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REPORTS

TO THE

LOCAL GOVERNMENT BOARD

ON

PUBLIC HEALTH AND MEDICAL SUBJECTS.

(NEW SERIES No. 53.)

Further Reports (No. 4) on Flies as Carriers of Infection:—

1. An Experimental Investigation on the Range of Flight of Flies: by Dr. Copeman, Mr. Howlett and Mr. Merriman.
2. Memorandum on the Result of Examinations of Flies from Postwick Village and refuse deposit: with a note on the occurrence of the Lesser Housefly at Leeds: by Mr. Austen.
3. The part played by Flies in the dispersal of the eggs of Parasitic Worms: by Dr. Nicoll.
4. Further observations on the ways in which artificially infected Flies carry and distribute pathogenic and other Bacteria: by Dr. Graham-Smith.

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Further Reports (No. 4) on Flies as Carriers of Infection.

ARTHUR NEWSHOLME,
Medical Officer,
11th May, 1911.

1.—AN EXPERIMENTAL INVESTIGATION ON THE RANGE OF FLIGHT OF FLIES. BY S. MONCKTON COPEMAN, M.D., F.R.S.; F. M. HOWLETT, B.A., F.E.S., SECOND IMPERIAL ENTOMOLOGIST TO THE GOVERNMENT OF INDIA; AND GORDON MERRIMAN, STUDENT IN MEDICAL ENTOMOLOGY, QUICK LABORATORY, CAMBRIDGE.

As indicated by one of us (S.M.C.) in the Memorandum* to the Board of July, 1909, determination of the possible range of flight of flies is obviously of considerable importance in relation to the whole question of the carriage of infection by these insects.

A preliminary search of the available literature afforded but little accurate knowledge on the subject, and consequently arrangements were made with Professor Nuttall and Mr. Merriman of the Quick Laboratory, Cambridge, for the carrying out of a series of carefully devised test experiments, on a large scale, in accordance with the plan described and illustrated in Report No. 3 on "Flies as Carriers of Infection." (New Series No. 40, pp. 45-47.)

Unforeseen circumstances unfortunately prevented further prosecution of this portion of our investigations at Cambridge, but, in the summer and autumn of last year (1910), opportunity presented itself for carrying out a somewhat similar series of experiments in Norfolk, the results of which are detailed below.

The only previous experiments on similar lines, with the common house-fly, appear to be those carried out by Dr. Arnold at the

* Reports to the Local Government Board on Public Health and Medical Subjects, New Series No. 16; Further Preliminary Reports on Flies as Carriers of Infection, 1909, *see* pp. 1-4.

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Monsall Hospital, Manchester, during August, 1906, to which brief reference is made by him in the last two paragraphs of the Monsall Hospital Laboratory Report for 1906. (*Vide* Annual Report of Dr. Niven, Medical Officer of Health of Manchester, 1907, p. 262.) The wording is as follows :—

> "Three hundred flies were captured alive : they were all marked with a spot of white enamel on the back of the thorax, and liberated. It had been previously shown, by keeping imprisoned a few so marked, that the energy of the fly was not affected, and the mark did not wear off rapidly.
>
> The flies were all liberated from a window of the administrative block, and instructions were issued that the fly-traps in the wards were to be watched for the marked flies. Out of the 300, five were recovered at distances varying from about 30 to 190 yards. The liberations were always in fine weather, and the recoveries were within five days."

In reply to enquiry, (December, 1910), Dr. Arnold states that the experiments were made on three successive Sundays—a hundred marked flies having been set free on each occasion. He adds that 190 yards was the greatest distance available in any direction, "so that the experiments cannot be taken as giving any hint of the limit of flight."

In connection with investigations on sleeping sickness, Dr. Bagshawe (Report of the Sleeping Sickness Commission of the Royal Society, 1908, No. IX. p. 45) carried out a number of experiments on the flight of *Glossina Palpalis* in order to determine whether "flies might be present in abundance at a spot far from their breeding place if a constant supply of animal food were there obtainable," and "how far flies may wander in the course of a river." These experiments, which must have involved an immense expenditure of time and patience, were carried out by snipping off a portion of one of the legs of a number of flies with scissors, which, as he says, afforded "a ready means of identifying a re-taken fly." These mutilated flies were released at various points, and 25 per cent. of them were subsequently re-taken at distances varying from 300 yards to (in one instance) 900 yards from their original place of capture.

Investigations at Postwick.

Early in July of last year (1910) it was ascertained that the inhabitants of Postwick—a small village situated about five miles East of Norwich, just off the main road to Yarmouth—were experiencing a "plague of flies" so unprecedented in extent as to constitute a serious annoyance, and possible danger to health.

As it appeared not improbable that enquiry into the local circumstances might afford useful information in relation to the Board's general investigation on "Flies as Carriers of Disease," sanction of the Board was obtained to enquiry into the causation of the unusual prevalence of flies at Postwick. For the purposes of this enquiry, the district was visited on July 14th, 1910 and on subsequent occasions, on one of which several days were spent in the village in order to obtain accurate information as to the local circumstances.

On the occasion of the first visit to Postwick (July 14th), the common house-fly (*Musca domestica*) was present in unusual numbers especially in the living rooms of cottages, and in the kitchens of the larger houses.

Through the agency of the village blacksmith, we had previously distributed a considerable number of wire-mesh "balloon" traps; and it was found that a London firm had also sent down to the village supplies of fly-papers and fly-tapes. By means of these appliances residents in the village had been successful in trapping large numbers of flies. In some instances where four or five tapes had been hung up in the same room only a few hours previously, all were found entirely covered with these insects, while some of the "balloon" traps were "humming" with the movements of hundreds of living flies.

There was, however, agreement on all hands, that the flies had considerably diminished in number during the previous fortnight, owing presumably to the lowered temperature and other changes in the meteorological conditions.

As some evidence of the excessive prevalence of the flies a week or two earlier, one woman informed us that she had had to drown the mass of flies caught in her "balloon" trap, several times a day; while another woman stated that, on one occasion, having left open the door on the sunny side of her cottage for about half-an-hour, the door-mat, in the course of that time, had become absolutely black with flies. All the residents interviewed affirmed that never in their experience, during previous years, had they known flies to be present in such abundance, and that great difficulty had been experienced in protecting articles of food, more especially milk and sugar, from their invasions.

Thanks probably to the continuance of cold and wet weather the number of flies more or less continuously diminished from the end of June, 1910. During a stay of several days at Postwick during the early part of August, one of us found the flies distinctly less numerous than on the occasion of former visits.

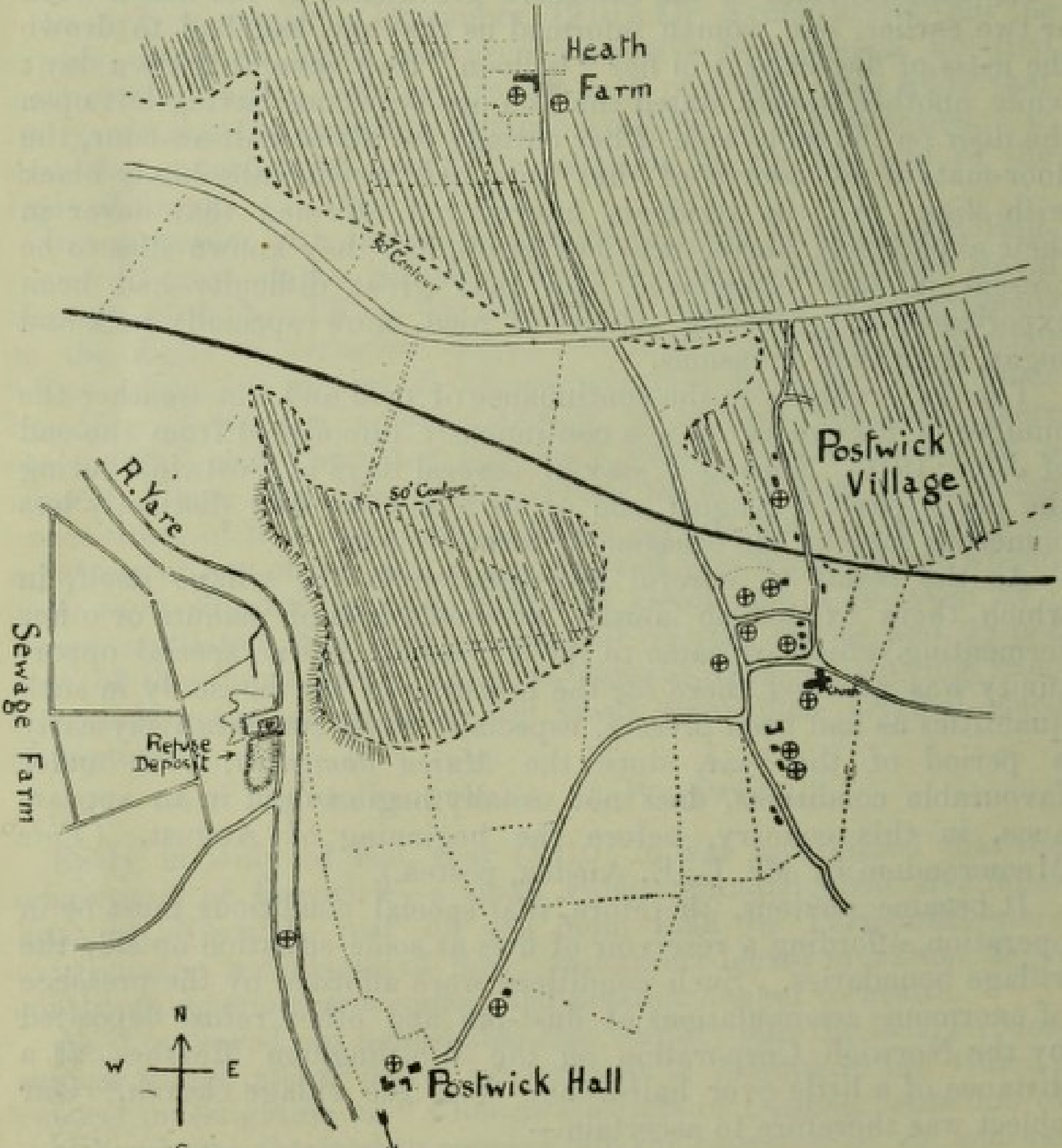
As the result of careful investigation in the village itself, in which there existed no unusual accumulations of manure or other fermenting refuse, we came to the conclusion that no special opportunity was afforded there for the breeding of the house-fly in such quantities as had been present, especially at so comparatively early a period of the year, since the *Musca domestica*, even under favourable conditions, does not usually begin to put in an appearance, in this country, before the beginning of August. (*Vide* Memorandum by Mr. E. E. Austen, postea.)

