A CRITICISM OF DR A. C. HOUSTON'S REPORT ON THE BIOLOGICAL CHARACTERS OF *B. COLI* ISOLATED FROM (1) RAW, (2) STORED RIVER WATER, AND (3) STORED AND FILTERED WATER.

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It may be safely stated that anything which Dr A. C. Houston writes on the subject of water bacteriology is received with very great interest and respect, and his recent report on the bacteriological characters of *Bacillus coli* in raw, stored and filtered river water (Metropolitan Water Board, 7th Report on Research Work) is no exception to the rule. The report is of very great interest to all engaged in the study of water bacteriology, particularly in England. We who are working on this subject in a tropical country have different conditions around us, and we naturally look at things from a somewhat different standpoint, so that a brief description of our experience, and a criticism of Dr Houston's results in the light of our ideas will probably be of interest to many who are engaged in this fascinating subject.

There are several points in Dr Houston's report upon which we propose to offer remarks.

Dr Houston commences his report as follows:

"For many years past I have pointed out the striking differences between the varieties of *B. coli* found in excremental matter and in waters of diverse origin. As regards the Metropolitan water supply, it has been shown that the proportion of the *B. coli* organisms which ferment lactose with gas production (lactose +) and also produce indol in peptone cultures (indol+) as compared with those which fail in one or other or both of these respects, is as follows:

Water	Samples	Specimens (Glucose+)	Typical "Lactose+ Indol+" (per cent.)	Non-typical "Lactose− Indol+" or "Indol− Lactose+ or "Indol− Lactose−" (per cent.)
Raw River Waters	3,546	12,744	81·2	18·7
Filtered Waters	35,696	18,960	50·8	49·1

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"These results have always been, and are now again, given simply as facts observed, when working with liquid primary media and not, as in any way, necessarily representing the actual ratio of typical to non-typical *B. coli* in the water originally.

"These findings have resulted from the adding of 100 c.c., 10 c.c., 1 c.c. and lesser amounts of water to one or other liquid primary medium, incubating at or about blood heat usually for forty-four hours, planting out the colonies on one or another solid medium, and picking off the suitable colonies, and studying their biological attributes in a variety of culture media.

"Two points are specially to be remembered in connection with the use of liquid primary media. (1) The original proportion between the various kinds of *B. coli* may be greatly altered by the multiplication process. Indeed, the method aims at encouraging the typical *B. coli* to multiply and oust the non-typical *B. coli* in the struggle for existence in a liquid nutrient medium. (2) The specimens of *B. coli* may and do pertain to different volumes of the water being tested."

In the quotation given above Dr Houston explicitly says that the figures obtained do not necessarily represent the actual ratio of the two kinds of bacilli in the original water. Now supposing that it can be definitely proved that there is a very great difference in the ratio of the classes between the actual water and these results, surely it must be admitted that the method is unsatisfactory. In other words, any method which can be proved to give a really reliable picture of the flora actually present in the water sample, must be superior, from the point of view of the water analyst, to one which cannot be relied on to do this. The main object of all analyses of water is to obtain an accurate picture of the actual bacteriological state of affairs in the sample at the time, so that if "the original proportion of the various kind of coli may be greatly altered by the multiplication process" the utility of this particular method appears to us to be very questionable. For many years Dr Houston has been using this "enrichment" method and presumably has been basing his opinion of the quality of water on its results; probably most water analysts besides ourselves believe that, when using a method very similar to his, they are getting a reasonably accurate picture of the flora of the sample in question so that it comes rather as a shock to us in India to read that Houston now says that this is not the case.

Since we commenced the study of water bacteriology in India in 1907, we have made, from time to time, a very exhaustive study of the

limitations and good points of all the media used in all our regular methods, so as to avoid fallacies and pitfalls as much as possible. Our work on this subject became the more necessary because we have always maintained that a thorough knowledge of the bacteriology of faeces, at any given time of the year, is necessary in order to interpret, with any degree of accuracy, the results obtained from the water. In fact the investigation of the subject of multiplication of coliform organisms in bile salt broth media was originally started in order to arrive at the best method of obtaining an accurate picture of the flora of faeces.

