ON THE INFLUENCE OF THE METAMORPHOSIS OF *MUSCA DOMESTICA* UPON BACTERIA ADMINISTERED IN THE LARVAL STAGE.

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A considerable amount of experimental work has been carried out by several workers to demonstrate that certain species of flies may infect food for varying periods of time after the fly is itself experimentally infected.

Till recently, the question as to whether pathogenic bacteria might be recovered from the interior of young flies, the larval stage of which had been contaminated with these bacteria, has not received much attention. During the past two years several investigators have attacked this question, but unfortunately their results are not strictly comparable owing to their having experimented with different species of flies and different organisms.

Bacot (1911) showed that if the larvae of *Musca domestica* be infected with *B. pyocyaneus* the resulting pupae and imagines undoubtedly remain infected with the bacillus.

Ledingham (1911) confirmed Bacot's results with *B. pyocyaneus* and then infected larvae with *B. typhosus*, but failed to recover the bacillus either in the grown larvae, pupae, or imagines. From the larvae were isolated other bacilli which had contaminated the ova and which he regarded as well adapted to the interior of the larvae, pupae, and imagines of *M. domestica*. Both the pupae and imagines were sometimes sterile but more often contained some of these persistent organisms, the most common being that described as "*Bac. A*" (Ledingham). After sterilization of the ova with lysol, however, and subsequent feeding of the young larvae with *B. typhosus*, this organism was recovered from the full-grown larvae and from the one pupa that he obtained.

Graham-Smith (1911) showed that if the larvae of the blow-fly (*Calliphora erythrocephala*) be infected with *B. anthracis* these bacilli may in a large proportion of cases be recovered from the young flies, but on the contrary he failed altogether to recover *B. typhosus*, *B. enteritidis*, *B. prodigiosus* or *V. cholerae* under the same conditions. He admits that "these experiments, however, afford no information as to the extent to which house-flies (*M. domestica*) bred from larvae fed on naturally infected excreta and similar materials are apt themselves to be infected."

Lucius Nicholls (1912) working independently in the Windward Isles has just recently published some interesting results. Using *Sarcophagula* he found that the number of organisms in the interior of pupae progressively diminished from the commencement of pupation to such an extent that if the number of bacteria in the grown larva be represented as 100, there was 0·4 left in the four-days old pupa. The absolute number of bacteria in the pupae of this fly, under more or less natural conditions, was less than 20 in seven pupae out of twelve.

Further, on breeding larvae of *Sarcophagula* in faeces infected with *B. typhosus*, *B. prodigiosus*, *Staphylococcus pyogenes aureus*, and a lactose-fermenting organism respectively, he found that these bacteria rapidly disappeared from the larvae if they were removed from their infected surroundings. From pupae developed from the larvae none of the bacteria were recovered.

Some pupae were watched and when about to hatch out, the imagines were seized, extracted with sterile forceps, and plated out. Of 12 imagines thus examined 2 were sterile and 10 gave only one or two colonies. A further batch of 12 imagines were allowed to emerge, placed immediately in a sterile vessel and kept for two days without food, after which every one was found to be sterile.

Of 12 imagines of another species (*Sarcophaga*), similarly treated, 10 were sterile.

Nicholls concludes that a freshly hatched fly may be considered as probably sterile and there is not much likelihood of its acquiring these organisms by contact with the material in which it bred.

The experiments which I will now detail were undertaken in order to confirm and elaborate certain points arising out of Ledingham's investigations summarized above. With regard to technique, Mr Bacot kindly supplied me with ova of *M. domestica* which were placed upon agar slopes to which a little fresh human blood was added together with

the organisms with which the young larvae were to be infected. Sometimes the ova were sterilized by washing in 3% lysol for two or three minutes. Larvae when full-grown were placed on sterile sand to pupate, and the pupae were stored in sterile test tubes till they were examined or till imagines emerged.

The method of examining the pupae was that described by Ledingham (1911), the blunt end being seared with a hot iron, a capillary pipette inserted and the contents sucked out and plated on MacConkey's neutral red lactose agar.

The imagines were examined either as soon as they were first noticed or after feeding on sterile cane sugar solution. The method used was to wash them separately in 2% lysol for seven to ten minutes, then in two or three successive tubes of broth, after which each was crushed in another small amount of broth and the latter plated out on several MacConkey lactose plates, so that the whole of each imago was bacteriologically examined. The broths used to wash the fly were also incubated in order to detect bacteria on the external surface, and sub-cultures were made from the last broth in which the fly was washed before being crushed and plated.

The ova, larvae, pupae and imagines were kept throughout at a temperature of 25° C

With regard to the bacteria met with and employed in this research, a few comments are necessary. The principal pathogenic organism on which larvae were fed was one of the mannite types of the dysentery bacillus, viz. the *Bac.* "*Y*" of Hiss and Russell.