It became obvious, therefore, that special conditions must be in operation, affording a reservoir of flies at some situation outside the village boundaries. Such conditions were afforded by the presence of enormous accumulations of dust-bin and other refuse deposited by the Norwich Corporation on the Whitlingham Marshes, at a distance of a little over half-a-mile from the village church. Our object was therefore to ascertain—

> Whether the plague of flies at Postwick was directly due to the presence of this refuse heap, and, if so, whether the flies were being attracted from the surrounding country by the very penetrating smell of the heap, or distributed from it as a breeding centre.

As stated, the tip consisted mainly of dust-bin refuse, and in this refuse fermentation was actively going on, the fresher portions steaming vigorously when the top layer was disturbed ; it was in fact an almost ideal breeding place for house-flies. The older parts of the heap were turfed over or covered with a layer of road-sweepings.

The refuse was brought up in closed barges from Norwich, along the river Yare, to a backwater situated on the north side of the tip ; it was then removed from the barges by means of a crane, and shot along the west side of the tip. On the north side, adjoining the backwater, is a workman's shelter-hut heated by a coke-stove, which was kept continuously burning day and night, and on the east side, where the river Yare flows close past the heap, is a small wharf raised on piles about 10 feet above the surface of the water. On the opposite side of the river is a steep bluff some 60–75 feet in height. The accompanying map shows the 50-foot contour line



Sketch Map showing relative position of Postwick Village and Norwich Corporation Refuse Deposit ; illustrating results of Experiments on Range of Flight of Flies. (Places at which "marked" flies were recovered indicated by ⊕).

Approximate scale, 500 yards to 1 inch.

and the general topography of the immediate neighbourhood; the village of Postwick lies roughly E. of the tip, and the prevailing wind is S.W.

It was difficult, if not impossible, definitely to prove that flies were not attracted from the leeward side of the refuse by the smell of the decaying matter, and it would be surprising if a certain number were not so attracted. Experiments carried out by one of us (F.M.H.) on the biting Muscid *Stomoxys calcitrans* and an Acalyptrate Muscid (*Dacus*) have shown that flies may be attracted by certain smells in a very definite way from considerable distances, but that the reaction to such smells is—to some extent at least—a sexual characteristic, the females responding more readily to the smells which advertise a place suitable for egg-laying. Hence any accumulation of refuse such as we had here to deal with, might, by analogy, be expected, if exerting any marked effect in this direction, to attract from the surrounding country a larger number of females than of males. Four lots of flies were caught at intervals of a month, in July, August, September and October. The sexes of the first lot were not noted, they being those sent to Mr. E. E. Austen for identification. The remaining three lots were examined and identified by one of us, and these were all caught under similar conditions, in balloon-traps placed in the workmen's hut. The results were as follows :—

	Males.	Females.
August	329	334
September	302	318
October	158	243

It is known that under normal conditions the sexes in the case of *M. domestica* are produced in approximately equal numbers.

The greater proportion of females in the last as compared with the very equal numbers of the two previous catches, is probably due to the fact that, in autumn, males tend to die off, and are not replenished by fresh broods, while the females live on and hibernate.

The great preponderance of *Musca domestica* is noteworthy. More than 99 per cent. of the flies caught in the village on fly-papers and tapes were *M. domestica*, the remainder mostly *Homalomyia canicularis*.

As shown by the map, there is on the west of the tip a sewage farm. If house-flies were attracted from the district by the smell of the tip it is not unreasonable to suppose, though it by no means follows, that a certain number might be attracted by the smell of the sewage effluent, which at times was almost equally penetrating though of a slightly different character. This, however, was not the case, for although great numbers of *Scatophaga* and *Sepsis* were seen about the effluent channels, *M. domestica* was practically absent.

The way in which house-flies congregated in the warm and rather ill-lit shelter-hut was very noticeable. On cold days it was often not easy to catch any flies on the tip itself, but they could be caught in any number in the shelter, where some sacking, which covered the wall and a bench near the stove, was sometimes literally "black with them."

Though the flies caught in the workmen's shelter, like those caught in houses in the village, were practically all *M. domestica*, this must be due rather to the habits of this fly than to the failure of other species to breed on the tip, since the collection of 200 larvæ and pupæ from fresh tip refuse gave the following results :—

Musca domestica	143
Muscina stabulaus	34
Calliphore erythrocephala	18
Pollenia rudis	3
Homalomyia canicularis	2

A considerable number of undetermined *Phoridæ* were also bred out from samples of the refuse which were kept as likely to be good for some of these larvæ.

We see, therefore, that there was a considerable variety among the flies breeding in the refuse ; just as on the sewage farm *Sepsis* and *Scatophaga* were the dominant species, so *Musca domestica* preponderated on the refuse heap ; but in most cases other species were also present.

The comparatively small number of flies usually observable on the actual refuse might possibly be taken as strengthening the case against their being attracted from the surrounding district by the smell of the refuse, since in that event we might not unreasonably expect that the refuse would be chosen as a resting-place as often as the hut, except perhaps on cold days. Although a few of the marked flies mentioned below remained in and about the hut for as long as a week in some instances, there was no evidence to show that they ever returned after having once left the refuse-heap.

The bearing of these minor observations on the question whether the tip was to be regarded primarily as an attracting or as a distributing centre may be summarised.

Flies were extremely abundant in the village from the third week of June, 1910, some two months earlier than the usual time of their appearance in this country. No unusual conditions were found in the village itself, but a refuse-tip half-a-mile away had been started in the previous January.

The suggestion that flies were attracted to the tip, and tarried in the village on their way thither, was rendered improbable by the facts that—

(1.) The number of flies actually to be seen at any one time on the refuse was not noticeably great. Marked flies which had once left the heap did not apparently return to it.

(2.) In spite of the great number of larvæ and pupæ in the refuse, it was very difficult to discover any eggs or even newly-hatched larvæ. (The period between the collection and the dumping of the refuse varied from 12 to 48 hours. In one case a considerable number of flies were seen to escape when the hatches of a barge were removed after being closed down for two days.)

(3.) The flies caught at the tip showed no preponderance of females such as might, *a priori*, be expected from the analogy of some other Muscid flies. In the case of *Homalomyia*, all of the first lot were males, which may encourage the presumption that they were bred on the spot.

Finally, the tip was shown in its true light as a centre of distribution by an examination of the fresh refuse brought up in the barges. This very frequently contained great numbers of larvæ and pupæ, and of these a considerable proportion developed into flies after the refuse was deposited, although it was usually covered with a layer of damped road sweepings three or four inches in depth.

