ON THE VARIETIES OF *BACILLUS COLI* ASSOCIATED WITH THE HOUSE-FLY (*MUSCA DOMESTICA*).

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The importance of flies in relation to the transmission of infectious diseases has been manifested in numerous instances; the incrimination of the house-fly, however, is as yet based upon by no means irrefutable grounds. Many observations have been made which must arouse grave suspicion, but the evidence hitherto adduced is far from conclusive. That house-flies can, and do convey infective material has been demonstrated both experimentally and under natural conditions, but the full significance of the house-fly as a disease-transmitter and the circumstances under which it assumes this rôle are still vague and indefinite.

The relation, in particular, of the house-fly to bacteria, pathogenic and otherwise, is not so thoroughly understood as might be desirable. It is well known that it may harbour a considerable variety of bacteria in its alimentary canal and carry an equal variety on the surface of its body, yet it is quite uncertain what organisms, if any, are particularly associated with the fly. It is not to be doubted that the bacterial flora of the fly's intestine may change under certain circumstances; at one time a particular group of organisms may be abundant and be replaced by others at some later period. The mutual relationship of these bacteria and their attitude towards organisms introduced into the intestine, are factors which must be considered in dealing with the problem of the house-fly as a carrier of pathogenic bacteria.

Only a very limited number of bacteriological examinations of flies under natural conditions has hitherto been made, and there has been no attempt to furnish a detailed account of such bacteria as have been found. One of the earliest investigators to consider the relation of bacteria to flies was G. Marpmann (1884), who examined 230 flies. His method was to press a drop of fluid from the proboscis and from the anus, and to examine these in stained preparations. He determined the presence of bacteria in every one of the flies, but beyond this he did not go. In 1903 W. H. Horrocks examined a number of flies in which he found lactose-fermenting bacilli which he considered to be *B. coli communis*, as they did not ferment saccharose. Graham-Smith (1909) made a series of examinations of flies for the presence of colon bacilli and classified the bacilli so found according to MacConkey's four groups. He found that out of 94 house-flies 21 % carried such bacilli, but only 4 % (out of 56) contained them in their intestine.

The present investigation, which is only preliminary in nature, follows the lines adopted by Graham-Smith, but the characters of the organisms isolated have been studied in greater detail in accordance with the methods proposed by MacConkey (1909). Many of the flies examined were obtained from houses in which epidemic diarrhoea had occurred[1], but various other sources were also drawn upon.

It has been shown very clearly by Graham-Smith (1910) that although many bacteria may adhere to the external surface of flies, they do not, in the case of non-sporing forms, survive there for more than a few hours. On the other hand, bacteria which have been ingested by the fly may remain in its intestine and be passed in its faeces for periods reckoned in days. The intestine must therefore be regarded as a more important vehicle of transit than the legs or surface of the body. In his experiments Graham-Smith found that, by feeding flies on pure cultures, *B. typhosus*, *B. enteritidis* (Gaertner) and *Vibrio cholerae* could be recovered from the intestine 6, 7, and 2 days later, respectively. Ficker (1903), Hamilton (1903) and Faichnie (1909a) have isolated typhoid bacilli from the intestine of flies caught in the vicinity of patients suffering from enteric fever. Faichnie (1909b), moreover, was able to recover typhoid bacilli from a fly which had become infected 16 days previously. These experiments and observations show that the typhoid bacillus may remain in the fly's intestine for a considerable period even under natural conditions.

Graham-Smith came to the conclusion that typhoid bacilli do not grow or multiply within the fly's intestine, and that they are more or less rapidly eliminated or destroyed. My own experiments in this

[1] I have to thank Dr G. Quin Lennane, M.O.H. Battersea, for affording me the opportunity.