Dr Houston not only says that "the original proportion between the various kinds of *Bacillus coli* may be greatly altered by the multiplication process" (in broth media) but "the method aims at encouraging the typical *Bacillus coli* to multiply and oust the non-typical in the struggle for existence in a liquid nutrient medium." In the first place, we wish to say that, within certain limits, we very much doubt the accuracy of both these statements, and, secondly, we should like to know on what experimental evidence this favouring of one variety of organism at the expense of others is actually based. No evidence of this kind is given in the report.

From a very careful study of the growth of mixtures of coliform organisms in broth, we have ascertained that a uniform chain of events follows inoculation and incubation with unfailing regularity. First of all let us describe what actually happens.

A flask of bile salt neutral red glucose broth is inoculated with a very small piece of faeces or drop of sewage and put in an incubator. From the commencement, multiplication of the bacilli goes on rapidly; and in about 24 hours there may be anything from 1 to 500 million bacilli present per c.c. During the process gas formation has occurred and a considerable quantity of acid is generated. For about 18 hours multiplication in the number of organisms and increase in the acidity goes on steadily. After this time there is a rapid falling off in the number of bacilli due to the poisoning action of the acid generated by the fermentation of glucose. At the end of 48 hours the 100 million organisms per c.c. present at the 24th hour, has now dropped down to half or one million organisms per c.c. At the end of 72 hours there are probably only 10–100 living organisms per c.c. or the broth itself may be entirely sterile. If absolute sterility has not been reached by 72 hours, it invariably is attained in 96 hours when glucose is the sugar used. Again, if an hourly estimation of the amount of acid generated in the broth is made, it will be found that there is a steady

increase up till about the 18–20th hour, when about 1–2 % of acid will be reached. From this point there is little or no increase in the amount of acid generated. The point of maximum acidity corresponds roughly with the point of maximum number of organisms in the broth.

This chain of events can be demonstrated perfectly plainly by anybody who cares to carry out experiments.

The explanation of this phenomenon is simple, viz. the coliform organisms go on growing steadily until the acidity, having reached a certain concentration, stops their growth. From this moment they begin to die off and in the case of a glucose broth the killing process is rapid.

In the case of a lactose broth, exactly the same phenomenon takes place, but the reduction in the number of organisms between 24 and 48 hours is very much less marked. It usually takes at least 5, 6, or even 7 days to kill off all the bacilli that have grown in a lactose broth.

Further chemical tests show that there is plenty of available sugar in the broth at all times, so that the sudden stop in the increase in acidity is not due to lack of sugar but to the cessation of activity of the organisms present. It may be well at this point to quote some actual experiments, in order to demonstrate these facts. For the sake of brevity the results are given in tabular form below.

These three results are absolutely typical. We have carried out a very large number of similar experiments with natural mixtures of organisms, that is to say, from polluted water, from fresh faeces, with mixtures of laboratory cultures, and with pure cultures of a single coliform bacillus. They all give almost identical results.

A careful study of the organisms taken at various times during the stage of increase, *i.e.* within 24 hours, shows that there is practically no selective or "enrichment" action in favour of any species of coliform bacilli. In the experiment quoted above (No. II), where five specimens of organisms were mixed together in equal proportions, there was no evidence of any one organism overgrowing the others. Indeed in lactose broth there seems to be no alteration in ratio during the period of increase or that of decrease. For, enumeration of the colonies after three days' incubation, still shows that the bacilli are roughly speaking in the same numerical relation to one another as they were when added to the broth. We, however, do not wish to push this statement too far. In the dying off process it is possible that one organism may prove more susceptible to the acid than another.

EXP. I. 10 c.c. Hughli water added to 100 c.c. of bile salt neutral red lactose and glucose broth (1 % of the sugar).

	Glucose		Lactose	
	Acidity[1] $\frac{N}{10}$ Na_2CO_3	No. of bacilli	Acidity	No. of bacilli
24 hours	7·3 c.c.	150,000,000	5·9 c.c.	30,000,000
48	7·9	2,000,000	5·9	6,000,000
72	7·8	900	5·9	3,000,000
96	7·8	sterile	5·9	2,000,000

EXP. II. One loopful of a watery emulsion of *B. coli communis*, *coscoroba*, *neapolitanus*, *lactis aerogenes*, and No. 67 were added to broths as above.