Together with Drs. Pattin and Nash, Medical Officers of Health for the City of Norwich and the County of Norfolk respectively, and Mr. Collins, the City Engineer, two of us visited and examined the site of the refuse deposit in question on the same date (July 14th, 1910) as that on which a first inspection of the village was made. Possibly for the reason that the day was cool, with a breeze blowing, and because more care had recently been taken to cover the refuse with a layer of soil and road-scrapings, the deposit, although representing many thousands of tons of refuse spread over a large area, was not specially offensive. Neither, on this occasion, were flies present in large numbers, except in the workmen's hut adjoining the refuse deposit, in which they swarmed, doubtless attracted by the warmth of the stove inside the hut, and by the remains of food and drink, especially sugar and beer.

Of these flies a number were caught with the help of a butterfly-net. These, together with a "balloon-trap" and its contents from the smithy cottage in the village, a long "tape" with its "catch" from the kitchen of Postwick Hall and a few larvæ and pupæ were subsequently forwarded to Mr. E. E. Austen, of the British Museum (Natural History), with a request that he would identify the particular species of fly present in each instance.

In order to determine definitely the relationship, if any, between the flies found at the refuse deposit and those in the village of Postwick, and so incidentally, the range of flight of these flies, large numbers of flies, caught in the workman's hut at the refuse deposit, were again liberated from the wharf alongside the river after having been marked in such a manner that they remained recognisable for several days afterwards. This marking was carried out by shaking the net, in which they were caught, in a stout paper bag containing a small quantity of finely-powdered coloured chalk (using, as a rule, a different colour each day, yellow and red being found to be the best) after the fashion suggested by Mr. Jepson (2nd Report on "Flies as Carriers of Infection," N.S. No. 16, pp. 4–9).

After a few preliminary trials with coloured flies, which showed that when thus liberated at the tip some of them subsequently appeared at the village (if the wind was not unfavourable), careful observations were made to determine, more accurately, the rate and direction of flight. Particulars of three successful flights are given in the table. (*Vide* page 10 of this Report.)

During the period over which these experiments extended the work was much hampered on occasion by the meteorological conditions which were, for the most part, unfavourable, the temperature remaining low, while rain showers were frequent, and high winds often prevailed.

Nevertheless, on each occasion on which several hundred marked flies were liberated, a certain number were subsequently recovered, within forty-eight hours or less, from human habitations in Postwick, at points of the compass which were apparently dependent, for the most part, on the direction of the prevailing wind, and at distances ranging from 300 yards to 1,700 yards (World's End Public House, Bramerton) from the refuse deposit. On one particular occasion on which chalk of a bright canary yellow colour was employed for marking the flies, the day being fine and sunny, with a gentle north-west breeze, the results obtained were specially interesting. Of these flies several were observed and captured by us along the stretch of river-bank between a point opposite the refuse heap and Postwick Hall, within half an hour of their liberation. Two other marked flies were caught in a large open-fronted summer-house on the lawn of Postwick Hall—a distance of 800 yards in a direct line from the point at which they were set free—one 35 minutes, and another 45 minutes after being liberated. Within the next four days, more than forty of these yellow-coloured flies were caught, on hanging fly-papers, in the kitchen and out-buildings at Postwick Hall, while isolated specimens were also trapped in various parts of the village, at greater distances, and at different points of the compass, from the refuse deposit. Of the marked flies caught on "tangle-foot" papers and tapes a certain proportion probably were overlooked, owing to the fact that the colour of the chalk soon gets considerably obscured by the glutinous material becoming spread over the bodies of the flies in their struggles to free themselves.

On the accompanying map, sketched from the 6-inch ordnance survey, the various points at which marked flies were caught during the progress of these experiments are indicated.

Many of our marked flies were seized and devoured by swallows, a number of which often appeared flying up and down the reach of the river, opposite the wharf-staging at the refuse deposit, shortly after the commencement of an experiment, even if there had been none to be seen just previously. According to local testimony swallows had been noticed in the village during the past summer in quite exceptional numbers, doubtless attracted by the excessive prevalence of flies. But it is interesting, from the biological point of view, that they should readily take flies of a brilliant canary-yellow colour such as they can never have seen before. A few of these coloured flies that happened to drop into the water were also seen to fall a prey to fish.

For the purpose of our experimental investigation these yellow-coloured flies proved most satisfactory. But owing to the fact that marked flies, after a longer or shorter interval of time subsequent to being freed, usually succeeded in removing the colour from their wings, eyes, legs and—except on the thorax—to some degree from their bodies also, they then presented the appearance of having been lightly dusted with pollen. And, although the house-fly rarely visits flowers, it appeared desirable, in order to meet possible criticism, to examine under the microscope the body surface, more especially the thorax, of some of these coloured flies which subsequently had been caught by means of traps in various situations in the village during the progress of our experiments. By this method of examination it was easy to see that the particles of coloured material on the bodies of these flies did not consist of pollen, but were of a mineral nature. On the thorax and body of a fly, the colouring material, under favourable circumstances, may remain perfectly recognisable for a week, or, on occasion, for as long as ten days.

As the outcome of these experiments we have not only obtained evidence, not previously available, as to the range and rapidity of the flight of flies between their breeding place and human dwellings at a distance, but, in the particular case under consideration, proof has been afforded that flies, captured in the village of Postwick, have made their way thither from the refuse deposit on the Whitlingham Marshes, notwithstanding the fact that the river Yare and, at Postwick Grove, a hill of moderate elevation, intervene.

S. Monckton Copeman.
F. M. Howlett.
Gordon Merriman.

TABLE giving details of Three Experiments on RANGE OF FLIGHT OF FLIES, at Postwick.

Date.	Wind.	Weather.	Time of liberation.	Colour.	Number of flies liberated (approx.).	Date of recovery of flies.	Number of flies recovered.	Where observed.	Direction of flight.	Distance travelled in yards.
Aug. 20	S. to S. W., Moderate.	Dull	12 to 2 p.m.	Blue	2,000	Aug. 21	1	Observed over river (S.M.C.) ...	S.	400
			3 to 4 p.m.	Blue	500	,, 22	1	Observed 4 p.m.	E.	1,000
				Orange	500	,, 21	1	Observed in church, 11.30 a.m....	E.N.E.	1,085
						,, ,,	1	Caught in Rectory Cottage, 5 p.m.	E.	980
,, 21	S.W. to W., Strong.	Bright	4 p.m.	Orange	1,500	,, 22	1	Caught at Rectory Cottage on fly paper, 1 p.m.	E.	980
						,, ,,	1	Caught in summer house, Postwick Hall, 3 p.m.	S.S.E.	800
						,, 23	2	Caught at Postwick Hall, 12 noon.	S.S.E.	850
						,, ,,	1	Caught at Bungalow adjoining Heath Farm.	N.N.E.	1,408
,, 22	N.W., Moderate breeze.	Bright	3 p.m.	Yellow	2,000	,, 22	4	On river bank opposite refuse deposit.	S.E.	300–700
						,, ,,	2	Caught in summer house, Postwick Hall, 3.35 p.m. and 3.45 p.m.	S.S.E.	800
						,, 23	10	Caught at Postwick Hall ...	S.S.E.	850
						,, ,,	1	Caught at Appleton's Cottage ...	E	1,050
						,, ,,	1	Caught at The Lodge	E.N.E.	1,027
						,, ,,	1	Caught at Bungalow, Heath Farm	N.E.	1,173
						,, 24	2	Caught at Post Office	E.	997
						,, ,,	1	Caught at Lodge Farm	E.N.E.	1,027
						,, ,,	6	Caught at Postwick Hall ...	S.S.E.	850
						,, ,,	1	Caught at Rectory Cottage ...	E.	980
						,, 25	1	Caught at Heath Farm	N.N.E.	1,408
						,, 25 and 26.	24	Caught at Postwick Hall (house and out-buildings).	S.S.E.	850

2.—Memorandum on the Result of Examinations of Flies, etc., from Postwick Village and Refuse Deposit; with A Note on the Occurrence of the Lesser House-Fly at Leeds. By Ernest E. Austen, F.Z.S.

The flies, etc., from Norwich and Postwick, forwarded July 15th, duly reached me on the 16th instant. Below, I give the result of my examination of this material, which, for convenience sake, I refer to under the receptacles in which the different portions of it were contained.

The net (flies caught at the refuse deposit) enclosed some 300 *Musca domestica*, and about 20 *Fannia* (*Homalomyia*) *canicularis*—the latter all males.

Tubes:—

"Workman's hut at refuse-heap. July 14th"—
3 *Musca domestica* (2 males), 1 female (latter arrived alive, males both dead).
1 *Fannia* (*Homalomyia*) *canicularis:* dead.

"Fishergate Depôt, Norwich, July 14th, 1910"—
1 *Musca domestica*, female (alive).

"Postwick Hall, dining room, July 14th, 1910"—
2 *Stomoxys calcitrans*, L. (both dead).

Boxes:—

"From refuse, fresh deposited, July 14th"—
2 live larvæ, not *Musca domestica* or *Fannia*, probably *Calliphora* or *Sarcophaga*.

