THE NUMBER AND VARIETIES OF BACTERIA CARRIED BY THE COMMON HOUSE-FLY IN SANITARY AND INSANITARY CITY AREAS.

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(With Plate V and 2 Charts.)

Introduction.

There have been in recent years a large number of experiments which prove that the common house-fly (*Musca domestica*), can carry living pathogenic bacteria both on and inside its body.

The most complete observations of this nature have been made by Graham-Smith (1910) who, *artificially* infecting flies, caught at random, with pathogenic bacteria was able to recover the latter alive after a long or short interval. He found the length of time varied with the kind of bacterium and its position on or in the fly.

Careful observations of their feeding habits on fluids led to the following conclusions:

"After feeding on liquid food flies habitually regurgitate from their crops drops of fluid through their proboscides. Sometimes these drops are deposited on the surface on which the flies are walking. When the

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infected food has been given the infecting organisms are usually found in great numbers in these spots or vomit. If allowed to feed on half dried materials or soluble solids the flies first moisten with vomit or saliva a small area, and then suck it dry. The importance of this habit in infecting dry food materials cannot therefore be over-estimated. Flies which have access to abundant food defaecate frequently." The number of spots (vomit and faeces) in 24 hours per fly was in three experiments 16·4; 20·4; and 30·7. "The rate of deposition seems to vary with the kind of food and the temperature." The faeces deposited by flies artificially grossly infected with non spore-bearing organisms "often contain these in considerable numbers for at least two days, and are frequently infective for much longer periods. Anthrax spores survived for many days on the exterior and in the alimentary canal."

Comparatively few experiments have been made to determine the kinds of bacteria carried by naturally infected, *i.e.* "wild" flies, and still fewer, probably none in this country, on the *number* of bacteria which may be carried by such flies as the result of feeding on refuse of all kinds. Some pathogenic bacteria have, however, been isolated from naturally infected flies:

(1) *B. typhosus* by Fickler (1903); Hamilton (1903); Klein (1908); Berterelli (1910) from flies caught in houses in which persons were lying ill of typhoid fever. Faichnie (1909) and Cochrane (1912) have also isolated *B. typhosus* from flies caught in various places where typhoid fever prevailed.

(2) *B. pestis* by Yersin (1894) in a dead fly found in his laboratory which contained many animals inoculated with plague bacilli.

(3) *V. cholerae* by Tizzoni and Cattani (1886) from flies caught in a cholera ward; by Simmonds (1892) from flies caught in a post-mortem room containing the bodies of persons who died of cholera; by Tsuzuki (1904) from flies caught in a cholera house.

(4) *Morgan's No.* 1 *Bacillus.* This is supposed to be a cause of summer diarrhoea in children and was isolated from "batches of flies which came for examination from infected and uninfected houses in Paddington, and from a country house situated many miles from London where no cases of diarrhoea had occurred, at any rate within a radius of two miles." (Morgan and Ledingham, 1908–1909.)

(5) *B. para-typhosus A.* by Torrey (1912) in America.

(6) *B. para-typhosus B.* by Nicoll (1911) in England.

(7) *B. dysenteriae* (Flexner), Graham-Smith (1909) in a preliminary note on examinations of flies for the presence of colon bacilli appears

to have obtained on two occasions an organism which corresponded "in its cultural reactions with *B. dysenteriae* (Flexner)." He does not say, however, whether serological tests were applied as well.

(8) *Colon group.* Graham-Smith (1909) examined 148 flies caught in various places at Cambridge and London, and isolated "35 lactose fermenting organisms of the colon group, 22 from the surface and 13 from the intestines."

Nicoll (1911) has also shown that flies may carry at least "27 varieties of the colon bacillus."

(9) *B. tuberculosis,* Hofman (1888) examined flies caught in the room of a phthisical patient and found tubercle bacilli in four out of six flies.

Variations in the number of bacteria carried by wild flies.

The only experiments we have been able to find as to the *number* of bacteria carried by flies caught under natural conditions are those of Jackson (1907), Esten and Mason (1908), and Torrey (1912), all in America.

Esten and Mason caught flies from several sources by means of a sterile net, introduced them into a bottle containing a measured quantity of sterile water, and then shook the bottle to wash the bacteria from their bodies, and so "simulate conditions obtaining when a fly fell into milk." They do not state how long the flies were shaken, besides shaking does not approximate to natural conditions, especially as the greatest number of organisms with which flies infect solids and liquids probably come from the vomit and dejecta rather than from their bodies.

These observers found that "the average for 414 flies was about 1,250,000 bacteria on each" but the flies from dirty areas carried a far greater number than those from clean areas. They classified their bacteria in three of their experiments as follows:

(1) Rapidly liquefying bacteria; (2) slowly liquefying bacteria; (3) *Bacterium acidi lactici* group; (4) *Coli-aerogenes* group. In these three experiments "the objectionable class, *coli-aerogenes* type, was two and one half times as abundant as the favourable acid type."

Jackson (1907) found as many as "100,000 faecal bacteria in a single fly, and as a general thing the nearer the flies were to the sewer outlets the more numerous was this class of bacteria." Torrey (1912) examined wild flies caught in his laboratory at New York not very far from low grade tenements and found: "Flies examined up to the latter

end of June were free from faecal bacteria, and carried a homogeneous flora of coccal forms." Faecal bacteria of the colon type were found in July, together with 'para colon' and paratyphoid A. Colon and 'para colon' were met with throughout August. The surface contamination varied from 570 to 4,400,000 per fly, and the intestinal bacterial content from 16 to 28,000,000. The colon group constituted 13·1 % of the total for the surface of fly, and 37·5 % of the total for the intestine. Thirty-nine cultures of lactose fermenters were isolated. They were distributed as follows: *B. acidi lactici* group, eight; *B. coli communis* group, ten; *B. coli communior* (*neapolitanus*), four; and *B. lactis aerogenes* group, seventeen." The predominance of the last group agrees with our observations in Liverpool, but not with those of Graham-Smith on London flies.

Summer diarrhoea in children.

It is generally recognised that infantile diarrhoea is most commonly caused by drinking milk infected with bacteria. Flies undoubtedly carry living bacteria and fall into or feed on milk.

Hence many authorities such as Fraser (1902); Newsholme (1903); Copeman (1906) consider that *M. domestica* is the chief carrier of diarrhoea-causing bacteria. Nash (1904) states: "My own opinion is that the usual seasonal circumstances during July, August, and the first half of September are in favour of contamination of food (especially milk) firstly and chiefly by flies and secondly by dust. I am quite convinced from continued observations that the common house fly is the principal agent in carrying diarrhoea-causing bacteria to food. I have always found that the district which have most diarrhoea are those which are most infected with flies." Vincent (1910) believes that it is especially the chemical poisons manufactured by various putrefactive bacteria growing in milk which produce the toxaemia characteristic of epidemic diarrhoea. These bacteria or their spores may be carried by flies.

Hamer (March 1910) on the other hand points out that "too great importance must not be attached to correspondences in the fly and diarrhoea curves of any one year." He has shown that "the London diarrhoea curve of 1907 showed marked decline at a time when the number of flies was still excessive."

Nash (1909) however argues that the actual gross number of flies may be of minor importance, and that their activity in relation to visits to infected material must be taken into account.