	Glucose		Lactose	
	Acidity[1] $\frac{N}{10}$ Na_2CO_3	No. of bacilli	Acidity	No. of bacilli
24 hours	6·6 c.c.	500,000,000	5·9 c.c.	400,000,000
48	6·6	2,000,000	5·9	80,000,000
72	6·7	10,000	5·9	4,500,000
96	6·8	sterile	5·9	2,500,000

10 colonies isolated after 72 hours' incubation in lactose broth, gave *Coli communis* 1, *coscoroba* 3, *neapolitanus* 2, *lactis aerogenes* 2, No. 67 2.

EXP. III. An emulsion of about equal quantities of *B. schäfferi*, *neapolitanus*, *coscoroba* and No. 67 was made and incubated in both broths.

	Glucose		Lactose	
	Acidity[1] $\frac{N}{10}$ Na_2CO_3	No. of bacilli	Acidity	No. of bacilli
24 hours	3·5 c.c.	1200,000,000	3·1 c.c.	1300,000,000
48	3·5	4,000,000	3·1	40,000,000
72	3·5	200	3·0	11,700,000
96	3·5	sterile	3·1	7,000,000
5 days	...	...	3·0	2,400,000
6	...	...	3·1	410,000
7	...	...	3·1	4,000
8	...	...	3·1	25

The lactose broth usually becomes sterile quicker when natural mixture organisms, such as are contained in a drop of sewage or 5 c.c. of polluted water, are used.

The point we wish to make is, that during the first 18 hours when apparently all organisms are growing steadily, there is no evidence whatever that any particular species overgrows the others. After the point of maximum acidity is reached alteration in ratio may take place, but in lactose it is very small.

[1] 10 c.c. of the broth titrated with $\frac{N}{10}$ Na_2CO_3. In Exps. I and II neutral red was used as an indicator, in III phenol phthalein.

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Stating that the point of maximum acidity is reached about the 18th hour does not preclude some variation as this would naturally be expected if the actual number of bacteria added to the broth was very great in one case and small in another. Adding enormous numbers does not appear to affect the result very much; if, say, 10 c.c. of crude sewage is added to one tube and a loopful to another, the acidity in the former appears earlier and developes much more rapidly but it reaches its maximum very little before the usual time, viz. the 18–20th hour.

But, if very few bacteria are added to the broth—possibly only one—as may be done by adding ·001 of a c.c. of a water sample, it may take 36–40 hours before acid and gas are formed in sufficient quantity to be visible. Our experience shows that by far the majority of these very dilute mixtures give the reaction in 20 hours and reach a maximum acidity in under 30, but occasionally delay does occur, so that all tubes are given 48 hours' incubation, so as to be quite certain of the negative results.

Again, if a mixture of two bacilli, such as *B. coli communis* Escherich (which is lactose+ glucose+) and our bacillus *P* (which is glucose+ lactose−) are mixed together in roughly equal proportions and inoculated into a glucose broth, it can be proved that within the first 24 hours there is little or no alteration in the relative numbers of the two classes; both bacilli fermenting glucose. But if the same mixture be inoculated into lactose broth, the lactose fermenters (*B. coli communis*) very rapidly overgrow the bacillus *P*, which does not ferment the sugar. Even in this case white colonies are usually found in the plate. As one would expect, the more bacilli *P* there are in the original mixture the less is the overgrowth of *B. coli* apparent, but in cases where the numbers are approximately equal at the time of inoculation, the *B. coli communis* undoubtedly very rapidly outgrows bacillus *P*. This is after all only what one would expect—a bacillus that does not ferment lactose would probably grow slower in a lactose medium than one that does—indeed it seems to us rather astonishing that a non-fermenter should grow so well in a sugar that it cannot alter in any way.