respect lead to the same conclusion. That other bacteria, however, amongst which some pathogenic to man, may grow and multiply inside the fly, is not without the range of possibility. During the course of my experiments, I have found that certain varieties seem to be able to establish themselves in the fly's intestine, to the exclusion, sometimes, of other forms, and to remain there for a considerable time. Amongst these may be mentioned Morgan's *Bacillus No.* 1 and *Bacillus paratyphosus β*. The former I have met with not infrequently and a few flies have yielded almost a pure culture of this organism. *B. paratyphosus β* I have found in two flies, which were being used for experimental purposes. They formed part of a batch of flies which were fed on a sample of faeces from a typhoid carrier. These two particular flies were examined 4 and 7 days respectively after they had fed on the faeces. *B. paratyphosus β* was recovered from the external surface and intestine of the first fly and from the intestine of the second, in each case in almost pure culture. The identity of the bacillus was confirmed by Dr F. A. Bainbridge. Bacteriological examination of the faeces on which the flies had fed failed to reveal the presence of *B. paratyphosus β*. Three subsequent examinations, as well as many previous examinations of faeces from the same patient gave negative results, so the conclusion cannot be avoided that the flies had become infected previous to the experiment. The flies had been kept under sterile conditions for a week before experiment. The source of infection could not be ascertained but it is apparent that the second fly must have carried the bacillus for at least 14 days, or that it had become infected from the first fly, which had carried it for at least 11 days. The point of importance is that at the end of that period the bacilli were still present in large numbers in the intestine. This, so far as I am aware, is the only record of this bacillus occurring naturally in flies. Morgan's bacillus No. 1 was obtained from flies by Morgan and Ledingham (1909) and appears to be a not uncommon inhabitant of the fly's intestine.

The numerous observations which have already been made on the subject clearly establish the fact that the house-fly may carry an enormous number and a great variety of bacteria and may inoculate those into materials on which it feeds. They have also shown that many of the bacteria pathogenic to man and animals may be carried in this way. It remains to determine the extent to which this means of transmission may occur in nature. To this end one of the most essential points appears to be a knowledge of the natural relation of flies to bacteria. A knowledge of the bacteria naturally occurring in flies,

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their relation to each other and their relation towards other, possibly pathogenic, bacteria, which may from time to time be introduced, can hardly fail to be of importance in dealing with the subject of fly-borne disease. It is evident that, before any general conclusions can be drawn from experimental work with flies in their relation to the transmission of pathogenic bacteria, or from chance observations on the natural occurrence of such bacteria in flies, the above mentioned points will require some consideration.

With regard to the natural intestinal flora, it is certain that all the bacteria which may be isolated from a fly under ordinary circumstances cannot be regarded as constituting its natural flora. Feeding as it does continuously or intermittently on contaminated material, its intestine contains a large number of bacteria derived from its food. It is more than likely that a considerable proportion of these are simply passing through and never become resident. This becomes apparent when flies are fed for a length of time on sterile food. The characteristically faecal bacteria, such as the colon bacilli, tend to disappear under such circumstances and to be replaced by other organisms, frequently non-lactose-fermenting. The common occurrence of such organisms towards the end of experiments has given rise to the suspicion that they perhaps represent to some extent the natural intestinal flora of the fly. This suspicion is strengthened by the observation of Graham-Smith (1911) that non-lactose bacteria commonly occur in recently emerged flies. Dr Ledingham has also had a similar experience in a series of experiments, the results of which are in course of publication[1]. I am indebted to him for his information on this matter.

The majority of the flies examined in the present investigation were obtained from the dwelling rooms of houses in London. Several were also examined from the various outhouses in connection with the laboratory. The procedure was almost invariably the same. The flies were first well washed in sterile broth, then in 2 % lysol or absolute alcohol for 10–20 minutes. They were then thoroughly washed in sterile water, dried over a flame and the whole alimentary canal was placed in broth. After incubation at 37° C. overnight the broth cultures were plated on MacConkey's bile salt medium, and from each plate about a dozen colonies were picked off and their characters determined. Altogether 145 specimens of *Musca domestica* were examined. 25 of these were examined individually, the rest in 23 lots of 5 to 7 each. Of the 96 plates which were made from these, 55 showed

[1] *Vide Journ. of Hyg.* XI. No. 3, 1911.

the presence of lactose-fermenting bacilli. 21 external cultures and 20 internal were negative. In 12 cases lactose-fermenting bacilli were found neither inside nor outside. Less than 75 % of the flies may therefore be regarded as carrying colon bacilli either in their intestine or on the surface of their body. In 3 cases no growth of any kind was obtained from the surface or the intestine. In 9 cases there was a growth from the surface but not from the intestine, and in 5 cases a growth from the intestine but not from the surface. At least 5 % therefore of the flies carried no aerobic bacteria capable of growing on MacConkey's medium.

