

(1) The methyl-red positive type ("M.R.+"), or the Voges-Proskauer negative type ("V.P.—"), and

(2) The methyl-red negative type ("M.R.—"), or the Voges-Proskauer positive type ("V.P.+").

The first is characteristic of excretal contamination, and is sometimes called the "Coli type," while the second is not characteristic, at any rate, of recent excretal contamination, and is sometimes called the "Aërogenes type," after its type organism, *B. lactis aërogenes*.

These tests commend themselves to the bacteriologist as suitable for routine work in the same way as the tests of Houston, by their simplicity, and are particularly to be welcomed by those in whose experience particular water supplies have yielded only atypical varieties of lactose fermenters according to Houston's definition.

Results show how small a proportion of the lactose fermenters of excreta belong to the "M.R.—" type, and how large a proportion are present in soil and grains. Occasionally a specimen of faeces contains a fairly large proportion, the high figure of 26% obtained by Rogers, Clark and Lubs was due to a single abnormal specimen, and to the employment of a method specially selected to favor the growth of this type, and does not represent the average numbers in which they were actually present.

Methyl-Red Negative Organisms Survive

Records of various observers strongly suggest that organisms of the "M.R.—" type are the more resistant, the more capable of surviving outside the animal body, and of finding a favorable environment in soil.

Winslow and Cohen carried out experiments on the relative viability of these two main types in water, and their results show that there is distinct evidence that the viability of the "M.R.—" type is greater than that of the "M.R.+" type.

Experiments show a decrease in the "M.R.+" type from 50 to 5%, and from 56 to 0% respectively (in sixty days). On the average, however, the reduction in the percentage was from 54 to 29.

As the methods employed by American bacteriologists differ in some respects from those usually employed in this country, it was thought advisable to investigate how frequently organisms of the "M.R.—" type were likely to be encountered in the routine examination of waters by English methods, and, by noting the association or absence of association with streptococci, to ascertain whether the presence of lactose-fermenting organisms of this type affords evidence of recent excretal contamination or not.

Observations were therefore made by the author of a large number of samples of water from various sources. Out of 200 samples in which lactose fermenters were present, organisms of the "M.R.—" type were encountered in 66, or 33%.

Lactose fermenters of the "M.R.+" type and streptococci were absent from practically every sample, strongly suggesting that in general no recent excretal contamination had occurred, for if it had streptococci would have been found in about 50% of these samples. [These samples were of water of good repute.]

Other samples from shallow wells, etc., were as follows:—

Samples from Shallow Wells

(1) Those containing no streptococci and no lactose fermenters of the "M.R.+" type, suggesting no recent contamination.

(2) Those containing streptococci and probably lactose fermenters of the "M.R.+" type also, though, since contamination was sufficiently proven, further search for this type was not made.

(3) Those containing streptococci and lactose fermenters of the "M.R.+" type, further search having been made for this type for the purpose of this investigation.

It may be asked, How is it that the "M.R.—" type are found in samples of groups 2 and 3 in which recent excretal contamination is proved? The explanation obviously is that a certain amount of self-purification has already taken place, but the contamination is sufficiently recent for organisms of

the "M.R.+" type and streptococci to be present also in larger quantities of the sample.

Of special interest was the occurrence of lactose fermenters of the "M.R.—" type in the deep wells in limestone. This supply consists of seven or eight wells, several miles apart, varying in depth from 250-450 ft. These wells yield a very pure supply, lactose-fermenting organisms being absent from 100 c.c. in 85% of all samples examined. In the spring of 1918 lactose fermenters were found in a much larger proportion, and all (with one exception) were found to belong to the "M.R.—" type. Streptococci were invariably absent. The inference is that the most resistant types only of the lactose fermenters reached the underground water, though why they were present in abnormal numbers at this particular time could not be discovered. The only exceptionally heavy rainfall occurred five months previously.

Viability of Capsulated Organisms

The large majority of lactose fermenters of the "M.R.—" type (about 85%) show capsule formation. Experiments by the present author show that some capsulated organisms display remarkable viability in boiled (or steamed) hard water. In one experiment the organism added to such water, containing no measurable trace of saline or organic ammonia, was isolated after a period of three and a-half years. It should be noted that it is only in boiled (or steamed) hard water that such remarkable viability is observed, though in unboiled water some of the capsulated types live longer than the non-capsulated (in one experiment fourteen weeks as compared with seven weeks). The phenomenon seems to be dependent upon the slow deposition of supersaturated carbonate of calcium or hydrate of magnesium, which takes place in the boiled water after filtration. Whether the necessary conditions for prolonged viability occur in limestone silt is a matter worthy of investigation.

Other experiments confirming this remarkable behaviour, one of which was carried out independently by Dr. W. G. Savage, are recorded in the *Journal of Hygiene* Vol. XVI, No. 3, January, 1918. In periods varying from five to eleven weeks, multiplication from fifty to one hundred fold was recorded.

Both strain T. and strain N.D. gave the Voges-Proskauer reaction, and belong to the "M.R.—" type. Strain N.D. produced indole and acid and clot in milk, and was therefore indistinguishable from Houston's "typical *B. coli*," except by the methyl-red and Voges-Proskauer tests.

Summary

(1) Although attempts to distinguish between human and animal excretal matter by the characters of lactose-fermenting bacilli present have so far been unsuccessful, some measure of success has attended attempts to distinguish between recent and less recent contamination by the identification of the less resistant and more resistant types.

(2) Clemesha based his differentiation on MacConkey's classification; Houston on the more simple indole test. Savage retains the milk-clot test, and notes marked weakness of lactose fermentation, having shown that these attributes tend to diminish with soil environment. American investigators have recently based their differentiation on the methyl-red and Voges-Proskauer tests.

(3) The more resistant the types, obviously, the less evidence there is of recent contamination in waters in which they are found.

(4) Most valuable information as to recency of contamination is afforded by the enumeration of streptococci by the simple method of Savage.

(5) Association or absence of association of lactose fermenters with streptococci can therefore be used as a control in judging the merits or demerits of any of the above methods of differentiation.

(6) Relative absence of association of the lactose fermenters of the "M.R.—" type with streptococci strongly supports the view that they are the more resistant types.

(7) Experiments show that, under certain conditions, the viability of capsulated organisms of the "M.R.—" type may be very considerable. Whether similar conditions occur in nature is a matter requiring further investigation.