Another series of experiments has been carried out in order really to ascertain whether the picture obtained from subculturing an 18-hour broth culture is reasonably like the original substance used for inoculation. A small piece of faeces of either human being or cow, was taken and was divided into two parts. One was carefully wiped over 4 or 5 bile salt neutral red lactose agar plates. The other was

inoculated into a bile salt lactose broth, and was incubated for 18 to 20 hours (inoculation was usually made in the afternoon and subcultured on the following morning). A careful comparison between a large number of colonies obtained by both methods, shows that the results are practically identical, the species of coliform organisms and their numerical relation to each other correspond to a most wonderful degree. The more colonies that are identified in each case the greater is the resemblance between the two results. For our experiments we took 50 colonies from both and we carefully identified each organism.

It will be observed that these experiments are infinitely more conclusive than those quoted by Dr Houston making use of raw, stored and filtered water. The possibilities of error in centrifugalisation, precipitation, evaporation, filter-brushing, etc., is extremely great: whereas in our experiments making use of faeces, all these very doubtful factors are eliminated.

From the above experiments it is obvious that the optimum time for incubation in a fluid medium is 18–20 hours and that if this period be adhered to, the results obtained give a reasonably accurate picture of the species of intestinal organisms present and their numerical relation to each other; that the longer the incubation is extended beyond the 18th or 20th hour—the point of maximum acidity—the greater is the possibility of alteration of this ratio, not because the organisms "oust one another in the struggle for existence," but because the acidity generated is killing off all varieties, and possibly affecting one more powerfully than another.

Dr Houston's method of water analysis and our own are almost identical in the first stage. For crude waters we inoculate both a lactose and a glucose broth with identical quantities of the sample, in order to find out the relative number of glucose and lactose fermenters present in the water. The full reasons for this procedure cannot be entered into here. For filtered waters we use a lactose broth only. Having done this, we take the tube that has received 20 c.c. of the sample, after 18 hours' growth in the incubator, and subculture the organisms present in this tube *only*. The various species of coliform organisms present are separated by MacConkey's method. We maintain that by this procedure we get a very accurate picture of the true bacteriological flora of 20 c.c. of the water under analysis, and it is not necessary to separate species in smaller quantities. The actual number of lactose or glucose fermenters present in all smaller quantities is ascertained by the first step.

The second point that we wish to discuss is in connection with the following statement made by Dr Houston:

"Strictly speaking the only completely satisfactory method of instituting comparison between the proportion of typical and non-typical bacillus coli in different waters is by direct culture on solid media without the intervention of liquid cultures (indirect method) but owing to the generally satisfactory quality of the filtered waters this is impossible in routine work as it involves some special concentration method (*e.g.* centrifugalisation, precipitation, evaporation, filter-brushing, etc.)."

Before dealing with the results obtained by the direct method it seems to us rather a pity that the technique employed in this process is not given in greater detail. Presumably, both raw and filtered waters were centrifugalised or concentrated in some way, before the bacilli were transferred to the solid medium. In order to form any

Comparison between direct and indirect method—figures taken from Houston's reports.

	Lactose +										Lactose + Indol +					
	Direct		Indirect								Direct		Indirect			
	1912		1908		1909		1910		1911		1912		1908	1909	1910	1911
	Colonies	%	Actual	%	Actual	%	Actual	%	Actual	%	Colonies	%	%	%	%	%
Raw	320	75·94	2710	90	2595	91·3	2427	88·7	2635	91·1	320	53·13	81·6	79·4	80	81·8
Filtered	225	69·33	3830	75·5	3164	68	3934	49·2	3522	69·6	225	34·22	45·5	42·8	35	49·8
Stored	232	57·33	...	...	...	...	...	...	...	...	232	46·12	...	...	...	...
											Raw:— monthly max.		94·3	86·6	87·6	
											monthly min.		75·5	68·7	68·0	
											Filtered:—monthly max.		79·2	68·6	59·4	
											monthly min.		35·3	26·2	20·7	

comparison with other results one should know how much water was used in obtaining the organisms for plating, because the results obtained from 1 c.c. of the water would obviously be different from those obtained from 20 c.c. A table has been prepared putting side by side the results from the direct method (given in the report under discussion) and those obtained by Dr Houston from the London river waters by the indirect method over a period of several years. It must be remembered that all these figures come from the same laboratory and they appear to us to be comparable, except that the indirect figures are collected from three rivers, whereas the direct all came from the Thames.