Peters, in his valuable monograph, reviews the whole question (XII. 1910) on the inter-relationship of diarrhoea, fly prevalence, and temperature. He points out that "all three curves are definitely correlated to one another; but the fly curve is more closely correlated to the temperature curve than the diarrhoea curve is," also that "the diarrhoea curve shows some independence of movement" when compared with the fly curve. This is due partly to the "inertia of case to case multiplication" and partly "to exhaustion of infection or of material to infect." He refers to "the correspondences of temperature, flies and diarrhoea, as being so extraordinary that the whole question merits the most thorough and laborious investigation."

Dudfield (1912) made a series of counts for three years in Paddington and found that the coefficient of correlation between flies and attacks of epidemic diarrhoea was as high as $+0{\cdot}75$ the probable error being $+$ or $-0{\cdot}06$, but he states that no "valid explanation has been suggested" why the "diarrhoea curve fades away earlier than that of flies."

Experiments in Liverpool, 1911.

In view of the prevalence of infantile diarrhoea in Liverpool, Dr Hope, Medical Officer of Health, suggested some bacteriological research upon flies might be of value. We therefore determined to investigate the following questions:

(1) Do flies caught from the town slums where disease especially infantile diarrhoea is common, carry more dirt, as measured by bacteria, than those from either the more sanitary or the suburban areas? If flies migrate from one locality of the city to another the number of bacteria should be approximately the same and should bear no constant relation to the quantity of street refuse or to the habits of the people.

(2) Do flies caught in the town slums more often carry pathogenic bacteria, particularly those liable to produce food poisoning?

(3) What is the approximate number of bacteria that flies are likely to deposit in food, milk, etc. by feeding upon or wallowing in it, and are such bacteria more numerous in flies caught in the insanitary parts of the city?

Technique.

(1) *Method of catching flies.* Wire balloon traps were sterilized in special tins and the latter carried unopened to the locality decided upon. A piece of sterilized wire gauze was placed over a small quantity of beer and sugar at the bottom of the trap; the fluid attracted flies into

the trap, but the gauze effectually prevented them touching it. (After remaining in one position for 24 hours, the traps now containing flies were replaced in the tins and conveyed to the laboratory.)

(2) *Method of estimating the number of bacteria carried by flies.* In order to remove the flies from the trap each batch was lightly anaesthetised with chloroform, and any showing signs of recovery were transferred with sterile forceps to a flask containing 125 c.c. of sterile tap water. The flies swam more or less vigorously on the surface of the water. The number examined from each locality and added to the water was 25, so that each c.c. contained the bacteria from 1/5 of a fly. At intervals of 5, 15, and 30 minutes, one cubic centimetre of this fluid, which contained the bacteria washed from the bodies of the flies and those from their excreta, was taken out and diluted with 9 c.c. of sterile tap water. One cubic centimetre of this mixture was then taken and progressive dilutions made up to one in ten thousand million. A tube of liquid gelatin and another containing nine cubic centimetres of bile salt glucose broth was inoculated with one cubic centimetre from each dilution. The gelatin tubes were solidified quickly by plunging them into melted ice. At the end of 30 minutes the 25 flies in the flask were killed, and then thoroughly ground up in a sterile pestle and mortar. The whole was then transferred to another flask and made up to 250 c.c., here 1 c.c. contained the bacteria from 1/10 of a fly. One cubic centimetre from the 250 c.c. flask was progressively diluted as described above, and 1 c.c. from the different dilutions, starting with the highest, distributed into gelatin and bile salt glucose broth tubes.

By making the flies swim in sterile water we were able

(i) To simulate as closely as possible the natural way flies pollute liquids if they fall into them, and to estimate the rate at which bacteria are given off.

(ii) To find whether flies from the more dirty areas set free a greater number than flies from cleaner areas.

By experiments with the whole fly pounded up we were able

(i) To find the gross number of bacteria carried both on and in a fly.

(ii) To ascertain whether the number carried inside a fly is always greater or less than the number set free even after struggling in a liquid for 30 minutes.

(3) *Method of isolating the different kinds of bacteria present.* After the flies had been struggling in the water for 30 minutes a portion of the fluid was spread over two agar plates and also over two plates

containing neutral-red-bile-salt-lactose-agar. In a like manner four plate cultivations on similar media were made from the fluid which contained the whole flies after being ground up.

The eight plates were incubated for 48 hours at 37° C., and a varying number of colonies transferred to agar slants. No attempt was made to remove any particular number of colonies, but as far as possible the colonies picked out were representative of the varieties on any pair of plates. We also usually prepared neutral-red-bile-salt-lactose-agar plates by adding 1 c.c. of the highest dilution from the 30 minutes and whole fly experiment. This was a precaution in case the highest dilution when incubated in the bile-salt glucose broth gave a positive result.

The method of preparing the 'sugar' media must be alluded to. The sugars were supplied by Merck and made up in 1 % solution in peptone (Witte) litmus broth, except in the case of dulcitol which was ½ %. Comparative experiments proved that ½ % solutions gave as good results as 1 %. This is a point of practical importance in the routine use of dulcitol when its high cost is considered.

Elsner and Huntoon (1909) often obtained different results in their fermentation tests with galactose and levulose broth according as it was sterilized once for 10 minutes at 100 or for 15 minutes on three consecutive days. They suggest that the contradictory results are due to the cleavage of the 'sugars' during prolonged sterilization, which is specially apt to occur when small quantities of alkali are present as Lobry de Bruyn and van Ekenstein (1897) have shown; the latter found 'glucose' is formed from 'levulose' while "galactose is converted into a number of isomeric sugars." All our sugar media were therefore sterilized by the filtration of a solution through a Massen porcelain filter, and added to an equal quantity of double-strength peptone litmus broth (reaction + 10 Eyre's scale) which had been sterilized by heat. The tubes were incubated for three days before use and with one or two exceptions were invariably sterile. There is no doubt that some of the discordant results obtained in the sugar fermentation tests are due to improper sterilization, particularly in the case of streptococci[1] and meningococci. With regard to the latter organism Gordon (1907) and Rundle and Stenhouse Williams (1907) have independently stated that it ferments galactose, whereas Elsner found that four strains of meningococci produced alkali in galactose,

[1] See the contradictory results obtained by M. H. Gordon (1903–4), Ainley Walker (1911) and Beattie and Yates (1911).

when subjected to short sterilization, but acid when sterilized in the usual manner intermittently for three days.

Finally, uniform results in the future with carbohydrate and similar media are more likely to be obtained, as is already recognised in America (see Gorham 1912), by an agreed method of standardisation of entirely synthetic media.

Areas investigated.

The localities where the flies were obtained for our experiments were as follows:

Milk Shop. (Table I.)

15. 9. 11. (*a*) In a clean suburban area, outside the infantile diarrhoea area. (Smithdown Road.)

16. 9. 11. (*b*) In a congested slum area, inside the infantile diarrhoea area. (Vauxhall Road.)

6.10. 11. (*c*) In a row of corporation dwellings which extend 400 yards on both sides of a street, and surrounded by slum property, inside the infantile diarrhoea area. (Hornby Street.)

5.10. 11. (*d*) Opposite the slaughter houses in a moderately congested area, on the edge of the infantile diarrhoea area. (Copperas Hill.)

Flies from each of these localities examined on one occasion only.

Shops with exposed food. (Table II.)

16. 9. 11. (*a*) Bread shop in moderately congested area, on the edge of the infantile diarrhoea area. (Northumberland Terrace.)