"Dead flies from workman's hut, July 14th"—
3 *Fannia canicularis* (all males).
1 *Musca domestica* (female).

"July 14th, 1910, from portion of heap deposited on July 11th"—
1 larva, 2 pupæ, not *Musca* or *Fannia*.
? *Stomoxys calcitrans*.

I may add that, as regards the material forwarded, on July 14th, by Mr. A. E. Collins, City Engineer, Norwich, the flies in a balloon trap, from the Smithy Cottage, Postwick, and those on a "tangle-foot" tape, from Postwick Hall Farm, were all *Musca domestica*.

Under ordinary circumstances *Musca domestica* does not begin to be numerous until August, and in the course of last week, on fly-papers sent to me from Leeds (*see* Note appended), *M. domestica* was outnumbered by *F. canicularis* in the ratio of about 8 to 1. I am, therefore, decidedly of opinion that the excessive predominance of *M. domestica* at Postwick and in the vicinity (*see* "The net" paragraph above) points to the existence of some unusual condition, which has favoured the breeding of the Common House Fly.

E. E. Austen.

A Note on the Occurrence of the Lesser House-Fly (*Fannia canicularis*, L.). By Ernest E. Austen, F.Z.S.

In the course of last year (1910) interesting evidence was received from Leeds, illustrating the well-known fact that the true house-fly (*Musca domestica*) season does not, as a rule, commence until the latter part of the summer. By direction of Dr. J. Spottiswoode Cameron, Medical Officer of Health, a series of fly papers (five in all) was exposed in the kitchen of a certain house in Leeds for varying periods during the two months commencing on May 19th and ending on July 18th. On removal, the papers were forwarded to the writer for examination, the result of which is shown in the appended table :—

Paper No.	Exposed.	Removed.	Flies captured.	
			Fannia canicularis, L.	*Musca domestica*, L.
I.	May 19.	May 26.	112	1
II.	?	June 18.	Number uncertain owing to condition, but all were *F. canicularis*.	None.
III.	July 7, 10.15 a.m.	July 11, 5.0 p.m.	70	18
IV.	July 11, 5.0 p.m.	July 14, 5.0 p.m.	92	12
V.	July 14, 2.30 p.m.	July 18, 3.0 p.m.	107	17

Total of flies captured { 48 *M. domestica*.
381* *F. canicularis*.

* This number should really be greater, since the flies on Paper II. were not counted.

Making an allowance for Paper II, and assuming that the five papers afford a fair average indication of the flies constantly present in this particular kitchen during the period covered, it will be seen from the above table that, from May 19th to July 18th, 1910, *Fannia canicularis* outnumbered *Musca domestica* in the ratio of about 8 or 9·5 to 1.

The kitchen used for the experiment abuts on a row of livery-stables, and therefore, since ammoniacal odours are strongly attractive to *Fannia canicularis*, the relatively large numbers of this fly are perhaps not difficult to understand. In the immediate vicinity, however, there are also two manure-pits, which, although emptied at frequent intervals, would be likely to cause an assemblage of *Musca domestica* (if present), and would thus tend to equalise the conditions.

E. E. Austen.

3.—On the Part Played by Flies in the Dispersal of the Eggs of Parasitic Worms. By William Nicoll, M.A., D.Sc., M.B., F.Z.S., Ernest Hart Memorial Scholar of the British Medical Association (from the Lister Institute of Preventive Medicine, London).

The somewhat scanty literature bearing on this subject has been summarised by Nuttall (1899), Nuttall and Jepson (1909), Hewitt (1909), and most recently by Galli-Valerio (1910). The greater part of it deals only with vague surmises, unsupported by actual observation or experiment. The only experimental observations which have hitherto been made are those by Grassi (1883), Stiles (1889), Galli-Valerio (1905), Calandruccio (1906), and Léon (1908).

It was early recognised that the house-fly is an insect with a penchant for visiting excremental matters for the purpose both of feeding and breeding. The fact, too, did not escape observation that the body and legs of the fly, by reason of their numerous bristles, are peculiarly adapted for carrying any débris which might adhere to them. It was a natural suggestion, therefore, that the eggs of parasitic worms, deposited in excrement, might adhere to flies and so be transported by them from place to place. A more secure and certain means of transport, however, was pointed out by Grassi, who demonstrated that flies could actually ingest the eggs of parasitic worms and carry them in this way for a considerable distance. He further showed that such eggs, when ingested by a fly, could pass through its intestine and be deposited in its fæces, apparently unaltered and undamaged. To this experiment Calandruccio has added an important observation showing that the eggs, after their passage through the fly, remained infective. A number of flies which had fed on infective material containing the eggs of a tapeworm (*Hymenolepis nana*) subsequently deposited a few of those eggs on sugar. A girl who had eaten some of this sugar was found twenty-seven days later to be infected with the tapeworm. The parasite is a very common one in southern Italy, but in this particular instance infection through other sources was carefully excluded, so that the observation appears to be perfectly trustworthy.

The observations of Galli-Valerio relate to the American hookworm (*Necator americanus*). (In his earlier paper he says *Ankylostoma duodenale*.) He found that flies could carry on the surface of their body not only the eggs but also the larvæ of this dangerous parasite. Neither eggs nor larvæ were found in the intestine of the flies.

Léon's experiment consisted in feeding flies on honey, with which were mixed the eggs of the tapeworm, *Dibothriocephalus latus*. He was able to recover the eggs from the excrement of the flies.

These comprise all the actual observations which have, so far, been published on this subject. The result of an experiment of a somewhat different nature was mentioned by Stiles in a personal communication to Nuttall. He allowed fly-larvæ to feed on egg-bearing female round-worms (*Ascaris lumbricoides*). On examining some of these larvæ he found eggs in their intestine. The remaining larvæ were allowed to develop into flies, which, on examination, also showed not only eggs but larval worms in their intestine. This possibly indicates an additional method by which flies may serve to disseminate the eggs of worm-parasites.

To sum up: The work of these observers has afforded more or less definite information on the following points :—

1. That flies can carry the eggs and larvæ of *Ankylostoma duodenale* (or *Necator americanus*) externally.
2. That they can ingest the eggs of *Taenia solium*, *Hymenolepis nana*, *Oxyuris vermicularis*, *Trichuris trichiurus*, and *Dibothriocephalus latus*.
3. That these eggs may pass through the flies' intestine and be deposited in their fæces.
4. That the ova of *Hymenolepis nana* may thereafter give rise to infection.
5. That fly-larvæ can ingest the eggs of *Ascaris lumbricoides*, and that the latter may undergo a certain amount of development in the fully-formed fly.

So far as they go these observations deal with the main points of the problem, but they are obviously too isolated to permit of general conclusions being drawn from them. The series of experiments about to be described is more or less preliminary in character and is far from being a complete treatment of the subject. Several points, however, are dealt with, which have hitherto not received attention.

Ten different species of parasites were experimented with, namely, the tapeworms *Taenia serrata*, *Taenia marginata*, and *Dipylidium caninum*, from the dog, *Hymenolepis diminuta* from the rat, *Moniezia expansa* from the ox, and the Nematodes *Trichuris trichiurus* from man, *Toxascaris limbata* and *Ankylostoma caninum* from the dog and *Sclerostomum equinum* and *Ascaris megalocephala* from the horse. Of these only *Trichuris trichiurus*, *Dipylidium caninum*, *Hymenolepis diminuta*, and *Toxascaris limbata* are human parasites. In certain respects the more extensive use of human parasites might have been desirable, but it was found impossible to rely on a supply of such material, and from a practical point of view the species employed served the purpose. *Taenia serrata* and *Taenia marginata* are two of the commonest parasites of the dog in this country, and they bear a very close resemblance to *Taenia solium* of man. The eggs of the three species are almost indistinguishable. The ankylostome of the dog is very like that of man, and the eggs of the two species differ only to a slight extent. *Moniezia expansa* is a common parasite of the ox and sheep, while *Sclerostomum equinum* and *Ascaris megalocephala* are frequently found in horses.

The main object of the experiments was to ascertain (*a*) to what extent, and (*b*) for how long a period flies could carry eggs by actually ingesting them and retaining them within their intestine, and (*c*) how great was the partiality they displayed towards feeding on infective material. Less attention was paid to transport of eggs on the body and legs of the fly which, although possibly the more usual means, is not likely to be of the same far-reaching consequence.

Nature and Life-History of Parasitic Worms.