The organism referred to as "*Bac. A*" was described by Ledingham and corresponds in its fermentation reactions with Morgan's *No. IV B*, a non-lactose fermenter from the faeces of children. "*Pseudo No. 1*" is a provisional name for a motile non-liquefying bacillus giving acid and gas in glucose media and alkalinity in litmus milk, like Morgan's *No. 1*. It differs from the latter (1) in fermenting adonite with gas production, (2) in not fermenting galactose, (3) in producing more marked alkalinity in litmus milk, (4) in producing acidity in saccharose in five days, (5) in a slower production of indol. In spite of these differences it is apparently more closely allied to Morgan's *No. 1* than to any other non-lactose fermenter. I have twice isolated it from human faeces as well as from flies and it is worth noting, in view of the fact that Morgan's *No. 1* has been said to be rather common in flies, that these two bacilli would not have been differentiated in all probability unless galactose or adonite media had been used.

Series I.

Technique.	*Bacteria found.*
Ova, not sterilized, placed on blood agar.	Contaminating the eggshells and young larvae were: Types of *B. coli*, Gelatin-liquefying cocci, "*Bac. A*" (Ledingham).
Two larvae, 2 days old, washed in lysol and plated out.	*Pseudo No. 1* bacilli found in each.
All larvae washed in lysol when 2 days old, and *Bac.* "*Y*" (Hiss and Russell) mixed with blood on fresh agar slopes on which the larvae were placed.	
One larva, 6 days old washed in lysol, and plated out.	"*Bac. A.*"
Remaining larvae placed on sterile sand.	
7 pupae examined. 1 kept and fly emerged.	6 pupae sterile. 1 pupa gave about 30 colonies, a mixture of *Pseudo No. 1* and "*Bac. A.*"

Summary of Series I. Of the numerous types of bacteria found in association with the ova and young larvae, only those called *Pseudo No. 1* and "*Bac. A.*" persisted, although 6 pupae out of 7 had become sterile. The mannite type of dysentery bacilli administered was not found either in larvae or pupae.

Series II.

Technique.	*Bacteria found.*
Ova, not sterilized, placed on blood agar.	Associated with 1-day old larvae Gelatin-liquefying cocci, *Pseudo No. 1* bacillus.
4-days old larvae washed with 0·25 % lysol (40 minutes) then with broth. Fed with blood and *Bac.* "*Y*" on agar for 5 days then placed on sand to pupate.	
3 pupae obtained.	1 pupa sterile. 1 pupa: *Pseudo No. 1* bacillus, Lactose fermenting gram-negative bacilli. 1 pupa: Gram positive cocci.

Summary of Series II. As before *Bac.* "*Y*" was not recovered from pupae. *Pseudo No. 1* bacillus again persisted in one pupa. One pupa sterile out of 3 examined.

Series III.

Technique.	*Bacteria found.*
Several ova incubated in broth and then plated out.	Lactose fermenters. "*Bac. A.*"
Remaining ova washed with 0·5 % carbolic for 3 minutes and placed on agar. Sterilization not effected for growth appeared in association with the young larvae.	
5 days old larvae washed with 0·25 % lysol (30 minutes) then with broth. Fed for 4 days with blood and *Bac.* "*Y*," then placed on sand to pupate.	
7 pupae examined.	4 pupae sterile.
	2 pupae contained both *Bac. A* and lactose fermenters.
	1 pupa "*Bac. A*" and a dulcite fermenter.
One fly found about 1 hour after emergence and allowed to walk over a MacConkey plate, on which a growth took place.	"*Bac. A.*" Gram positive cocci.

Summary of Series III. "*Bac. A*" persisted as in Series I. "*Bac. Y*" not recovered from pupae or fly. Four pupae sterile out of 7.

Series IV.

Technique.	*Bacteria found.*
Ova washed with 3 % lysol for 2–3 minutes, then with normal saline. Larvae hatched and remained sterile being fed with fresh blood. When 3 days old fed with "*Bac. Y*" and blood.	
When 9 days old and full grown placed on sand to pupate.	
4 pupae examined.	3 pupae sterile.
	1 pupa contained about 60 colonies, all non-lactose-fermenters, 4 of which were further examined and found to be *Bac. Y.* This pupa was 4–7 days old when examined.
1 pupa left and a normal imago emerged but was not examined.	

Summary of Series IV. Larvae hatched from sterilized ova were bred and afterwards fed in contact with a dysentery bacillus which was recovered from 1 pupa out of 4.

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Series V.