16. 9. 11. (*b*) Bread shop in congested slum area, inside the infantile diarrhoea area. (Vauxhall Road.)

12. 9. 11. (*c*) Greengrocer's shop in a clean suburban area, outside the infantile diarrhoea area. (Allerton Road.)

26. 9. 11. (*d*) Greengrocer's shop in a congested slum area, inside the infantile diarrhoea area. (Currie Street.)

26. 9. 11. (*e*) Eating house opposite the slaughter house in a moderately congested area, on the edge of the infantile diarrhoea area. (Gill Street.)

Flies from each of these localities examined on one occasion only.

Dwelling Houses and Office Room. (Table III.)

(*a*) Dwelling rooms in a number of different corporation houses extending some 400 yards on both sides of Hornby Street, and surrounded by slum property.

(*b*) Dwelling rooms in a number of different houses from Upper Beau Street which on each side is made up of condemned insanitary courts. These courts are frequently polluted with human excrement.

(*c*) The office of a refuse destructor situated in the offensive trades area.

Flies from each of these localities examined on three *different* days, 5. 9. 11, 13. 9. 11, and 21. 9. 11.

The population of Upper Beau Street was 863 and two deaths occurred there from infantile diarrhoea, and the population of Hornby Street was 1223 and the number of deaths five. Unfortunately the number of cases is unknown, and the population of Upper Beau Street was a rapidly diminishing one, owing to removals.

Variations in the weather.

The experiments up to and including those on September 12 were made with flies collected at the end of the long hot summer of 1911. After September 12 the weather became broken and gradually colder up to the last experiment on October 6.

Interpretation of results.

The progressive dilution method particularly in the higher dilutions is rather inaccurate, for a stray bacillus may be carried too far, especially when dealing with uneven suspensions like pounded flies. Again an error in the ninth dilution for example means an error of about a thousand million. It was frequently noted in the gelatin tubes that the reduction in the number of colonies was not mathematically proportionate to the dilution. Occasionally also one tube was sterile, but growth occurred in the higher dilution; this most frequently happened in bile salt broth cultures. These points are well illustrated by the following example.

Gelatin tubes.	1st, 2nd, 3rd dilutions 1 in 10 to 1 in 1000 innumerable colonies.								
	4th ,, 1 ,, 10,000						14 ,,		
	5th ,, 1 ,, 100,000						3 ,,		
	6th ,, 1 ,, 1,000,000						0 ,,		
	7th ,, 1 ,, 10,000,000						1 ,,		
	8th ,, 1 ,, 100,000,000						1 ,,		
	9th ,, 1 ,, 1000,000,000						0 ,,		
Bile salt broth tubes.	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
	a & g	a & g	a & g	a & g	a	—	a & g	—	—

In order to minimise the errors alluded to, we decided to base our calculations in the case of the gelatin cultures on the tube containing the lowest number of colonies of bacteria which could be easily counted, and in the case of the bile salt cultures on the tube giving acid and gas in the highest dilution of an unbroken series.

Thus in the example quoted the figures work out as follows:

Gelatin tubes: $14 \times 10{,}000 \times 5 = 700{,}000$.

Bile salt broth tubes: $10{,}000 \times 5 = 50{,}000$.

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Occasionally the figures, if based upon the cultures of the highest dilution in which any growth occurred, gave incredible numbers varying from many thousand millions to a million million.

In order to further justify our method of interpreting our results and to demonstrate the absurdity of the high figures just quoted, we have attempted very roughly to estimate the maximum number of bacteria a common house-fly can possibly carry. Taking a colon bacillus $3\mu \times \frac{1}{2}\mu$ as the average type of bacterium we proceeded as follows: assuming the shape of the bacillus is a cylinder with hemispherical ends, the total length being 3μ, the diameter $\frac{1}{2}\mu$, the length of the cylindrical portion $2\frac{1}{2}\mu$, then the radius of the hemispherical end is $\frac{1}{4}\mu$, and the volume of the bacillus is equal to that of the cylinder and the two hemispheres, or

$$2\tfrac{1}{2}\mu\pi\tfrac{1}{16}\mu^2 + \tfrac{4}{3}\pi\tfrac{1}{64}\mu^3$$
$$= \pi\mu^3\left[\tfrac{5}{32} + \tfrac{1}{48}\right]$$
$$= \pi\mu^3\tfrac{17}{96} = \mu^3 \times \tfrac{17}{96} \times 3{\cdot}14157 = {\cdot}556\mu^3.$$

If the specific gravity of the bacillus be taken as equal to water, then, since one cubic millimetre equals one milligramme, and one cubic μ equals $\frac{1}{10^9}$ milligramme, the weight of a colon bacillus is therefore equal to ${\cdot}556 \times \frac{1}{10^9}$ mg. We have found by experiment that the weight of a house-fly is about 20 milligrammes. The number of colon bacteria which is equal to the weight of a fly is therefore $\frac{20 \times 10^9}{{\cdot}556}$ = about 32,500,000,000.

We found by actual experiment that *Musca domestica* can easily fly off with a weight of 5 milligrammes, but can scarcely lift 10 milligrammes. Even making allowance for the bacteria carried in the intestine it is clear that the figures 1,200,000,000,000 and 100,000,000,000 obtained from the knacker's yard are too high (see Table III).

Results.

Our results are given in Tables I, II, and III, also in Charts 1 and 2, while Figs. 1—4 (Pl. V) are photographs of some of the houses etc. in which the fly traps were set.

The figures in Tables I, II, and III have been represented graphically in Charts 1 and 2. The former contrasts the relative numbers of bacteria carried by flies in the sanitary and insanitary dwellings of the