In order to indicate the precise relationship which flies and other insects may bear to the dissemination of parasitic worms, it may be advisable here to recapitulate briefly the principal facts which are known concerning the mode of life and means of transmission of these worms. They belong to three principal classes, namely, Trematodes or flukes, Cestodes or tapeworms, and Nematodes or round-worms. These worms in their adult state live for the most part in the alimentary canal of vertebrate animals. Practically speaking all tapeworms live in the intestine, the great majority of round-worms and flukes live in the intestine, stomach or œsophagus, but certain varieties live in the lungs, the liver, the kidneys, the bladder and the blood and lymphatic vessels. In whatever situation they live, however, they all possess the common characteristic of being unable to multiply without some intermediate external influence. They all produce a large number (in many cases an enormous number) of eggs, but before the latter can grow into adult worms, they must pass part of their life outside their host under certain definite conditions which vary according to the particular kind of worm. In most cases the eggs are conveyed out of the body in the fæces, in some cases in the urine or the expectoration. Some tapeworms throw off a segment containing eggs, which may pass out independently of the fæces. The chief exceptions to this method are the *Filaria* worms and their allies. With these, however, the present investigation is not concerned. The conveyance of the eggs to the outside is only a short stage in the life of the parasite. Thereafter a more or less lengthy and complicated career awaits them before they are suited to reinfect their original host. This portion of their life history follows one of two broad lines: 1. The egg develops and in time produces a larva, which may be retained in the egg shell or set free, but in either case the larva is ready to reinfect. 2. The egg gives rise to a larva which enters an animal (intermediate host) different from that in which the egg was produced. In this second animal it passes part of its life and, in the event of this animal being devoured by the first, the parasite is enabled to complete its life-cycle and cause infection again.

The avenue by which the first animal is infected is in the great majority of cases its food; in a few instances it may be infected by the larva penetrating its skin. It is evident, therefore, that there must be some means by which the food is contaminated with excremental matter in the first of the above-mentioned categories and, in the second, some means by which the eggs of the parasite are conveyed to the second animal. Water is by far the most important vehicle of transit. A certain amount of moisture is necessary for the development of the eggs although many of them can resist drying for long periods. In closely-associated communities, however, mechanical transit on the feet of individual animals is a common mode of dispersing the eggs. It is here that the agency of flies has to be reckoned with, especially such flies as divide their attention between excremental matter and food-stuffs. The common house-fly is well known to have such habits and it is thus with reason that it has been suspected of conveying the eggs of parasites from fæcal material to food. There are many flies, other than *Musca domestica*, which display like habits and though they do not bear such a close relationship to man several of them are commonly associated with the domesticated animals.

With regard to their life-history the parasites of man may be divided into two classes, excluding the *Filaria* worms, and mentioning only the best known species, as follows :—

I. Those not requiring an intermediate host.

a. Those in which the larval worm remains within the egg-shell.

Ascaris lumbricoides.
Toxascaris limbata.
Belascaris mystax.
Oxyuris vermicularis.
Trichuris trichiurus.
Hymenolepis nana.

b. Those in which the larva is liberated from the egg-shell and spends its life in water.

Ankylostoma duodenale.
Necator americanus.
Schistosomum haematobium.
Schistosomum japonicum.

II. Those requiring an intermediate host.

a. Those encysting in animals which are commonly eaten by man.

Trichinella spiralis in the pig.
Taenia solium in the pig.
Taenia saginata in the ox.
Dibothriocephalus latus in fresh-water fishes.

b. Those encysting in animals which may be swallowed accidentally by man.

Fasciola hepatica in pond snails and eventually on grass.
Dicrocoelium lanceatum in slugs and pond snails.
Hymenolepis diminuta in fleas and other insects.
Dipylidium caninum in fleas.

There are several other common human parasites of which the life history is entirely unknown. Of those in the above list, the mode of infection of *Schistosomum haematobium* has not been definitely proved, but it is probably similar to that of *Schistosomum japonicum*, the free-swimming larva of which is capable of infecting directly. *Trichinella spiralis* is peculiar in being viviparous and its larvæ are shed into the blood stream or the lymphatics. The house-fly can therefore take no part in its dissemination. The species in II. b. are, as might be expected, but very occasional parasites of man.

In addition to the above-mentioned worms of which man is the final or adult host, there are a few others for which man figures as the intermediate host. By far the most important of these is *Taenia echinococcus*, a tapeworm of the dog, the larva of which gives rise to hydatid disease. Man is also an intermediate host of *Taenia solium* and *Trichinella spiralis*.

It is evident from this short summary of the life histories that the only parasites with which it is possible that flies can directly infect man are those in Section I., along with *Taenia echinococcus* and *Taenia solium*. In the other cases and in the case of *Taenia solium* also, the infection is conveyed to some other animal, *e.g.*, pig, ox, etc. The nature of the problem is therefore not the same in every instance.

The Nature and Size of the Eggs of Parasitic Worms.

These factors have an important bearing on the present question, for it is obvious the eggs must bear some definite proportion to the vehicle which carries them. In most cases the eggs are ovoid in shape. Sometimes they are more elongated, and become almost spindle-shaped. Frequently they are nearly globular. Occasionally they are cuboid while a few other shapes occur more rarely. Appendages are not uncommon. They may take the form of slight roughnesses or small knobs scattered irregularly over the surface of the shell. There may be a small spike projecting from one end, or from the side. In some cases one end of the shell tapers to a point and is prolonged as a spiral filament, which may be much longer than the egg itself. In certain other cases there may be button-like projections from either end of the shell.

The shell may be of various degrees of thickness and hardness. In some cases it is quite thin and transparent, in others it is much thicker and opaque. In most cases it possesses a considerable amount of flexibility so that it can be compressed to a certain extent without breaking. In this way a globular egg can be pressed into an ovoid shape. This is of importance in relation to the ingestion of eggs by flies.

The general surface of the shell may be either smooth or slightly roughened. It is usually covered by a layer of mucus when it is being extruded by the worm, and it may receive an additional coating of mucus in the intestine of the host. This imparts to it a certain degree of adhesiveness. In some cases the eggs are laid in batches surrounded by a gelatinous or mucous investment.

The size of the eggs varies in different worms. They are rarely smaller than ·01 mm. or larger than ·15 mm. in length. The breadth is most commonly half to two-thirds the length. Even in the same worm the eggs vary considerably in size although they usually approximate towards a definite size and shape, which is more or less characteristic for the species. On that account the eggs of most species of human parasites can be recognised very readily. In the following list the parasites of man are arranged according to the size of their ova, the sizes given being approximate averages. They are divided into three sections, the first of which contains those whose eggs do not exceed ·045 mm. in both diameters, the second comprises those with eggs of which the breadth is less than ·045 mm., while in the third the minimum diameter exceeds ·045 mm. The reason for this division is, as will appear later, that *Musca domestica* is apparently unable to ingest particles of larger size than about ·045 mm.

		Length.	Breadth.
I.	Opisthorcis felineus	·030	·010
	Clonorchis sinensis	·025	·015
	Heterophyes heterophyes	·030	·015
	Opisthorcis noverca	·035	·020
	Oxyuris vermicularis	·050	·020
	Taenia saginata	·035	·025
	Trichuris trichiurus	·050	·025
	Taenia solium	·035	·030
	Taenia echinococcus	·035	·030
	Dicrocoelium lanceatum	·040	·030
	Hymenolepis nana	·040	·040
	Dipylidium caninum	·040	·040
	Davainea madagascarensis	·040	·040
II.	Ankylostoma duodenale	·060	·040
	Ternidens deminutus	·060	·040
	Necator americanus	·065	·040
	Trichostrongylus subtilis	·065	·040
	Schistosomum japonicum	·075	·040
	Ascaris lumbricoides	·060	·045
	Eustrongylus gigas	·065	·045
	Dibothriocephalus latus	·070	·045
	Schistosomum haematobium	·115	·045
III.	Diplogonoporus grandis	·065	·050
	Dibothriocephalus cordatus	·075	·050
	Hymenolepis diminuta	·070	·065
	Paragonimus westermanni	·090	·065
	Belascaris mystax	·075	·070
	Toxascaris limbata	·080	·070
	Gastrodiscus hominis	·150	·070
	Fasciolopsis buski	·125	·075
	Fasciola hepatica	·130	·080

The resistance of eggs to external conditions is also of importance. All do not withstand similar conditions equally well. For many, moisture is absolutely essential, and in its absence they rapidly die. This is true for the eggs of practically all Trematode worms. Many of these can survive only in sea-water, others only in fresh water, and changes in the composition of the water affect them injuriously. Drying is usually fatal within a few hours; the shell becomes crumpled and shrivelled up, the embryo dies and the subsequent application of moisture does not resuscitate it. On the other hand, eggs of some tapeworms and round-worms can survive desiccation for comparatively lengthy periods. For instance, I have kept the eggs of *Hymenolepis diminuta* in dry powdered fæces for as long as 17 days, at the end of which time many of them were still alive. In the presence of even a small amount of moisture, other conditions being suitable, the eggs of most parasitic worms will remain alive for a great length of time. Thus it is stated by Davaine that the eggs of *Trichuris trichiurus* may remain alive for as long as five years. With regard to other conditions, temperature is probably the most important. A fairly high temperature (80–90° F.) hastens development, but temperature much above that will destroy the eggs in many cases. Continued exposure to cold is also fatal, although freezing, if not too prolonged, is not necessarily fatal. Little information, however, is available on this point except in regard to a few species.