Technique.	*Bacteria found.*
Ova washed with 3 % lysol for 2 minutes then with normal saline. Larvae sterile as before and fed with blood and *Bac. Y* from the second day till mature. Then placed on sand.	
5 pupae examined.	3 pupae sterile. 1 pupa gave 1 colony of *Bac. Y.* 1 pupa gave 2 colonies of *Bac. Y.*
1 pupa washed with 3 % lysol for 7 minutes and placed on a sterile plate. An abnormal imago emerged and lived for 36 hours and was then crushed and plated.	Fly sterile.
1 pupa treated as above but no imago appeared. After about 10 days the contents which were semi-solid were plated.	1 pupa about 10 days old contained 50 colonies (*Bac. Y*). (Possibly this pupa was dead and the organisms had afterwards multiplied.)

Summary of Series V. Dysentery bacilli found to persist though in very small numbers in 2 pupae out of 5. 1 fly found to be sterile.

Series VI.

A. One batch of eggs was washed with lysol as before and the sterile larvae were fed with *Bac. Y* and fresh blood.

1 pupa was obtained and found to be sterile.

B. A second batch of eggs was treated with lysol but the larvae were contaminated and were allowed to mature without administering other bacteria.

4 pupae were obtained, all sterile.

Summary of Series VI. Batch B shows that some bacteria will resist treatment with lysol but yet fail to persist in the metamorphosis from larva to pupa.

Series VII.

Technique.	*Bacteria found.*
A. One batch of ova washed with lysol as before and the sterile larvae fed with *Bac. A* (Ledingham).	
1 pupa examined.	About 170 colonies of *Bac. A* (identified).
B. Another batch treated in the same way.	
1 pupa examined (4 days old).	About 500 colonies of *Bac. A* (identified).
1 pupa left and the emerging imago washed in 2 % lysol for 7 mins. and then in successive broths, then crushed and plated out.	Fly sterile.
Another imago fed for 3 days on sterile sugar solutions. This fly died and was plated out.	Fly sterile.
Another imago was plated out a few hours after emergence after washing with lysol and broths.	Fly gave very numerous colonies, not further identified as *Bac. A* other than that they gave the peculiar sour odour of *Bac. A* and were non-lactose-fermenters.

Summary of Series VII. "*Bac. A*," found naturally associated with flies, was recovered from 2 pupae examined in large numbers, and almost certainly from 1 fly out of 3.

Series VIII.

Ova were sterilized as before and fed with blood and *Bac. Y.* The pupae obtained were placed in sterile test tubes and the flies examined as soon after emergence as possible. If alive they were chloroformed, then washed in 2 % lysol for about 5 minutes and finally in successive broths. The fly was then crushed in broth and plated out on several MacConkey plates.

Fly 1. Broths clear and plates sterile.

Fly 2. Broths clear and plates sterile.

Fly 3. Broths clear. Three plates used, each showed a number of pale colonies, one of which was taken and identified as *Bac. Y.*

Fly 4. Broths clear. One plate showed numerous pale colonies and a few sarcina-like colonies.

Fly 5. Broths clear and plates sterile.

Fly 6. This did not develop its wings and died soon after emergence. The broths used for washing became turbid and the plates were thickly covered with pale colonies, one of which was identified as *Bac. Y.*

Summary of Series VIII. Two flies of normal appearance were found to contain a dysentery bacillus which had been administered to larvae reared from sterilized ova.

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Series IX.

Ova treated with lysol as before and some larvae fed with *Bac. A* and some with *B. pseudo No. 1.*

I am unable to give the results separately, but of 8 flies examined 6 were sterile whilst 2 gave numerous colonies, the broth washings being clear.

Series X.

A. A batch of larvae from sterilized eggs were fed with *B. typhosus*. The one fly obtained proved to be sterile.

B. From a similar batch of larvae hatched from sterile ova and fed with *B. typhosus* 15 flies were obtained, but *B. typhosus* was not recovered in any one of them.

C. In a further batch 6 pupae and 16 flies were examined, all negative for the *B. typhosus*.

Summary of Series X. *B. typhosus* failed to persist in the metamorphosis from larva to imago in every case examined.

It must be remembered that the conditions in many of these experiments have been highly artificial. *Musca domestica* does not specially seek human excrement for the purpose of depositing its eggs, and in human excrement only are the bacilli of dysentery and typhoid fever likely to be found in any number. Numerous other types of bacilli are associated in nearly all cases with these pathogenic bacteria, and it has been shown by several workers that in the case of the *B. typhosus* and, in some of the above experiments, in the case of a dysentery bacillus, that where larvae are bred in association with a mixed flora, that is in more or less natural conditions, these pathogenic bacteria can rarely be recovered from the larval interior and certainly not from the pupae. The possibility then of an emerging fly being already infected with either of these bacilli seems to be very remote. Lucius Nicholls working with other species found that emerging flies were under natural conditions either sterile or contained one or two colonies only.

