as to allow of the liquid being poured in, and taking care to avoid infecting the nutrient, by using sterilized forceps for removing the cotton-wool from neck of flask, the lip of which must also be gently heated in Bunsen flame. Before the medium has solidified varying quantities of the water samples are introduced by means of the sterilized pipettes. Convenient amounts are 1 cc., $\frac{1}{2}$ cc., $\frac{1}{4}$ cc., $\frac{1}{10}$ cc., and $\frac{1}{100}$ cc., but with very bad water $\frac{1}{1000}$ cc. (or even less) will be sufficient. These smaller quantities are measured by first preparing dilutions of the original water with 99 or 999 times its volume of sterilized water. The water and medium have now to be thoroughly mixed by tilting the dishes backwards and forwards several times, and then set at rest in a cold place (refrigerated during hot weather) until thoroughly set, when they are transferred to an incubator and maintained at about 20° C. In those dishes which contain a sufficiently small portion of the water, each individual organism is separated from the others by mixing with so large a proportion of medium, and when this solidifies each one is kept in its place, consequently when it multiplies it in time produces a "colony" sufficiently large to be seen by the naked eye or a pocket lens. The dishes are therefore examined from time to time during several days. By the end of the second day, but frequently much earlier than this, some colonies will be seen, and these will increase in number until all are developed, when they must be counted. If few in number this may easily be done, but, if many, a special counting apparatus will be required. That usually employed is Wolfhugel's, which consists of a blackened plate, upon which the dish is placed, and covered with a glass plate divided into squares. The dish is viewed through this divided plate, and the number of colonies in five of the squares (diagonally) is counted. From this the number in the whole dish may be easily calculated. The following figures are given as an instance of results actually obtained:

$\frac{1}{2}$ cc.	contained	230	col's	= 460	bact.	per cc.
$\frac{1}{10}$ cc.	"	45	"	= 450	"	"
$\frac{1}{10}$ cc.	"	47	"	= 470	"	"
$\frac{1}{100}$ cc.	"	5	"	= 500	"	"
$\frac{1}{1000}$ cc.	"	1	"	= 1,000	"	"

In such a case, where the number of organisms is comparatively small, it is evident that the figures obtained from the smaller quantities are less reliable than those from the larger, and in calculating results it is well to omit them, the actual number to be certified in the above example would be 460, the mean of the first three plates. On the other hand, when the number is very large, the results from smaller quantities are more reliable than from larger ones, where the difficulty of accurate counting is a very real one.

Having thus ascertained the number of bacteria in the water before and after filtration, a simple calculation gives the percentage removed. When working well, sand-filters, as used by most water companies, will remove from 95 to 99.9 per cent. of the bacteria. This remarkable result is explained by the fact that the bacteria at first arrested by the upper surface of sand there multiply, forming a slimy coating, which serves as a very efficient filtering medium for the water. It follows, therefore, that a newly-constructed filter does not purify the water well, but requires a few days to attain its maximum of efficiency. It also follows that the rate of filtration becomes slower as the slimy coating increases in thickness—so much so that from time to time the upper surface of fine sand must be scraped off to the depth of about ⅛ to ¼ inch.

The vast majority of bacteria present in natural waters are of a harmless character, but there is no reason to believe that pathogenic organisms, such as cholera and typhoid germs, will behave in any way differently from the ordinary "water bacteria," in fact, the experience of Hamburg during the cholera epidemic of 1892 shows that they behave similarly. The towns of Hamburg and Altona both drew their supply from the river Elbe. The former received the water from a point above the outfall of the town sewers, and did not filter it before supplying, whilst the latter took its supply below the outfall of sewers of both Hamburg and Altona. The neighboring town of Wandsbeck received water from an inland lake after filtration. The deaths from cholera in the three towns were as follows:

Hamburg	12.28	per thousand.
Altona	2.34	"
Wandsbeck	2.15	"

2. THE GENERAL EXAMINATION OF WATER AS TO SUITABILITY FOR DRINKING PURPOSES.

The first step in this examination is the same as already described, but it is evident that the bare determination of the total number of organisms present is of practically little value, because the great majority will usually be harmless forms, and the *purest* natural waters are capable of sustaining the life of vast number of bacteria; moreover, as has been before observed, a water naturally very pure bacteriologically becomes teeming with bacterial life a few days after collection, by multiplication. There are, however, other considerations of much greater value, viz., the number of bacteria which cause liquefaction of the gelatine, and the number of different species present. When examining the dishes for the number of colonies it will be apparent that great differences exist between them, some appear within thirty-six hours or even earlier, whilst others do not become visible for several days; moreover, some are found only upon the surface of the gelatine, others are entirely embedded beneath the surface; the color may vary, the commonest being yellowish or white, reddish, brown, or gray. One very important difference is that while some forms cause rapid liquefaction of the gelatine, others liquefy it but slowly; others, again, do not possess this power at all. This liquefaction may extend far beyond the visible circumference of the visible colony, or may be confined to a small radius; the liquid gelatine may be thin or viscid, transparent or turbid. The form of the non-liquefying colonies is also important—round, oval, or irregular, or of characteristic form.

These liquefying bacteria are very frequently the cause of putrefaction, and produce unpleasant odors consequent upon the decomposition of the gelatine; moreover, they are not able to live and multiply in pure waters to anything like the extent that the non-liquefying species do, hence their presence in large numbers is a very bad feature.

A water which becomes polluted by sewage and other household filth is thereby contaminated not only by great *numbers* of bacteria, but by many *species*, whereas a water drawn from a deep spring or other source remote from polluting influences contains comparatively few, and very few, perhaps only three or four, species of bacteria. The gelatines cultures having been made as usual, they are carefully examined, and a minute portion of each form of colony is removed by means of a needle (previously sterilized by heating in the Bunsen flame, and cooled), and transferred to test-tubes of gelatine or other culture media. The inoculation of test tubes is carried out either by "stroke" or "puncture" as follows:

For stroke culture test-tubes are used, containing gelatine, agar-agar, or sterilized potatoes. Agar-agar culture medium is prepared in the same way as gelatine-peptone, substituting for the gelatine 20 grams of agar-agar, which, however, requires prolonged heating to ensure solution. Potatoes are difficult to sterilize; they are first washed, soaked in solution of bichloride of mercury (1-1000) for an hour or two, and then peeled; again placed in bichloride solution for five minutes, washed with sterilized water, and cut into slices about one-third of an inch thick, with a sterilized knife. The slices are then put into sterile cultivation plates or test-tubes (plugged with sterilized wool), and placed in the steam sterilizer for an hour longer.