The Feeding of Flies and their Larvæ in Relation to the Ingestion of Eggs.

The observations which I have made on the general feeding habits of flies correspond to a great extent with those of Graham-Smith (1910, p. 5–11), and need not be detailed here. It is necessary, however, to refer to some points which have a direct bearing on the present investigation and which were not touched upon by Graham-Smith.

It is a matter of common observation that fresh and moist fæces attract flies much more readily than old and dried fæces. Flies feed on warm fresh fæces with considerable avidity, and they will do so even although they have been previously feeding on other material. To flies which have not fed for some time the presence of fresh human fæces acts as an immediate source of attraction, and in some of my experiments the eagerness with which they attacked it was most striking. When the portion of fæces was so small that all the flies could not find standing room upon it or around it, they struggled together and pushed each other aside, and more than once I have seen them so closely packed together that each fly could find room for only the tip of its proboscis, the flies on top practically "standing on their head," supported by the bodies of those around. Their behaviour towards older fæces, however, is very different. When the material has become cold it does not attract flies nearly so readily. So long as it remains moist it continues to attract and does so quite as much as moist bread, although very much less so than moist sugar. When it has become dry it possesses little or no attraction, but this is increased when it is moistened again. It is evident therefore that the presence of moisture plays an important part in a fly's attitude towards fæces as an article of food.

When the alternatives of fresh fæces, sugar and bread were offered, the flies did not confine their attention to any one of these articles, but made repeated excursions from one to the other.

Some interesting observations were made in regard to flies feeding on segments of tapeworms. As already mentioned, such segments may be deposited along with fæces or independently, and in the case of several tapeworms their eggs are conveyed to the exterior in this way instead of being shed singly into the gut. In the course of the present experiments it was rather surprising to find that such segments possessed a great attraction for flies. When an intact segment of a tapeworm (*Taenia serrata*, *Taenia marginata*, *Dipylidium caninum*) mixed with moderately fresh fæces was presented to some flies they appeared to select the tapeworm and feed upon it in preference to the fæces. The observation was made on several occasions. Further when an isolated tapeworm segment, some fæces and some sugar, were separately introduced into the fly cage, the flies showed a decided preference for the tapeworm, which they attacked with much assiduity. This trait was displayed not only when the tapeworm was in a fresh state, but even when it had lain for a day or longer. Tapeworms usually possess a faint characteristic, musty odour, but whether it is this or simply the juicy nature of the body which proves the attraction, it is, at present, impossible to say.

The action of flies feeding on tapeworms was studied in close detail. Applying their proboscis to the surface of the worm they suck with considerable vigour for as long as half a minute on end. Having selected a spot they continue there for some time. From time to time a small drop of fluid was seen to be extruded from the proboscis, and this apparently was used to moisten the surface of the worm. The vigorous sucking efforts were kept up, with intermissions, for several hours. Flies examined within two or three hours after the beginning of such an experiment were found to have very little fluid in their crop, which contained, however, numerous large bubbles of air. Later, when the flies had been feeding on the tapeworm for 5—10 hours their crop was found greatly distended with white milky juice recognisable as the juice of the tapeworm. In such flies, too, several tapeworm eggs were found in the intestine. It is evident, therefore, that house-flies, although they possess no piercing or biting mouth-parts, are able in course of time to penetrate the fairly tough external covering of tapeworms and to extract the internal contents. In this they are helped to a considerable extent by the fact that tapeworms undergo a process of decomposition (autolysis) independent of putrefaction. This is further hastened by the action of putrefactive bacteria. Dead tapeworms will remain soft and "juicy" for 48 hours after exposure to the air. Later their fluids evaporate and they begin to shrivel up and become dark brown in colour. Living tapeworms or their segments will remain alive on exposure to air for two or three days, and in suitable media (saline solution, &c.), they may be kept alive for over a week. There can be little doubt, therefore, that living tapeworm segments when expelled from their host may remain a source of attraction to flies for several days.

Similar observations were made in the case of round-worms (*Toxascaris limbata*, *Ascaris megalocephala*). These appeared to possess much less attraction for flies. Not infrequently they were attacked with some readiness and in the same manner as tapeworms. The extremely thick cuticular investment of round-worms, however, is much more resistant than the covering of tapeworms, and in no case were the flies able to penetrate this even after the lapse of three or four days, by which time the worms had become dry and shrivelled up.

It seems worthy of note that solid particles were rarely found in the crop of flies dissected in the course of these experiments. The intestine, however, except in flies which have been feeding for several days on nothing but fluid food, invariably contains a large number of particles. After feeding on fæces, for instance, the intestine becomes filled with the debris of which the fæces are composed, but none of this is found in the crop. The particles met with are of various sizes and irregular shapes, but they rarely exceed ·04 mm. in diameter. It would thus appear that when flies feed on fluids or soluble solids such as sugar, the food is first sucked into the crop, and when this is full it passes into the intestine directly, as noted by Graham-Smith. Insoluble solid food, however, is probably taken directly into the intestine. This suggests that the fly is able to exercise some voluntary control over the passage of food into the crop or the intestine respectively.

The feeding of fly larvæ was studied specially in regard to their behaviour towards round worms. The voracity with which larval flies feed is well known, and the increase in their size from day to day is remarkable. They appear to be as omnivorous as adult flies. When fresh round-worms were offered to the larvæ, they were at once attacked. The larvæ swarmed over the worms, nibbling at them with great vigour. They seemed, however, quite unable to penetrate the tough cuticle unless there were a crack or a small tear, and, if other food were not provided, the larvæ died. On the other hand, when the worms were cut or broken before being introduced, the larvæ devoured the internal parts with extraordinary rapidity. Starting at one end of a broken piece, they would eat their way right through to the other end leaving nothing but a tube of cuticle. In this way half a dozen larvæ would devour, within two or three days, a large worm 20 or 30 times their own bulk. On examining larvæ which had fed on female egg-bearing worms, large numbers of eggs were found surrounding the larvæ but not actually sticking to them. On examining the intestine of the larvæ no intact eggs were ever found, but numerous fragments of shells were always recognisable. No embryonic worms in any stage were seen. From these experiments I am convinced that even full-grown larvæ are unable to swallow unruptured eggs as large as those of the worms used (·07 mm.).

Methods.

The common house-fly, *Musca domestica*, was used almost exclusively. Only a few experiments were made with the lesser house-fly, *Fannia canicularis*, and the blow-fly, *Calliphora erythrocephala*. In most cases the flies were obtained from the surrounding locality, but artificially-reared flies were used on several occasions.

For the experiments a large cage was constructed. All the sides were made of perforated zinc, except one, which consisted of a large sheet of plate-glass; this was fixed in two grooves and could be removed. The bottom was of plain zinc. The dimensions of the cage were 3 ft. × 1½ ft. × 1 ft. It was divided into two by a partition of perforated zinc in which was a sliding panel, by which communication could be made between the two compartments. Each compartment was further furnished with a sliding door at the bottom of one side for the admission of flies, and another door at the top, through which they could be removed. For the latter purpose a small square cage with a sliding door was used. This fitted the door in the top of the large cage, and the two sliding panels could be drawn out simultaneously. The object of the cage was to afford the flies as much space, light and air as possible, and it was found that they could be kept alive in it for over a month. The plate-glass side was of use in allowing the experiments to be accurately watched. A few experiments were conducted in a large bell-jar, and in a considerable number of cases glass chimneys, similar to those described by Graham-Smith (1910) were employed.

Infective material was offered to the flies in four different ways: 1. Fæces containing ova. 2. Complete worms or intact parts of them. 3. Broken or damaged segments of worms. 4. Suspensions of ova in water.

The experiments were commenced in the middle of November, 1909, and were continued without intermission throughout the whole winter and the following year. No difficulty was experienced in obtaining an adequate supply of flies during the winter from the warmer parts of the Institute, such as the animal house and the crematorium, except on a few of the colder days in March and April, 1910.

Experimental Observations.

The experiments which are detailed here represent only a small proportion of those actually performed. Each experiment was repeated at least once, and in most cases several times. In some the flies were allowed to feed on the infective material throughout the whole experiment; in others the material was removed after a certain length of time.

The flies which were removed for examination were killed with chloroform vapour. Their bodies and legs were then examined for eggs. The legs and wings having been removed, the body was carefully washed to get rid of any adhering eggs. The intestine, ventriculus and crop were then dissected out and examined separately. No eggs were ever found in the crop, so that the positive results in the following records refer to the intestine or ventriculus only.