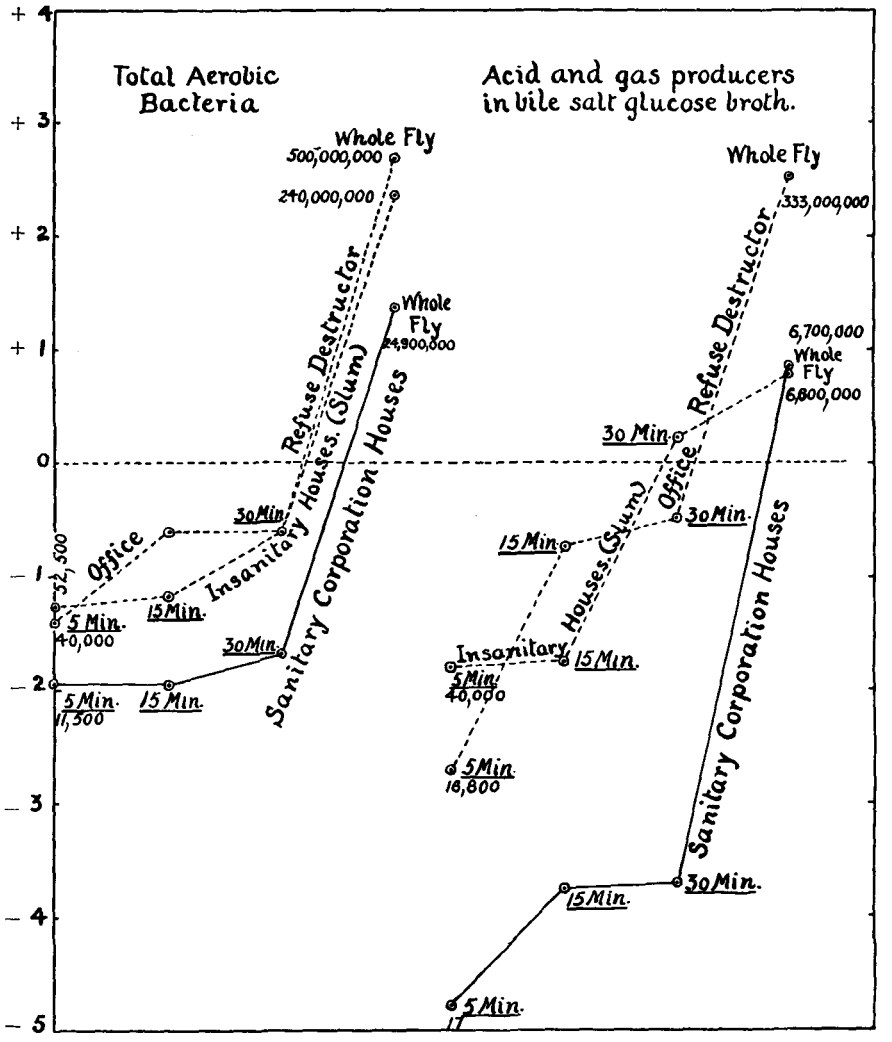

Chart 1. Chart of relative number of bacteria carried by flies caught in sanitary and insanitary town dwellings and in office of refuse destructor, showing:

1. The number washed off flies in 5, 15, and 30 minutes.
2. Total number carried inside and outside flies, *i.e.* whole fly.

Each curve represents the average of three experiments.
For method of graphing see foot of p. 299.
The maximum and minimum numbers are given on each curve.

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city; the latter the relative number carried by flies caught in shops in the sanitary and insanitary areas. As the numbers range from 17 to 500,000,000 a great difficulty was experienced in charting them so we adopted the following method. The number of bacteria in each case was divided by 10^6 and the log. of the resulting quantity was plotted;

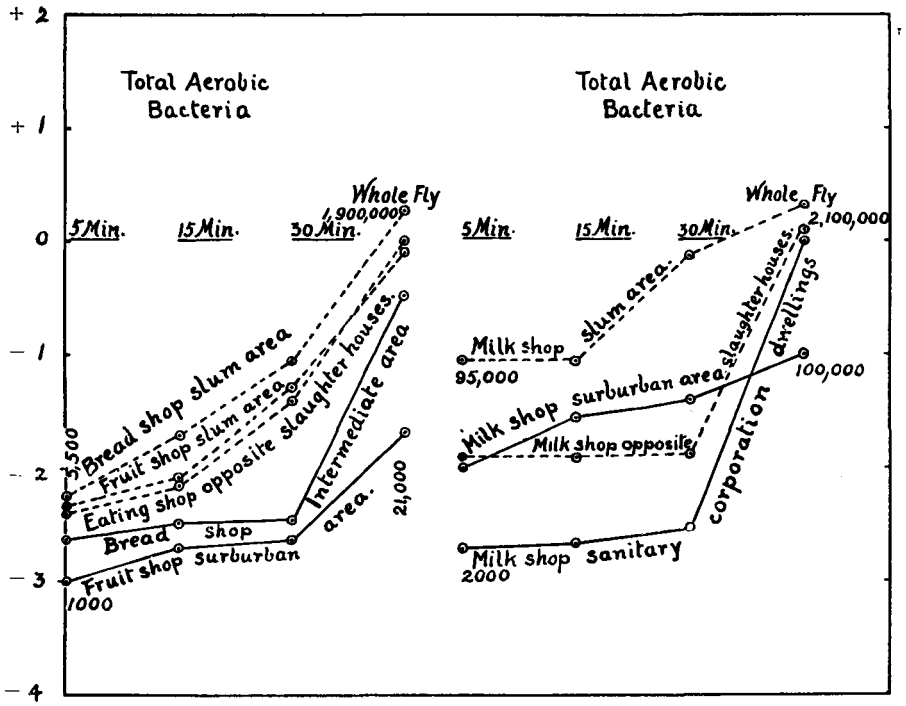

Chart 2. Chart of relative number of bacteria carried by flies caught in shops with exposed food in sanitary and insanitary areas, showing:

1. The number washed off flies in 5, 15, and 30 minutes.
2. Total number carried inside and outside flies, *i.e.* whole fly.

Each curve represents one experiment.

For method of graphing see foot of p. 299.

The maximum and minimum numbers are given on representative curves.

therefore the logs. of numbers below a million are negative quantities, and those above a million are positive quantities. The quantities were then plotted above and below the abscissae, the ordinates showing the log. value.

Our experiments show:—

1. The number of bacteria derived from house-flies whilst struggling in a liquid, and which can be taken as a measure of their capacity

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to pollute food by vomiting, defaecation, etc., may be very large, and increases with the time they remain in the liquid; but the number of bacteria carried inside the fly is much greater with one exception.

2. Flies caught in congested areas always carried and contained more aerobic bacteria including those of the intestinal group, than flies from cleaner areas.

3. Flies caught in the dwelling rooms of different corporation houses forming two sides of a street about 400 yards long, and a sanitary oasis in the midst of a slum area, carried and contained less bacteria of all kinds than those from the dwelling rooms of a street with insanitary court property on each side.

4. Flies caught in the office of a Refuse Destructor, situated in the Offensive Trades Area, carried and contained an enormous number, as many as 500,000,000 bacteria of all kinds, and 333,000,000 acid and gas fermenters of glucose bile salt broth, per fly. These figures from the Refuse Destructor, which are the average of three experiments, and others from the insanitary court property are much bigger than any we have yet seen. Much larger figures have been obtained from the slaughtering room of a knacker's yard. We have already discussed (p. 299) why such figures (over a million million) are probably theoretically impossible, but this locality was undoubtedly the most unsavoury (see Table III).

5. Flies caught in milk shops apparently carry and contain more bacteria than those from other shops with exposed food in a similar neighbourhood. The reason of this is probably because milk (when accessible) especially in the summer months, is a suitable culture medium for bacteria, and the flies first inoculate the milk and later reinoculate themselves, and then more of the milk, so establishing a vicious circle.

6. On one occasion we compared the number of bacteria carried by house-flies caught in an eating house opposite the slaughter houses with the number carried by blue-bottles. The latter as might be expected was far larger.

7. The fact that flies from congested and relatively insanitary areas of the city carry more bacteria than those from cleaner areas, may be explained by the lower standard of general cleanliness in the house, the yard, the street, and the alley: human excrement is frequently found in the courts of the slums.

It might have been imagined that flies were constantly migrating from one street or locality of the city to another, and consequently the number of bacteria carried by them would be approximately the same

and bear no relation to the amount of street refuse and the habits of the people.

Our observations, however, prove that such migrations from one area to another do not occur to any great extent.

Professor Newstead, moreover, informs us, that though flies may travel considerable distances, especially in the country, yet when food is abundant as obtains in towns, they do not migrate far from the locality in which they were bred. This is also proved by actual experiments in a recent paper by Hewitt (1912).

8. Though the flies in a sanitary oasis in Hornby Street carried less bacteria than in the slums of Upper Beau Street, yet the number of deaths from infantile diarrhoea was rather higher in proportion to the population. Unfortunately, however, we have no data regarding the number of cases, and the population of Upper Beau Street was a rapidly diminishing one.