Any one surveying this table, as a whole, cannot fail to be struck with the general similarity of the results between the direct and the indirect method. Do these figures, as a whole, demonstrate the truth of the statement that there is a serious alteration due to the action of nutrient broth in the numerical ratio between the varieties of coli? Admitting for the sake of argument the theoretic superiority of the direct method over the indirect, is the difference in result likely to lead to serious error in drawing conclusions from the latter? The answer to this query must undoubtedly be in the negative. It appears to us that the figures of the table might equally be quoted as demonstrating the general similarity in results obtained by the two methods.

Besides this, the following points must be carefully borne in mind:

(1) The great difficulty and general complexity of the direct method, necessitating centrifugalisation, precipitation, evaporation, filter-brushing, etc. Surely with a method of this complexity the possibilities of altering the numerical ratio of coli present by a few per cent. is very considerable. Again, all accustomed to work with bacilli plated on solid media know that some species of colonies influence the growth of their neighbours, especially when common water organisms grow alongside intestinal bacilli. Might this not introduce a possible factor of error? Might not this be the explanation of the lower percentages in raw waters in the direct method?

(2) As already stated, we are not told from how much of the water these organisms were obtained—whether from 10, 20 or 100 c.c. The same amount could not be used for a polluted Thames water as was necessary for a good stored and filtered water.

(3) We are not told at what time of year the samples for the direct method were obtained. Houston's own figures (*vide* tables in annual reports) show that the ratio of typical and atypical coli in crude waters may vary between 95 and 60 % and in the filtered water from 80 to 35 %, according to the time of year. Obviously, therefore, a difference of 5 or 10 % between the direct and the indirect method might be accounted for by the time at which the samples were obtained.

(4) In using the direct method 44 hours' incubation were allowed, whereas we have already shown it would have been better to allow 18–20 hours.

(5) Compare the number of colonies used in the direct and the indirect method. In the indirect method the percentages are worked

out at usually 2000 or 3000 samples. In the direct method the percentages are 200 or 300 colonies. This factor might easily account for the slight differences.

Taking these various factors into consideration, all of which would tend more or less to influence the percentages, we consider that it may reasonably be concluded that the figures quoted above do not demonstrate that the direct method is superior to the indirect in giving a picture of the flora of the water samples. On the other hand, these figures seem to us to show, that, making allowance for the above variable factors, the indirect method is approximately as satisfactory as the direct.

The third point that we wish to discuss rises from the following quotation:

"Lactose+ indol+ microbes are typical of excrement inasmuch as they are present therein in an enormous number and are the predominant microbes in this material. Waters not recently contaminated with excremental matters contain none or very few of these bacteria."

This short paragraph is taken from the general remarks (p. 6), not from the report itself, so we are not quite sure if Dr Houston means it to apply to London waters *only*. If so, we can take very little exception to it. But we know that some analysts hold that this dictum is almost universally true, therefore we propose to discuss it from a wider standpoint.

In the first place we consider that the first sentence in this paragraph is rather too strongly stated. There is no doubt whatever that the true faecal organism is a lactose fermenter and it is true that the majority of faecal bacilli also give the indol reaction. We would, however, point out that we have shown that there is a considerable variation at various times of the year in the flora of faeces in India; "epidemics" of one particular organism are fairly common. Consequently, this being the case, it is obvious that too much reliance cannot be placed on a very wide statement such as this. Supposing we had four faecal organisms *A*, *B*, *C* and *D*, three of which gave indol and one did not, it is clear that if the numerical ratio between *A*, *B*, *C*, *D* varies considerably, due to climatic or other influences, it is possible that the number of organisms which are lactose+ indol+ may be subject to considerable alteration.