The figures in brackets in the positive cases refer to the number of eggs found in each fly.

I.—*Feeding Experiments with Adult Flies.* (*M. domestica, unless otherwise stated.*)

a. Hymenolepis diminuta. (Ova ·07 by ·065 mm.)

1. Dec. 9th.—11 flies fed on contents of cæcum of rat, containing numerous eggs.

„ 11th.—4 flies examined. No ova in intestine or crop.
Fæces of remaining flies examined. Negative.

„ 13th.—3 flies examined ... ... „
Fæces of remaining flies examined „

„ 14th.—2 flies examined ... ... „
2 flies examined ... ... „

2. Feb. 14th.—12 flies fed on fæces of rat containing numerous ova.
  „ 15th.—3 flies examined ... ... Negative.
    Fæces of remaining flies examined „
  „ 16th.—4 flies examined ... ... „
    Fæces of others examined ... „
  „ 17th.—5 flies examined ... ... „

3. Jan. 14th.—6 flies fed on ripe segments of *Hymenolepis diminuta*, containing numerous ova.
  6 hours later.—3 flies examined ... ... Negative.
  24 „ —3 flies „ ... ... „
    Fæces of flies examined ... „

4. Jan. 15th.—6 flies fed on emulsion of the tapeworm in water, containing numerous ova.
  2 hours later.—3 flies examined ... ... Negative.
    Fæces of other flies examined... „
  24 „ —2 flies examined ... ... „
  4 days later.—1 fly „ ... ... „

From this series it is evident that *Musca domestica* is quite unable to ingest eggs as large as those of *Hymenolepis diminuta*.

b. Toxascaris limbata. (Ova ·08 by ·07 mm.)

1. Dec. 3rd.—8 flies fed on dog fæces, containing numerous eggs.
  „ 4th.—Fæces of flies examined ... ... Negative.
    Flies all examined ... ... „

2. Mar. 22nd.—6 flies fed on intact female worm.
  „ 23rd.—2 flies examined ... ... Negative.
    Fæces of other flies examined ... „
  „ 24th.—4 flies examined ... ... „

3. Apr. 8th.—6 flies fed on broken female worm, containing numerous ova.
  2 hours later.—2 flies examined ... ... Negative.
    Fæces of other flies examined ... „
  10th.—4 flies examined ... ... „

c. Ankylostoma caninum. (Ova ·06 by ·04 mm.)

1. Dec. 3rd.—8 flies fed on dog fæces containing numerous ova.
  4th.—5 flies examined ... ... Negative.
    Fæces of other flies examined ... „
  5th.—3 flies examined ... . „

d. Trichuris trichiurus. (Ova ·05 by ·025 mm.)

1. Nov. 20th.—12 flies fed on human fæces containing a few ova.
  21st.—4 flies examined ... ... Negative.
    1 fly examined ... ... Positive (2).
    Fæces of other flies examined.
    1 Positive, rest ... ... Negative.
  22nd.—7 flies examined ... ... „

None of the samples of human fæces which I was able to obtain were very heavily charged with eggs of this parasite. The small number of eggs contained in the fæces reduced the chance of any being swallowed by the flies.

e. Dipylidium caninum. (Ova ·04 by ·04 mm.)

1. Feb. 21st.—3 p.m.—(4 *Musca*, 1 *Fannia*) fed on unbroken ripe segments.
   „ 22nd.—11 a.m.—Segments removed.
   1 *Fannia* examined .. Negative.
   6 p.m.—1 *Musca* „ ... Positive (3).
   7 p.m.—1 „ „ ... Negative.
   „ 23rd.— 1 p.m.—1 „ „ ... Positive (2).
   6 p.m.—1 „ „ ... Negative.

2. May 3rd.—10 a.m.—6 flies fed on dog fæces containing ova.
   4 p.m.—fæces removed.
   2 flies examined ... 1 Positive (3).
   „ 4th.— 3 p.m.—2 „ „ ... Negative.
   „ 5th.—11 a.m.—2 „ „ ... 1 Positive (1).

This series shows that flies can suck the eggs out of the tapeworm and that they can carry them for at least 43 hours in their intestine.

f. Taenia marginata. (Ova ·035 by ·035 mm.).

1. Mar. 14th.—1 fly (*Calliphora*) fed on mashed up segments containing numerous ova.
   „ 16th.—Examined ... ... Positive (1).

2. „ 11th.—11 flies (8 *Musca*, 3 *Fannia*) fed on ruptured segments containing numerous ova.
   6 hours later. 1 *Musca* and 1 *Fannia* examined ... ... Negative.
   Segments removed.
   14th.—1 *Musca* examined ... ... Positive (5).
   1 *Fannia* „ ... ... „ (2).
   1 *Musca* „ ... ... Negative.
   19th.—3 *Musca* „ ... ... „
   21st.—2 *Musca* and 1 *Fannia* examined „

3. Apr. 11th.—4 flies (1 *Musca*, 3 *Fannia*) fed on emulsion of ripe segments.
   12th.—1 *Musca* examined ... ... Positive (10).
   2 *Fannia* „ ... ... Negative.
   13th.—1 *Fannia* „ ... ... „

These experiments show that eggs of this size are fairly readily ingested not only by *Musca* but also by *Fannia canicularis*, and that they may be found in their intestine after an interval of nearly three days.

g. Taenia serrata. (Ova ·035 by ·035).

1. Feb. 18th, 2 p.m.—5 flies fed on emulsion of ripe segments in water.
   4 p.m.—3 flies examined ... Positive (500).
   „ 19th, 11 a.m.—1 fly „ ... Negative.
   5 p.m.—1 fly „ ... „

2. Feb. 22nd, 2 p.m.—7 flies fed on ruptured segments containing numerous ova.
4 p.m.—2 flies examined ... Positive (400).
„ 23rd, 12 noon.—2 flies „ ... Negative.
5 p.m.—1 fly „ ... „
„ 24th, 2 p.m.—1 fly „ ... Positive (2).
1 fly „ ... Negative.

3. Feb. 23rd, 2 p.m.—6 flies fed on intact segments containing numerous ova.
5 p.m.—2 flies examined ... Negative.
„ 24th, 11 a.m.—1 fly „ ... Positive (4).
4 p.m.—1 fly „ ... Negative.
„ 26th, 11 a.m.—3 flies „ ... „

4. Feb. 21st.—4 flies (3 *Musca*, 1 *Fannia*) fed on dried segments.
„ 22nd.—2 *Musca*, and 1 *Fannia* examined ... Negative.
„ 23rd.—1 *Musca* examined ... ... „

5. Mar. 3rd, 10.0 a.m.—7 flies fed on intact segments containing numerous ova.
12.30 p.m.—1 fly examined ... Negative.
3.0 p.m.—Segments removed.
6.0 p.m.—1 fly „ ... Positive (1).
7.0 p.m.—2 flies „ ... Negative.
„ 4th, 10.30 a.m.—1 fly „ ... „
Fæces of other flies examined ... Positive (1).
3.0 p.m.—1 fly „ ... Negative.
„ 5th, 11.0 a.m.—1 fly „ ... „

6. Mar. 3rd, 11.0 a.m.—6 flies fed on emulsion of segments containing numerous ova.
12.0 noon—Emulsion removed.
5.0 p.m.—1 fly examined ... Positive (1).
5.30 p.m.—1 fly „ ... „ (22).
7.0 p.m.—1 fly „ ... „ (312).
Fæces of other flies examined ... „ (200).
Rabbit (A) fed with ova recovered.
„ 4th, 11.0 a.m.—1 fly examined ... Positive (171).
Rabbit (B) fed with ova recovered.
6.0 p.m.—1 fly examined ... Negative.
„ 5th, 11.0 a.m.—1 fly „ ... Positive (1).
Fæces examined ... „ (4).
„ 15th.—Rabbit (A) dead. No cysticerci.
May 3rd.—Rabbit (B) killed. 23 *Cysticercus pisiformis* found in mesentery.

The rabbits were less than a month old and were obtained from an isolated stock. They were themselves removed and isolated as early as possible. They were, therefore, in all probability uninfected to start with, and the fact that Rabbit (A) was found uninfected supports this presumption. *Cysticercus pisiformis*, the larva of *Taenia serrata*, takes about four weeks to develop.

7. Mar. 9th.—10 flies fed on fæces containing ruptured mature segments. Piece of sugar also introduced. Fæces kept moist.

„ 11th.—Sugar examined ... ... Positive (4).
Another piece of sugar introduced.
„ 14th.—Sugar examined ... ... Negative.
„ 18th.—1 fly „ ... ... Positive (1).
1 fly „ ... ... „ (2).
„ 21st.—2 flies „ ... ... Negative.
Sugar „ ... ... Positive (3).
„ 22nd.—1 fly „ ... ... „ (2).
1 fly „ ... ... „ (1).
3 flies „ ... ... Negative.
„ 23rd.—1 fly „ ... ... Positive (10).