9. We have shown that the amount of dirt carried by flies measured in terms of bacteria, bears a definite relation to the habits of the people and the state of the streets. This demonstrates the vital necessity of efficient municipal and domestic cleanliness; and cleanliness is the essence of sanitation.

The typhoid incidence in Liverpool, and its relation to flies.

Dr Stallybrass, Assistant Medical Officer of the Port of Liverpool, has unknown to us been conducting an investigation into the incidence of typhoid in Liverpool, and kindly supplied us with the following notes from a paper which he is at present writing.

During the year 1911, 181 cases of typhoid occurred in Liverpool. In 86 of these the probable cause was ascertained with some certainty, infection occurring during residence out of Liverpool by direct contact, by consumption of shell fish, etc. The remaining 95 cases could not be so accounted for. These latter were classified into two groups, according as they occurred in the outer parts, or in the central area of the city. A distinctive feature of the central area is firstly the presence of some 600 courts, the great majority of houses in this area being within one or two hundred yards of a court; and second, the frequency of midden-steads attached to horse stables.

The population of the outer area is approximately four-fifths of that of the city and 44 of the 95 cases of typhoid occurred in it, *i.e.* 0·073 per thousand, whereas the population of the central area is approximately one-fifth and 51 cases occurred here, or 0·34 per thousand.

It was further noted first that the cases in the outer area occurred in small foci which in several instances were in close association with a group of courts; second that no marked seasonal fluctuation occurred in the cases in the outer area, but the monthly incidence of those in the central area exhibited a well-marked epidemic curve resembling that obtained in Liverpool during past years.

Dr Stallybrass conducted inquiries into the prevalence of flies in the central and outer parts of the city in order to ascertain whether they might partly account for the differences in the incidence of typhoid. The answers to his questions revealed a marked difference between the two areas. The following were typical replies received from August to October from persons living in houses within the central area in which typhoid had occurred. "A perfect plague of flies," "spent eightpence a week on fly papers, had three over the bed," "something shocking couldn't get our food." Such replies were only obtained twice in the outer area and in each case horse stables were in close proximity. There are several means by which flies could be infected with typhoid bacilli, for although no privies now exist in Liverpool, many of the courts are provided with trough-closets (usually common to several families), and emptied once a day. Further the courts themselves are frequently used by the younger inhabitants for the double purpose of play grounds and common conveniences, and even the closest attention of the sanitary staff must fail to prevent such sources of infection. The danger of courts acting as centres of infection is one that is rapidly diminishing with the demolition of these insanitary dwellings. During the last 16 years their number has been reduced from 1600 to 600, and of those which remain many have been greatly improved and there has been a commensurate reduction of typhoid during that period, the coefficient of correlation being $+ \cdot 935$, and the error only + or $- \cdot 002$.

Classification of the bacteria isolated from Liverpool flies.

In the separation of intestinal bacilli it is unfortunately necessary to use a large number of the so-called 'sugars.' We have already mentioned the way we sterilized these media (p. 296) and must briefly explain their arrangement in Tables IV, V and VI.

Most observers do not seem to have any definite arrangement. We have attempted one based on the chemical relationship between the various substances. By this method of grouping we hoped that some might be found redundant and therefore unnecessary.

The three substances which usually give identical reactions are d. fructose (levulose), d. glucose, and d. galactose, but reference to Table V will show that no absolute rule can be maintained. In our arrangement we would draw attention to

1. The names of the substances. We have retained for those which are polyhydric alcohols the suffix 'ol.'

2. In front of the names are the letters i, l and d, which signify according to Fischer's system of nomenclature genetic relationship and not necessarily the sign of optical rotation.

3. The list of substances is divided into groups of carbohydrates, polyhydric alcohols and glucosides. All those in the first two groups are arranged in a series according to the number of carbon atoms in the molecule. Thus on the left the first is i-erythrol having four carbon atoms, the others following in a regular order up to dextrin which has the greatest number of carbon atoms.

123 strains of bacteria were isolated in pure culture.

1. The Micrococci were:

(*a*) Two *Streptococci*, allied to the so-called *S. salivarius*, and *S. fecalis* of Andrews and Horder.

(*b*) Six strains of albococci (*Staphylococci*) each differing slightly from the other by variations in their sugar reactions.

(*c*) Two lemon-yellow *Sarcinae* and one white one. These did not ferment any of the sugars, starches or glucosides.

2. The bacilli may be divided into two main groups—those which were small Gram-negative non-spore bearing rods, and those which were not. The former numbered 106 and fall chiefly by their sugar reactions, into the following groups:

(*a*) Chromogenic group; (*b*) Colon group; (*c*) Salmonella (paratyphoid) group; (*d*) Morgan's Infantile Diarrhoea group; (*e*) Group producing acid in lactose and sucrose (saccharose); (*f*) Proteolytic group; (*g*) Miscellaneous group.

(*a*) *Chromogenic group.*

Two strains of a motile Gram negative bacillus were obtained from a knacker's yard which produced considerable quantities of a blue colouring substance soluble in chloroform, and giving a red water-extractible body upon treatment of the chloroform solution with an acid. Milk was clotted and peptonised. These bacilli are therefore in all probability *B. pyocyaneus*. Their reactions agree with a strain isolated from a human subject.

Another chromogenic bacillus from a bread shop was a short Gram negative non-motile non-indole producing organism, which on agar appeared as an opaque gamboge coloured growth, liquefied gelatin and produced an alkaline reaction in milk without any previous acidification. Fructose, mannitol, galactose, dextrin, salicin and amygdalin were fermented with acid production; there was no action in the other substances used (erythritol, adonitol, arabinose, glucose, sorbitol, dulcitol, maltose, sucrose, lactose and raffinose).

(*b*) *Colon group.*

The colonies were picked off the plates haphazard and the 41 strains so isolated may be classified according to McConkey as follows:

8	Group I	("Saccharose"	–	"Dulcit"	–)	or 19·5 %	*B. acidi lactici* type
6	,, II	(,,	–	,,	+)	,, 12·2 %	*B. coli communis* type
8	,, III	(,,	+	,,	+)	,, 19·5 %	*B. neapolitanus* type
19	,, IV	(,,	+	,,	–)	,, 46·4 %	*B. lactis aerogenes* type.

The predominance of the last group agrees with the observations of Torrey (1912) on American flies but does not agree with those of Graham-Smith (1909) on London flies.

(*c*) *Salmonella*[1] *or Paratyphoid group* (see Table IV).

We have not been so fortunate as some observers who have isolated either Para B or Para A. One of our organisms, No. 78, which came from a knacker's yard is exactly similar as regards its sugar reactions and morphology to the *B. enteritidis* of Gaertner, except after prolonged growth on agar it has rather a crinkled appearance. Unfortunately, however, although subcultured for over two months, it was not agglutinated by Para B, *B. enteritidis* or *B. suipestifer* serums kindly supplied to us from the Lister Institute. The absence of an agglutination reaction with the last serum also shows that this bacillus isolated from flies is not "Bacillus F," an organism obtained by Stenhouse Williams, Leith Murray, and Rundle (1910) from cases of epidemic summer diarrhoea in Liverpool in 1908 and 1909, and closely allied to *B. suipestifer*, if not identical with it.