Excluding the numerous cases in which two or more colonies of the same organism were picked off the same plate, colon bacilli were met with 77 times. These represented 27 different varieties and most of them possessed characters corresponding with those of some organism in MacConkey's list. Seven varieties not agreeing with any in that list were met with, but without exception only in isolated cases.

The chief difficulty encountered in classifying these organisms was with regard to the liquefaction of gelatine. The great majority have shown no trace of liquefaction at the end of a year. Some, however, have shown the first signs after six months. Only a few produced marked liquefaction within four months. The latter proved to be *Bacillus cloacae*, or Nos. 36 or 102 in MacConkey's list.

The indole test was performed twice in every case and found to be constant. The Voges and Proskauer's test was repeated in several doubtful cases. The motility was always investigated in a six hours' broth culture. The sugar fermentation reactions were found to be remarkably uniform. With only one exception, acidity and gas production were apparent within 48 hours in the case of glucose, mannite, saccharose and dulcite, and in the negative cases, no further change was noticed at the end of six days. The exception was a strain of *B. vesiculosus* which fermented dulcite after four days. With regard to lactose, fermentation was almost invariably slower, but only in two cases was it delayed beyond the fourth day. The same applies to adonite. With regard to inulin the result was negative even at the end of ten days except in one case, in which fermentation occurred within four days.

The following table shows the characters and numbers of the organisms investigated. They were all Gram-negative, gave acid and gas in lactose, glucose and mannite and produced acid and clot in litmus milk. They are numbered and named according to MacConkey's list.

TABLE.

	Gelatine	Motility	Indole	Saccharose	Dulcite	Adonite	Inulin	Voges and Proskauer	Numbers obtained: External	Internal	Total
GROUP I.											
1.	−	+	+	−	−	+	−	−	1	1	2
2. *B. acidi lactici*	−	−	+	−	−	+	−	−	0	1	1
4. *B. Grünthal*	−	+	+	−	−	−	−	−	3	2	5
5. *B. vesiculosus*	−	−	+	−	−	−	−	−	2	0	2
7.	−	+	−	−	−	−	−	−	2	2	4
8. *B. coli mutabilis*	−	−	−	−	−	−	−	−	2	2	3
				Total					10	8	17
				Number of varieties					5	5	6
GROUP II.											
34. *B. coli communis*	−	+	+	−	+	−	−	−	5	10	14
35. *B. Schafferi*	−	−	+	−	+	−	−	−	1	1	2
36.	+	+	−	−	+	−	−	−	1	0	1
33*a*.	+	+	+	−	+	+	−	−	0	1	1
36*a*.	−	+	−	−	+	−	−	−	1	1	1
				Total					8	13	19
				Number of varieties					4	4	5
GROUP III.											
65. *B. oxytocus perniciosus* ...	+	−	+	+	+	+	+	+	0	1	1
66.	−	−	+	+	+	+	−	−	0	1	1
67.	−	−	−	+	+	+	−	+	1	2	2
71.	−	+	+	+	+	−	−	−	6	7	11
72. *B. Neapolitanus*	−	−	+	+	+	−	−	−	2	0	2
74.	−	+	−	+	+	−	−	−	0	1	1
75.	−	−	−	+	+	−	−	+	1	1	2
66*a*.	−	+	+	+	+	+	−	−	0	1	1
				Total					10	14	21
				Number of varieties					4	7	8
GROUP IV.											
101.	−	−	+	+	−	+	−	−	0	1	1
102.	+	+	−	+	−	+	−	+	2	4	6
104. *B. gasoformans non-liquefaciens*	−	−	−	+	−	+	−	−	2	2	3
106.	−	+	+	+	−	−	−	−	1	2	3
108. *B. cloacae*	+	+	−	+	−	−	−	+	0	3	3
109.	−	+	−	+	−	−	−	−	1	1	2
106*a*.	+	+	+	+	−	−	−	−	0	1	1
108*a*.	+	+	+	+	−	−	−	+	1	0	1
				Total					7	14	20
				Number of varieties					5	7	8
			Total in all the groups						35	49	77
			Total number of varieties						18	23	27

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Comparing the above table with that given by MacConkey (1909) it is apparent that a considerable similarity in respect of the colon bacilli exists between the bacterial flora of flies, and the bacteria met with in the faeces of man and other animals. The most striking feature is the marked preponderance of the characteristic faecal organisms *B. coli communis* and MacConkey's bacillus No. 71.