Again the indol reaction does not give very constant results. It depends how the reaction is carried out. The "Benzaldehyd" method is superior to the old process, and Rivas (*Centralblatt*, 1912) has

shown that by converting the peptone to tryptophan, by the use of trypsin, a better reaction is obtained. It is easy to show that these three methods give very discordant results.

To the second sentence of the quoted paragraph, viz. "Waters not recently contaminated with excremental matters contain none or very few of these bacteria" we must take very grave exception. Waters are not infrequently met with in India, in lakes and storage reservoirs, which we know have received no pollution for probably two, three or even six months, yet the majority of these may contain as many as 1 to 10 lactose+ indol+ organisms per c.c. The reason for this is, that all but one of the common varieties of lactose fermenters that are capable of surviving many month's exposure give the indol reaction.

Another point must be mentioned. It is obvious that if both parts of the quotation are true, one would be perfectly justified in concluding that the pollution was recent in any sample of water in which lactose+ indol− bacteria were plentiful; and if lactose+ indol+ only were present, the pollution was old or less recent. If, as in India, the second remark is incorrect, although the first may be true in point of fact, the whole statement is valueless from the water analyst's point of view; no conclusion can possibly be drawn from it. It would be impossible to tell from the results whether the pollution was six hours or six months old.

The great objection we have to statements of this nature is that they are unscientific. Dr Houston admits that there are many varieties of coli, some of which give indol and some do not. If the quotation is to be taken as universally true, it follows that the numerical ratio of all these varieties must remain constant for all animals, for all places and for all seasons. Even if it is applied to London only it is still objectionable, for it takes no account of the various natural characteristics of the individual species and their behaviour in natural surroundings under different environment. It is tantamount to describing the various types of inhabitants of a large country by the single statement that all the men living in a certain town had red hair and blue eyes.

This brings us to the next point.

In the general remarks on the Report Dr Houston says:

"The considerable number of apparent varieties of bacillus coli described in the report is of scientific interest. No doubt the list can be extended still more by the application of further tests, serum reactions, etc. *It must not be supposed, however, that it is possible to*

assign to these real or apparent varieties any definite place in a scale designed to measure their relative significance as indicators of dangerous pollution. Our inability to do so forces us after separating them to bring them together into a group or groups as otherwise all continuity of record is apt to be lost."

We are perfectly prepared to admit the truth of this statement, provided that the *only* facts that we know concerning any given species of faecal organisms are its fermentation reactions, but we wish very strongly to emphasize that there is another entirely different set of characteristics which may be of very great importance to the analyst; and although it is very unlikely that a scale of the relative importance of *all* faecal bacteria as indicators of pollution could ever be made, yet any well authenticated natural characteristic of any individual may be of considerable use to the sanitarian. The best way to acquire this knowledge is to study carefully the bionomics of the process of self-purification of polluted water.

It is of little value to anybody to know that bacillus *A* (*B. cloacae*) ferments lactose, does not give the indol reaction, and liquifies gelatine; but on the other hand, supposing from a careful study of its life history, it is discovered that the bacterium is to be found chiefly at the bottom of lakes, where it multiplies and where it will live for six months, in spite of the tropical sun, this fact has a practical application.

Again, organism *B* (*B. lactis aerogenes*) is lactose, saccharose, adonite, Vosges and Proskauer+; inulin, dulcit, indol, motility−, and it may be recognised by these tests. But it is rare in faeces of man and animals—in lakes 7–12 days after pollution, and rivers during the rains bacillus *B* simply swarms—in two or three weeks, if no more pollution takes place, it has disappeared[1]. Would not information of this kind render organism *B* a useful indicator of pollution? Surely, these facts are of very great importance.

Again, in faeces there may be 50 varieties of coliform organisms. Now is it likely that all these 50 varieties are going to behave in exactly the same way when they pass into water? If they are, then Dr Houston is right, and there is absolutely no use in attempting to isolate individuals, and the fact that 50 such varieties exist is of academic interest only. In India we have demonstrated beyond all shadow of doubt that a certain number of these 50 varieties have a characteristic life history and behave in a way different from the other members of the group[1]. Consequently, it is necessary and advisable to

[1] *Vide* Clemesha, *Bacteriology of Surface water in the Tropics.*

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do just as much separating of bacteria as will allow one to recognise certain individuals. An example of what we mean is to be found in Dr Houston's own report.