In Table IV the reactions are given of four other organisms which fall into this group. One from a fruit shop, except for an inability to

[1] The salmonella group includes *B. paratyphosus* (A) and (B); *B. enteritidis* (Gaertner), *B. suipestifer* or *aertrycke*. Bainbridge, Milroy Lectures, 1912.

ferment dulcitol is also similar in all its other cultural reactions to *B. enteritidis* or *para-typhosus B.* Nos. 38, 43, and 2 may be classified as atypical paratyphoids. They all ferment raffinose but fail to ferment dulcitol and sorbitol. The first three came from a milk shop, No. 2 from the office of a refuse destructor.

(*d*) *Morgan's infantile diarrhoea group* (see Table V).

We have isolated a number of bacilli with reactions either similar to those described by Morgan and by Torrey or differing slightly by their behaviour in one or more of the sugars. The group reaction that is common for all our bacilli is: litmus milk first turned acid but later an intense alkaline, gelatin not liquefied, indole produced, and arabinose, sorbitol, dulcitol, lactose, dextrin, inulin, and amygdalin unfermented. By these reactions this group is obviously allied to the dysentery group.

It can, however, be divided into two:

Sub-group A. Consisting of bacilli exhibiting identical reactions with Morgan's No. 1 bacillus, and others which differ slightly (see Table V).

This sub-group is quite distinct from the dysentery group by the production of acid and gas in fructose (levulose) and glucose.

Sub-group B. Exhibits acid without gas in erythritol, adonitol, fructose (levulose), glucose, galactose, and raffinose. It is more closely related to the dysentery group and to the bacilli described by Morgan as No. 3 and No. 4. It differs from the dysentery group by the production of acid in erythritol (15 days), adonitol, and raffinose (15 days), and an inability to ferment any of the monosaccharids. Further some of the bacilli are motile.

(*e*) *Acid lactose-sucrose* (*saccharose*) *group* (see Table VI).

There appears to be a well-defined group of bacteria which exhibit the following group reactions: small Gram negative bacilli, no liquefaction of gelatin in six months, litmus milk turned acid in 24 hours and remaining acid at the end of 15 days, indole production negative, fermentation of adonitol, fructose (levulose), glucose, mannitol, galactose, sucrose, lactose, and raffinose with the production of *acid* only, and no fermentation in erythritol, arabinose, sorbitol, dulcitol, maltose, dextrin, inulin, salacin or amygdalin.

This is a group of intestinal bacteria which has been, so far as we are aware, but little studied. It is possible that one or more of its

numbers plays a part in the production of infantile diarrhoea, or may even be associated with some of the obscurer intestinal disorders. Bacilli resembling this group by their reactions in five sugars (no observations in litmus milk) have been obtained from human faeces by McConkey (1909).

(*f*) *Proteolytic group* (see Table VI).

Although we have not found a typical *Bacillus proteus*, a number of bacilli peptonising milk were isolated from our flies. They fall into two classes, and typical reactions are given in Table VI.

(*g*) *Miscellaneous group.*

Finally there are a number which cannot be placed in any of the above groups. They illustrate the extraordinary diversity which obtains amongst these bacteria, though it is possible that some would say, especially in America, that the slight differences are due to equally slight differences inevitable in the production of our media.

Summary and Conclusions.

1. Over 450 naturally infected or wild flies (*Musca domestica*) were caught in Liverpool during September and the first part of October 1911 from different parts of the city. The number and kinds of bacteria carried and contained by them have been investigated.

2. The number of bacteria coming from house-flies whilst struggling in liquid may be very large, varying from 2000, the lowest figure in 5 minutes, to 350,000, the highest figure in 30 minutes. This number may be taken as a measure of their capacity to pollute liquid with their vomit or excrement, or by wallowing in it. The number of bacteria carried inside the fly is very much greater.

3. Flies caught either in insanitary or congested areas of the city carry and contain far more bacteria than those from the more sanitary, less congested or suburban areas. The number of aerobic bacteria from the former varied from 800,000 to 500,000,000 per fly, and from the latter from 21,000 to 100,000.

4. The number of intestinal bacteria as indicated by glucose bile salt fermenters is also greater in the insanitary or congested areas, the numbers varying from 10,000 to 333,000,000, than in the more sanitary areas where they carried from 100 to 10,000.

5. Pathogenic bacteria and those allied to the food poisoning group were only obtained from the congested or moderately congested areas and never from the suburban areas.

6. We have examined the morphological characters and cultural reactions of 123 strains. Among those identified were two *Streptococci*, and several *Staphylococci* and *Sarcinae*. 106 were small Gram negative non-spore bearing bacilli, and have been grouped as follows:

Chromogenic group. Two strains of *B. pyocyaneus* were isolated from a knacker's yard; for the first time, we believe, from wild flies.

Colon group. 41 colonies of this group were picked off haphazard and classified according to McConkey as follows:

B. acidi lactici type	19·5 %.
B. coli communis type	12·2 %
B. neapolitanus type	19·5 %
B. lactis aerogenes type	46·4 %

Salmonella group. One bacillus gave identical reactions to *B. enteritidis* of Gaertner except that serological tests were negative.

Morgan's infantile diarrhoea group. One identical to Morgan's No. 1, and many others closely resembling it and Morgan's Nos. 2 and 3 were obtained.

Others fall into Proteolytic, Acid lactose-sucrose (saccharose), and Miscellaneous groups.

7. Flies caught in milk shops apparently carry and contain more bacteria than those from other shops with exposed food in a similar neighbourhood. The reason of this is probably because milk when accessible, especially in the summer months, is suitable culture medium for bacteria, and the flies first inoculate the milk and later reinoculate themselves, and then more of the milk, so establishing a vicious circle.

8. On one occasion we compared the number of bacteria carried by house-flies caught in an eating house opposite the slaughter houses with the number carried by blue-bottles; the latter, as might be expected, was far larger.

9. In cities where food is plentiful flies rarely migrate from the localities in which they are bred, and consequently the number of bacteria they carry depends upon the general standard of cleanliness in that locality. This is well indicated by the fact that flies caught in a street of modern fairly high class workmens' dwellings forming a sanitary oasis (Hornby Street) in the midst of a slum area, carried far less bacteria than those caught in the adjacent neighbourhood.

10. It is clear that flies from the suburbs where infantile diarrhoea is rare carry far less bacteria than those in the city where it is common. It was, nevertheless, impossible in the time at our disposal to correlate exactly the number or varieties of bacteria carried by flies in the city with the number of cases and deaths from infantile diarrhoea in individual streets.

11. As the amount of dirt carried by flies in any particular locality, measured in terms of bacteria, bears a definite relation to the habits of the people and the state of the streets, it demonstrates the necessity of efficient municipal and domestic cleanliness, if the food of the inhabitants is to escape pollution, not only with harmless but also with occasional pathogenic bacteria.

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Fig. 1. Shop with exposed food (Currie Street). (See Table II and Chart II.)