Under the artificial conditions where the ova were sterilized and larvae only allowed to come into contact with the organism administered it was found in the case of the dysentery bacillus used that a large number of pupae and imagines were sterile, but that in some cases a few bacilli persisted through the metamorphosis. On the other hand, *B. typhosus* was never recovered in either pupae or imagines though it is worthy of mention that Ledingham recovered a few bacilli from one pupa under similar experimental conditions.

The general impression that one gathers from the work already published and from these experiments is that during the metamorphosis a marked inhibition is exhibited towards certain bacteria ingested in the larval stage. It does not seem to take effect upon all bacteria; for example, Bacot found *B. pyocyaneus* appearing very constantly in the pupae and imagines.

The factors affecting bacteria in a larva commencing to pupate may vary according as to whether the organisms are in pure or mixed culture. In the latter case it is possible that one variety of organism may injuriously affect the growth of the other and this inhibition will then be superadded to the definite bactericidal action of metamorphosis itself, however produced; on the other hand, if only one species of organism is present, a symbiotic effect cannot take place. An attempt was made to extract bactericidal substances from larvae. A large number of larvae were crushed in saline in a mortar and the emulsion passed through a Berkefeld filter. An emulsion of *Bac. Y* (of dysentery) was then added to different dilutions of the larval extract, but the extract was not found to be so prejudicial to this organism as normal saline itself.

Ledingham concluded that certain bacteria found commonly in flies had a special adaptability to the metamorphosis and were able to persist in the insects throughout their metamorphosis. Not even these types, however, are always able to withstand the inhibitory effect though they do so far more successfully than do such bacteria as *B. typhosus* and *Bac. Y* of dysentery. I found that the latter bacillus does not grow so well at the temperature of a fly (room temperature 60°–75° F.) as does *Bac. A* of flies, so that temperature may play some part in increasing the relative number of this latter bacillus. Whether the normal flora of the house-fly exerts a direct inhibitory effect on the growth of pathogenic bacilli was not thoroughly investigated, but it was found in one series of experiments that where *Bac. Y* and *Bac. A* (of flies) were inoculated in about equal numbers into broth and incubated at 37° C., in 24 hours *Bac. Y* had diminished to 36 %, and in 48 hours to 25 %. Possibly the truth lies in a combination of these explanations, viz. that the metamorphosis of the fly exerts a very marked inhibitory effect on certain bacteria as, for example, the pathogenic species above mentioned, and that where other bacteria are present, which are better adapted both to their environment and temperature, this prejudicial effect is still more marked, accounting for the result that by the time the pupa is formed none of these pathogenic bacteria can be recovered.

SUMMARY AND CONCLUSIONS.

(1) Pathogenic organisms such as *B. dysenteriae* (type "*Y*") cannot be recovered from pupae or imagines reared from larvae to which these organisms have been administered.

(2) When the larvae have been bred from disinfected ova and are subsequently fed on *B. dysenteriae* (type "*Y*"), this organism may be successfully recovered from the pupae and imagines in a small proportion of cases.

(3) Under similar conditions *B. typhosus* was not recovered in a single case from pupae or imagines.

(4) In those cases in which *B. dysenteriae* (*Y*) was successfully recovered from pupae, the colonies on the plate were invariably fewer than those obtained from pupae and imagines after administration to the larvae of more adaptable organisms such as "*Bac. A*" (Ledingham).

(5) When organisms such as "*Bac. A*" were administered to larvae bred from disinfected ova, or non-disinfected ova contaminated with this organism, it was in many cases possible to recover the organism from pupae and imagines.

(6) In no series of pupae examined after administration to the larvae of either *B. dysenteriae* or "*Bac. A*" was it possible to recover the organisms in every instance. A certain proportion of pupae in both cases proved sterile, so that the process of metamorphosis is undoubtedly accompanied by a considerable destruction of the bacteria present in the larval stage.

(7) The temperature at which the larvae develop (19°–25° C.) has probably an important bearing on the survival of pathogenic organisms such as "*Bac. Y*" of dysentery, administered in association with organisms such as "*Bac. A*," in view of the fact that the latter grows far more luxuriantly at this temperature. Even when grown in broth at 37° C. (the optimum temperature for *Bac. Y*) together with "*Bac. A*," the "*Bac. Y*" was found to form after two days only one quarter of the total number of bacteria present in the mixed growth.

(8) There was no evidence that the larval juices contained substances bactericidal for *Bac. Y*. The bacilli died more rapidly in normal saline solution.

(9) The possibility of flies becoming infected from the presence of pathogenic organisms in the breeding ground of the larvae may be considered as very remote.

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