From the varied sources from which his organisms were derived, MacConkey found that those belonging to group III were greatly in excess of those belonging to the other groups and constituted 45 °/₀ of the whole. Those of group IV were only about a third as frequent. From purely faecal sources the disproportion between these two groups is still more marked. In a general way, therefore, it may be considered that the bacilli of group III are more characteristically faecal organisms than those of group IV. The latter were obtained for the greater part from non-faecal sources. The varieties in groups I and II were more frequent in faeces than in other materials. Again in human faeces groups I, II and III were all about equally common, while group IV was comparatively rare. An examination of my table will show that each of the groups is about equally represented. There is not the comparative rarity of group IV characteristic of excremental material nor the rarity of groups I and II characteristic of other sources. This would indicate that the colon bacilli found in flies comprise a mixture derived both from faecal and other sources. Such a conclusion is in accord with preconceived notions.

With regard to the individual varieties, those most frequently met with by MacConkey were No. 71, No. 34 (*B. coli communis*) and No. 5 (*B. vesiculosus*), and these were the three organisms which occurred most frequently in excremental matter. They were also most frequent in human excrement. From other sources, however, the most common varieties were Nos. 71, 73, and 108 (*B. cloacae*). *B. coli communis* and *B. vesiculosus* were much rarer. Now in *Musca domestica* the most common varieties were 34 and 71 followed by 102, 4 and 7. The great frequency of *B. coli communis* can hardly be regarded as other than direct evidence of faecal contamination. The same may be said of No. 4, which was never found by MacConkey apart from faeces.

It is impossible to tell to what extent the flies in the present investigation have acquired contamination from human excrement. The number of bacterial organisms which have from time to time been isolated from human faeces and which appear capable of living for a longer or shorter period in the human alimentary canal is very great.

The same holds good in the case of several animals, and from the very incomplete evidence at present in existence, the intestinal florae of various mammals have very much in common. Differences and distinctive features have however not passed unnoticed, and with fuller knowledge the presence of a certain few bacteria might be sufficient to warrant the diagnosis of excremental matter from a particular animal or class of animals. This would demand more intimate acquaintance with the bionomics of bacteria, and in particular of those which are parasitic in man and animals. Modern methods have shown that bacteria under artificial conditions have very varied physiological requirements, and there is no reason to suppose that their requirements under natural conditions are any less varied. If such is the case, it follows that for each class or group of bacteria and for individual species or varieties of that group, there will be some environment which is an optimum and in which that particular species thrives and develops to the fullest extent. There can be no question that a great variety of physiological environment does exist in the alimentary canal of animals. Some organisms are known to require an extremely specialised environment, while others can accommodate themselves to a wide variety. For this reason the distribution of some organisms is extremely wide, while that of others is restricted. From biological considerations it may be assumed that each variety of environment will call forth the existence of a corresponding variety of organism and that the latter is conditioned by the former. There should therefore be just as many species or varieties of organisms as there are variations in environment. The different environments obtaining in the intestine of man and other animals should be sufficient to differentiate distinctive groups of bacteria and these groups would probably be readily isolated in the faeces were it not for the fact that a large number of bacteria probably simply pass through the intestine with the food, not actually growing and multiplying, but at the same time not being killed off in the process. It ought however to be possible by suitable experiment to elucidate this matter and I hope to be able to do so to some extent in the case of flies.

SUMMARY.

1. A study of the natural bacterial flora of the house-fly appears to be essential in forming a correct estimate of the part played by flies in transmitting pathogenic bacteria.

2. The house-fly may carry at least 27 varieties of *Bacillus coli*, by

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far the most frequent of which are *B. coli communis* and MacConkey's bacillus No. 71.

3. As far as can be judged from the character of these colon bacilli the house-fly derives its bacterial flora equally from excremental matter and from other sources.

4. Certain non-lactose fermenting bacilli appear to be capable of multiplying in the intestine of the house-fly. Of these Morgan's bacillus No. 1 is a not infrequent inhabitant of the fly's intestine and *B. paratyphosus* β has been found on two occasions.

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