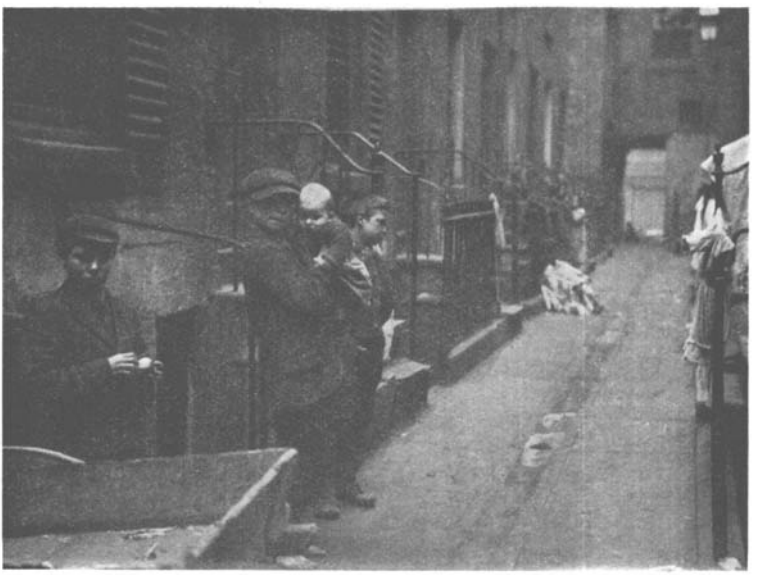

Fig. 3. Condemned, insanitary court dwellings (Upper Beau Street). (See Table III and Chart I.)

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Fig. 2. Yard and office of Refuse Destructor. (See Table II and Chart I.)

Fig. 4. Corporation Dwellings (Hornby Street). (See Table III and Chart I.)

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TABLE I. *Showing number and kinds of bacteria from flies caught in four milk shops.*

Date	Locality		No. of aerobic bacteria per fly after struggling in water for 5 minutes	15 minutes	30 minutes	Total No. of aerobic bacteria per fly after being ground up	Type of organisms isolated
Exp. I 15. 9. 11	Milk shop	1. Gelatin, 4 days at 18° C.	10,000	30,000	40,000	100,000	Colon group.
	Suburban area ...	2. Acid & Gas in glucose bile salt broth, 2 days, 37° C.	500	500	5,000	10,000	
Exp. II	Milk shop	1. Gelatin ...	95,000	95,000	750,000	2,100,000*	Salmonella (paratyphoid) group.
16. 9. 11	Congested area ...	2. Acid & Gas ...	Nil	500,000	500,000	10,000,000*	Morgan's infantile diarrhoea group. Acid lactose-saccharose group.
Exp. III	Milk shop	1. Gelatin ...	2,000	2,500	3,000	1,000,000	Colon group.
6. 10. 11	Corporation dwelling	2. Acid & Gas ...	Nil	Nil	Nil	100	
Exp. IV	Milk shop opposite	1. Gelatin ...	13,000	13,000	13,500	1,300,000	Morgan's infantile diarrhoea group.
5. 10. 11	Slaughter house	2. Acid & Gas ...	50	50	500	100,000	Colon group. Proteolytic group.

* For discussion on these figures see page 299.

TABLE II. *Showing number and kinds of bacteria from flies caught in shops with exposed food.*

Date	Locality		No. of aerobic bacteria per fly after struggling in water for 5 minutes	15 minutes	30 minutes	Total No. of aerobic bacteria per fly after being ground up	Types of organisms isolated
16. 9. 11	Bread shop ...	1. Gelatin, 4 days at 18° C.	2,500	3,500	3,500	350,000	Colon group.
	Intermediate area ...	2. Acid & Gas in glucose bile salt broth, 2 days at 37° C.	Nil	Nil	Nil	100,000	
16. 9. 11	Bread shop ...	1. Gelatin ...	5,500	20,000	90,000	1,900,000*	Colon group.
	Congested area ...	2. Acid & Gas ...	Nil	500	50,000	10,000,000*	Morgan's infantile diarrhoea group.
12. 9. 11	Greengrocer ...	1. Gelatin ...	1,000	2,000	2,500	21,000	Colon group.
	Suburban area ...	2. Acid & Gas ...	Nil	Nil	Nil	1,000	
26. 9. 11	Greengrocer ...	1. Gelatin ...	5,000	8,500	55,000	800,000	Colon group.
	Congested area ...	2. Acid & Gas ...	50	500	500	10,000	
26. 9. 11	Eating house ...	1. Gelatin ...	4,000	8,000	50,000	1,000,000	Colon group.
	Slaughter-house area house flies	2. Acid & Gas ...	50	50	50	1,000	Morgan's infantile diarrhoea group.
26. 9. 11	Same eating house ...	1. Gelatin ...	90,000,000	130,000,000	75,000,000	400,000,000,000	
	Blue bottles ...	2. Acid & Gas ...	500,000	500,000	100,000,000	100,000,000	

* For discussion on these figures see page 299.

TABLE III. *Showing number and kinds of bacteria from flies caught in corporation dwellings, condemned insanitary houses, office of a refuse destructor and the slaughtering room of a knacker's yard.*

Date	Locality		No. of aerobic bacteria per fly after struggling in water for 5 minutes	15 minutes	30 minutes	Total No. of aerobic bacteria per fly after being ground up	Types of organisms isolated
	Corporation dwellings	1. Gelatin, 4 days at 18° C.	11,500	11,500	21,300	24,900,000	Colon group.
5. 9. 11 13. 9. 11 21. 9. 11	(Average of 3 experiments)	2. Acid & Gas glucose bile salt broth, 2 days at 37° C.	17	183	200	6,700,000	Morgan's infantile diarrhoea group. Acid lactose-saccharose group.
	Condemned insanitary houses	1. Gelatin ...	52,500	64,700	256,000*	240,000,000	Colon group.
5. 9. 11 13. 9. 11 21. 9. 11	(Average of 3 experiments)	2. Acid & Gas ...	16,800	18,300	1,660,000*	6,600,000	Morgan's infantile diarrhoea group. One identical with Morgan's Group No. 1.
	Office of Refuse Destructor	1. Gelatin ...	40,000	252,000	260,000	500,000,000	Salmonella (paratyphoid) group.
5. 9. 11 13. 9. 11 21. 9. 11	(Average of 3 experiments)	2. Acid & Gas ...	2,000	184,000	334,000	333,000,000	Colon group. Morgan's infantile diarrhoea group.
26. 9. 11	Slaughtering room ...	1. Gelatin ...	950,000	55,000	350,000	1,200,000,000,000*	Salmonella (paratyphoid) group.
	Knacker's yard ...	2. Acid & Gas ...	50,000	5,000	5,000	100,000,000,000*	Colon group. Bacillus pyocyaneus.

* For discussion on these figures see pages 299—302.