Year after year Dr Houston has reported the fact that during the winter the number of true coli in crude Thames water is greater, relatively, than during the summer. He has also reported that the number of true coli in filtered (and therefore stored) water is about half of what it is in raw water. Hence, it is perfectly apparent to everybody that there are some forces at work which cause a rearrangement of varieties, and a departure from the true faecal conditions of the pollution. These forces act more powerfully in summer. As the process is one of general reduction of numbers, it is obvious that amongst the 50 varieties of coli, some disappear more rapidly than others, or some varieties grow while others disappear. In none of Dr Houston's many reports is there a word of explanation of this phenomenon. Furthermore, we are prepared to state that as long as Dr Houston remains satisfied with the meagre statement that (in London waters) the typical excremental organism is a lactose+ indol+ microbe, he will never solve this very common problem. The only way in which a solution could be found is by a careful study of the individual species present at different stages of the reduction process.

In order to exemplify what we mean, we will give a very brief account of the discovery of the true explanation of this phenomenon in Indian waters. In India we have observed that in fresh faeces 96 % of the organisms are lactose+ glucose+. The other 4 % are glucose+ lactose−. In water that has been exposed to the sunlight for many weeks or months the reverse is true, viz. 80 to 90 % of the organisms present are glucose+ lactose− and the other 10 are glucose+ lactose+. From these facts we thought that the class glucose+ lactose− was more resistant to natural forces inimical to bacteria than the class glucose+ lactose+.

Careful experimentation showed that this was entirely wrong; speaking of classes only, both are equally susceptible, but we discovered that in the class glucose+ lactose− (which as far as we can ascertain contains about 10 fairly common varieties) *one organism or perhaps two*, the commoner of which we call bacillus *P*, multiplies very rapidly in water after the second day; it goes on increasing in spite of the action of the sunlight, and will remain alive for many months, apparently perfectly happy in its environment. All other members of this class disappear within about one to two days. This is the

explanation of the phenomenon in Indian waters; we do not say that this applies in every detail to the London waters.

There is yet another step in this argument. In the report under consideration Dr Houston has carefully studied the characteristics of the various colonies that he has obtained by the direct method, using MacConkey's scheme for separating out these species (he has added the fermentative reaction in salicin, raffinose, and inosit—this latter has also been used by MacConkey, but not by us). We have gone very carefully through these results and have rearranged the organisms into two tables, (1) those which are glucose+ lactose+, and (2) those which are glucose+ lactose−. We find it advisable to separate these two groups of bacilli in our work in India. A tabular statement of Dr Houston's results is given below with his percentages.

We have, however, calculated the percentage prevalence of these organisms in their own class (*vide* the third column). We have also entirely neglected the filtered water results, because water that has once been through filter cannot be said to be a strictly natural product, because it has been subjected to the action of an arrangement that does not exist in nature.

A careful scrutiny of these figures shows one or two very striking points. It will be remembered that both Dr Houston's work, and ours, shows that this particular class (glucose+ lactose−) very largely predominates over the class (glucose+ lactose+) in waters that have been exposed to the sun for many weeks. We have explained that in India this increase is due to the rapid growth of one particular bacillus, viz. our bacillus *P*.

In the first place our bacillus *P*, which is No. 13 in the table, is present in English waters but is comparatively rare; there is no numerical increase in stored water; hence the explanation of the altered ratio between the classes in England is not exactly the same as in India.

Secondly, observe the enormous increase in stored waters of variety No. 1 and variety No. 46. In the case of No. 1 there are three times as many in stored waters as there are in the crude, and in the case of No. 46 there is a large increase. These two varieties account for 78 per cent. of all organisms present. It appears to us highly probable that the relative increase in the class (glucose+ lactose−) over the class (lactose+ glucose+) in stored water is due to the multiplication of one or both of these two bacilli simultaneously with a steady dying off of the lactose+ glucose+. If this surmise is incorrect it devolves on Dr Houston to

The Bacteria that are lactose− glucose+ from Houston's Report.

Serial No.	Saccharose	Dulcite	Adonite	Inulin	Vosges & Pros.	Indol	Motility	Salicin	Raffinose	Inosite	Raw: Actual	Raw: Dr Houston's percentage	Raw: Percentage in this class	Stored: Actual	Stored: Dr Houston's percentage	Stored: Percentage in this class	
3	−	−	−	−	−	+	−	−	−	−	4	1·25	5·19	6	2·59	6·06	
1	−	−	−	−	−	−	−	−	−	−	10	3·12	12·98	**42**	**18·1**	**42·42**	
6	−	−	−	−	−	−	−	+	−	−	3	·94	3·89	3	1·29	3·03	
79	−	−	−	−	−	−	−	−	+	−	0	...	...	0	...	...	
52	−	−	+	−	−	+	−	−	−	−	1	·31	1·29	0	...	...	
20	−	−	−	−	+	−	−	−	−	−	2	·62	2·59	1	·43	1·01	
31	−	−	−	−	+	−	−	−	+	−	0	...	...	0	...	...	
51	−	−	−	−	+	−	−	+	+	−	1	·31	1·29	0	...	...	
23	−	+	−	−	−	−	−	+	−	−	0	...	...	0	...	...	
26	−	+	−	−	−	+	−	+	−	−	3	·94	3·89	1	·43	1·01	
38	−	+	−	−	−	+	−	−	−	−	1	·31	1·29	1	·43	1·01	
29	+	+	−	−	−	+	−	−	−	−	2	·62	2·59	0	...	...	
48	+	+	−	−	+	−	−	+	+	−	9	2·81	11·68	1	·43	1·01	
68	+	+	−	−	−	−	−	+	+	−	2	·62	2·59	0	...	...	
80	+	+	−	−	−	−	−	−	+	−	0	...	..	0	...	...	
12	+	−	−	−	−	−	−	+	+	−	5	1·56	6·49	1	·43	1·01	
19	+	−	−	−	−	−	−	−	−	−	3	·94	3·89	1	·43	1·01	
41	+	−	−	−	−	−	−	−	+	−	1	·31	1·29	0	...	...	
62	+	−	−	−	−	−	−	+	−	−	1	·31	1·29	0	...	...	
46	+	−	−	−	−	+	−	−	−	−	19	5·94	24·67	**35**	**15·09**	**35·35**	
78	+	−	−	−	−	+	−	+	+	−	0	...	...	1	·43	1·01	
13	+	−	−	−	+	−	−	+	+	−	7	2·19	9·09	4	1·72	4·04	Bac. P
27	+	−	−	−	+	−	−	+	−	−	2	·62	2·59	1	·43	1·01	
30	+	−	−	−	+	−	−	−	−	−	0	...	...	0	...	...	
40	+	−	−	−	+	−	−	−	+	−	1	·31	1·29	0	...	...	
75	+	−	−	−	+	+	−	−	−	−	0	...	...	1	·43	1·01	

show in what way these facts fail to explain the condition reported by him, and what is the correct explanation.

These facts demonstrate beyond all possible doubt (*a*) the necessity for studying the natural history of organisms outside the body, and (*b*) that generalisations regarding the natural characteristics of groups of organisms, based on fermentative reactions, are hopelessly wrong, and (*c*) that separation of individual species is necessary if any progress is to be made.

What water analysts require is the simplest laboratory procedure to enable them to identify certain well-known species of faecal bacilli, because, as we have already shown, certain of these have very definite

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characteristics and, when passed into water, this information is of great importance.

Hence we may conclude that it is possible to assign a very definite place to certain varieties of faecal organisms as a measure of their relative significance as indicators of dangerous pollution, but the data which render this possible are not solely the fermentative reactions observed in the laboratory, but also any facts relating to the life history of these bacteria, and these can only be obtained by a careful study of the self-purification of water.

As long ago as 1905 MacConkey recommended the separation of faecal organisms into separate species, because by so doing we should probably obtain valuable information as to their significance as indicators of pollution. We maintain that the truth of the contention has been amply proved.