TABLE IV. *Salmonella Group.*

	Gram – or +	Motility*	Gelatin liq. or non-liq.†	Litmus milk 1, 3, 15 days	Indole	Tetrahydric alcohol: i. Erythritol	Pentahydric alcohol: Adonitol	Pentose: l. Arabinose	Hexoses or Monosaccharids: d. Fructose (Levulose)	d. Glucose	d. Galactose	Hexahydric alcohols: d. Mannitol	d. Sorbitol	Dulcitol	Disaccharids: Maltose	Sucrose (Saccharose)	Lactose	Trisaccharid: Raffinose	Poly-saccharids: Inulin	Dextrin	Glucosides: Salicin‡	Amygdalin‡
Paratyphoid B. §	–	+	–	sa – alk	–	–	–	+	+	+	+	+ alk	+ alk	+	+	–	–	–	–	–	–	–
B. enteritidis of Gaertner §	–	+	–	sa – alk	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
78	–	+	–	a alk alk	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–
85	–	–	–	a alk alk	–	–	–	+ alk	+	+	+	+	–	–	+	–	–	–	–	–	–	–
38	–	–	–	a alk alk	–	–	–	+ alk	+	+	+	+	–	–	+	–	–	+	–	–	–	–
43	–	–	–	sa – alk	–	–	–	+ alk	+	+	+	+	–	–	+	–	–	– +	–	–	–	–
2	–	–	–	a sa	–	–	–	+ alk	+	+	+	+	–	–	+	–	–	a +	–	–	+	–

– = no reaction. sa = slight acid. a = acid reaction. alk = alkaline reaction. + = acid and gas. Sugar reactions after three days incubation at 37° C. Where two reactions appear, the lower is at the end of 15 days. * Motility tested after six to eight hours growth in peptone water; when doubtful examined by dark ground illumination. † Time limit for gelatin six months. ‡ Both these are β-Glucosides, *i.e.* they are hydrolised by emulsin, not by maltase. § These strains were obtained from Kral.

TABLE V. *Diarrhoea Group.*

Bacilli resembling Morgan's No. 1.

	Gram – or +	Motility*	Gelatin liq. or non-liq. †	Litmus milk 1, 3, & 15 days.	Indole	Tetrahydric alcohol: i. Erythritol	Pentahydric alcohol: Adonitol	Pentose: d. Arabinose	Hexoses or Monosaccharids: d. Fructose (Levulose)	d. Glucose	d. Galactose	Hexahydric alcohol: d. Mannitol	d. Sorbitol	Dulcitol	Disaccharids: Maltose	Sucrose (Saccharose)	Lactose	Trisaccharid: Raffinose	Poly-saccharids: Inulin	Dextrin	Gluco-sides: Salicin	Amygdalin
Morgan's No. 1 ††	–	–	–	– alk	+	–	…	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
No. 11	–	+	–	– s alk alk	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
31	–	+	–	a v sa alk	+	–	a +	–	+	+	–	–	–	–	– a	– a	–	–	–	–	–	–
29	–	–	–	a sa alk	+	–	a +	–	+	+	–	–	–	–	a	a	–	–	–	–	–	–
40	–	+	+ 6 ms.	– sa alk	+	–	a +	–	+	+	–	–	–	–	–	– a	–	– a	–	–	–	–
72	–	–	–	s a alk alk	+	–	a +	–	+	+	–	–	–	–	a	a	–	a	–	–	–	–
71	–	–	–	– alk alk	+	–	–	–	+	+	+	–	–	–	–	–	–	a	–	–	–	–
88	–	–	–	– alk alk	+	–	a +	–	+	+	–	–	–	–	–	– a	–	a +	–	–	–	–
69	–	–	–	– s alk alk	+	–	+	–	+	+	–	–	–	–	–	– a	–	a	–	–	–	–

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TABLE V (*continued*).

Bacilli related to Morgan's Nos. 3 and 4 and the Dysentery Group.

Morgan's No. 3‡	–	–	–	a a –	+	–	...	a	a	a	a	a	a	–	a	–	–	–	–	a	–	–
„ „ 4‡	–	..	–	sa – alk	–	–	...	a	a	a	a	a	–	–	–	–	–	as	–	–	–	–
Bacillus Y§	–	–	–	sa – alk	+	–	...	– a	a	a	a	a	–	–	a	–	..	– a	–	–	–	–
46		–	–	a sa alk	+	– a	a	–	a	a	a	a	–	–	–	–	–	– a	–	–	–	–
48	–	–	–	a sa alk	+	– a	a	–	a	a	a	–	–	–	–	–	–	– a	–	–	–	–
11 A	–	–	–	a salk alk	+	– a	a	–	a	a	a	a	–	–	–	–	–	– a	–	–	a	–
53	–	–	–	a a alk	+	– a	a	..	a	a	a	a	–	–	–	–	–	– a	–	..	–	–
73	–	–	–	sa sa alk	+	– a	a	–	a	a –	a	– a	–	..	–	–	–	– a	–	–	–	..
64	–	–	–	sa salk –	+	– a	a	–	a	a	a	a	–	..	–	–	–	– a	–	–	–	–
62	–	–	–	.. alk alk	+	– a	a	..	a	a	a	a	–	–	..	–	–	a	–	– sa	a	–
119	–	–	–	– alk alk	+	sa a	a	–	a	a	a	–	–	–	–	–	–	–	–	alk	–	–

– = no reaction. sa = slight acid. a = acid. + = acid and gas. alk = alkaline. s alk = slight alkaline. Reactions noted at end of three days. Where two given, lower is at end of 15 days. * Motility tested after six to eight hours in peptone water; when doubtful examined by dark ground illumination. † Time limit for gelatin six months. ‡ Reactions of Morgan's Bacilli Nos. 1, 3, and 4, from *Brit. Med. Journ.* I. 1906, p. 910. § This strain was obtained from the Lister Institute.

Acid Lactose-Sucrose (Saccharose) Group.

	Gram − or +	Motility*	Gelatin liq. or non-liq.†	Litmus milk 1, 3, 15 days	Indole	i. Erythritol	Adonitol	d. Arabinose	d. Fructose (Levulose)	d. Glucose	d. Galactose	d. Mannitol	d. Sorbitol	Dulcitol	Maltose	Sucrose (Saccharose)	Lactose	Raffinose	Inulin	Dextrin	Salicin	Amygdalin
Bacillus Human‡ Faeces. McConkey	...	+	~	... a	−	...	−	...	...	...	...	...	...	a	...	a	a	...	...	...	...	...
Nos. 27, 34, 44	−	−	−	a a a	−	−	a	−	a	a	a	a	−	−	−	a	a	a	−	−	−	−
41	−	−	−	a a a	−	−	a	−	a	a	a	− a	−	−	− a	a	a	a	−	−	−	−
39 & 60	−	+	−	a a a	−	−	a	−	a	a	a	a	−	−	−	a	a	a	−	−	.	−
67	−	+	−	a a s clot	−	−	−	a	a	a	a	a a	alk	a	a	a	a	a	−	−	a	−
									Proteolytic Group.													
36, 101 & 109	−	+	+	− or ac alk or pep pep	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
100, 103, 110	−	−	−	? alk sa pep	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
									Miscellaneous Group.													
74	−	−	−	− sa alk	−	−	− a	−	a	a	− +	+	−	−	a	+	−	− a	−	−	a +	a
5 C	−	+	+	a a ac	+	−	−	−	−	+	+	−	−	−	+	a	−	a	−	−	−	−
120, 121	−	− and +	−	sa alk alk	−	−	+	−	+	+	−	−	−	−	−	− a	−	a	−	−	−	−
90	−	−	−	sa alk alk	−	−	+	−	+	+	−	−	−	−	−	− +	−	− a	−	−	−	−
70	−	−	−	− ac ac	−	sa alk	+	−	+	+	−	−	−	−	−	− +	−	−	sa	sa alk	−	−

− = no reaction. sa = slight acid. a = acid. + = acid and gas. alk = alkaline. ac = acid and clot. pep = peptonised. Reactions noted at end of three days. Where two given, lower is at end of 15 days. * Motility tested after six to eight hours in peptone water; when doubtful examined by dark ground illumination. † Time limit for gelatin six months. ‡ Reactions of this bacillus from *Journ. Hyg.* 1909.