This constitutes the longest and most important series, and shows unmistakably that *Musca domestica* can quite readily ingest the ova of *Taenia serrata*, not only from fæces, but also from intact segments of the worm. Experiments 1 and 6 show that the eggs, when suspended in a liquid, may be ingested in enormous numbers. No. 6 also shows that large numbers can remain in the intestine of the fly for one or two days, without undergoing any visible change, and that they remain capable of infecting for at least one day. No. 7 shows the very important fact that fæces containing tape-worm segments may continue to be a source of infection, from which food such as sugar may be contaminated, for as long as a fortnight.

II.—*Feeding experiments with larval flies.*

a. With *Taenia serrata.*

1. Apr. 11th.—Several larvæ placed on dog fæces containing ripe segments.

„ 13th.—3 larvæ examined ... ... Negative.
3 „ „ ... ... Positive (5).
„ 15th.—2 „ „ ... ... Negative.
„ 17th.—2 „ „ ... ... „
„ 19th.—2 „ „ ... ... „

By this time the remaining larvæ had pupated. Several pupæ were examined with negative result. Two flies were hatched and on examination gave a negative result. The ova found in the larvæ on the 13th were in every case broken, and fragments of shell were found throughout the intestine.

b. With *Toxascaris limbata.*

1. May 12th.—Several larvæ put on dog fæces containing mature female worms.

„ 13th.—3 larvæ examined ... ... Negative.
„ 14th.—5 „ „ ... ... „
„ 17th.—2 „ „ ... ... „
Remaining larvæ pupated.
„ 18th.—2 pupæ examined ... ... „
„ 22nd.—3 flies emerged ... ... „

No evidence of embryos or larval worms could be detected.

c. With *Ascaris megalocephala.*

1. Oct. 25th.—Several larvæ put on horse fæces containing female worms with numerous eggs.		
„ 26th.—4 larvæ examined	... ...	Negative.
„ 27th.—3 „ „	... ...	„
„ 28th.—3 „ „	... ...	„
„ 29th.—2 „ „	... ...	„
Remaining larvæ pupated.		
„ 31st.—3 pupæ examined	... ...	„
Nov. 2nd.—2 flies emerged...	... ...	„

It is evident from these experiments, which were repeated several times, that the eggs are not transmitted through the larvæ to the fly. The results are entirely at variance with the already quoted observation of Stiles in the case of *Ascaris lumbricoides.* The larvæ and pupæ were kept at a fairly high temperature (77–80° F.). It may be noted that the eggs of *Ascaris lumbricoides* are slightly smaller than those of the two Ascarids used in those experiments, but they are much larger than those of *Taenia serrata.*

III.—*The body and legs of flies as carriers of eggs.*

As already mentioned, the surface of the body and the legs of the flies used in the foregoing experiments were always carefully examined before dissection. In addition, separate experiments were made to determine the length of time during which eggs might adhere. These were performed for the most part with the eggs of *Hymenolepis diminuta* and *Taenia serrata.*

In this connection the habits of flies immediately after feeding are worthy of study. When feeding even on very foul material flies do not usually soil the surface of their body to any great extent. When the food material is firm and fairly dry they alight upon it and walk all over it. The body, however, is kept well raised above the surface. When the material is sloppy and wet, flies are loth to venture upon it. They prefer to stand round about and feed at the edges. In this way only the proboscis and the first pair of legs come in contact with the material. With a material much to their liking, such as syrup, they are not infrequently tempted to venture too far in with the result that they may get their legs stuck together. In their struggles to extricate themselves the body is invariably very much soiled.

When they have finished feeding, flies usually walk a short distance away to a convenient dry spot, or, especially if disturbed, they fly off to the nearest place of safety. As far as I have been able to observe, they do not fly to any great distance under such circumstances. As soon as they have settled they proceed to clean themselves. The proboscis is passed along the anterior pair of legs; the legs are rubbed together briskly, and from time to time they are drawn over the eyes, with an action very similar to that of a cat in passing its paw behind its ear. The second and third pairs of legs are also rubbed against each other and the third pair are rubbed together in the same way as the first. The hind pair also vigorously brush the abdomen, belly and rump, the legs passing over the back in a scissor-like fashion and being drawn forward and backward. This process is usually continued for a considerable time and may be very lengthy if the fly has been badly soiled. It is undoubtedly a process of cleansing and by its vigorous application adhering particles of any size are quickly removed. On the other hand small sticky particles may be transferred from the proboscis to the legs, from one leg to the other and so to the surface of the body. In this way the body may to a certain extent become soiled, although it has not been in actual contact with the infective material.

There remains the further consideration of the *breeding habits* of flies in connection with contamination of their body-surface. Practically all kinds of excrement are made use of by flies as material in which to lay eggs. The latter are generally deposited not actually on the surface but at some distance below. On this account the body of the female fly is usually more soiled during ovo-position than while simply feeding. The larvæ wander all over the fæces but usually pupate in some dry place near the surface. According to Hewitt, however, it occasionally happens that the larva pupates below the surface, in which case the fly, on hatching, has to burrow its way out. It is obvious that under such circumstances its body must become grossly soiled.

From what has been said in regard to the habits of flies after feeding it must appear that foreign particles of large size are not likely to remain any length of time on the surface of the body or legs unless they are particularly adhesive. This supposition is confirmed by actual observation. On flies caught during the act of feeding, or immediately afterwards, I have found numerous particles of fairly large size, and in many instances the eggs of parasites as large as those of *Ascaris megalocephala*. These were found chiefly on the distal segments of the legs and on the proboscis. When, however, the flies were allowed to clean themselves before being examined, few particles were found and only occasionally were eggs observed. On examining the spot where the flies had rested while cleaning themselves eggs were found quite frequently. Apparently, therefore, the eggs are got rid of at the spot where the fly first alights after feeding. How far flies can convey eggs in this way depends on the distance they may traverse in their first flight after feeding. In the experimental cage I was only able to demonstrate this up to a distance of three feet.

There is the possibility, however, that some of the eggs which have been rubbed off may again adhere to the fly before it leaves its first resting place, and so be carried on to a second or even a third spot. To test this point I allowed a few flies, confined in a glass chimney, to feed on fæces containing eggs of *Hymenolepis diminuta*. After they had cleansed themselves, they were made to fly into a second clean chimney. Shortly afterwards they were transferred to a third and finally to a fourth vessel. Eggs were obtained on washing out the first and second chimneys, but none were got from the other two. Only a very few eggs were found in the second. In several other similar experiments eggs could not be found in the second chimney. The flies were all examined at the end of each experiment, and in two cases a single egg was found adhering to a fly which had been transferred to the third chimney.

In some experiments the flies were allowed to remain in the first or second chimneys for varying lengths of time. The longest interval after which eggs were found adhering to them was about three hours.

The possibility of flies in this way contaminating food was demonstrated by allowing some to feed on fæces containing eggs of *Hymenolepis diminuta*, and subsequently affording them access to some moist sugar placed at the other end of the experimental cage. After 24 hours the sugar was examined and found to contain a few eggs. Now, as has already been shown, the eggs of *Hymenolepis diminuta* are too large to be ingested by the fly, so that in this experiment they must have been carried on the legs or body.

In the case of such eggs as can be ingested, *e.g.*, those of *Taenia serrata*, it is possible that, on occasion, the legs of the fly may be contaminated from its own excrement at some time subsequent to the fly having fed on infective material. Experiments to determine this point were, however, not undertaken.

Summary and Conclusions.

The foregoing observations serve to confirm the results of previous workers, in particular those of Grassi and Calandruccio, and from them certain general conclusions may be drawn, with the reserve that they require corroboration from observations under natural conditions.

The well-known habit of the house fly of feeding in turn upon excremental matter and human food-stuffs, taken in conjunction with the fact that the spread of infection with parasitic worms depends in a large number of cases upon the dissemination of egg-laden excrement, constitutes a strong reason for suspecting the house-fly of aiding in the spread of such infection. Flies feed readily upon infective material and even upon evacuated worms.

Flies may convey eggs from excrement to food and they may do so in two ways, namely, on the external surface of their body and in their intestine.

The latter mode is practicable only in certain cases, where the eggs are of small size (under ·05 mm. in diameter). On the external surface eggs of larger size (up to ·09 mm. in the experiments) may be conveyed.

Eggs adhering to the external surface are usually got rid of by the fly within a short time, but those which are taken into the intestine may remain there for two days or longer.

The eggs which are conveyed in either of these ways, may remain alive and subsequently cause infection.

There are only certain parasitic worms in the dissemination of the eggs of which flies are likely to play an important part. This is determined in the individual cases by the nature of the parasite's life-history and the resisting powers of its eggs.

Material containing eggs of parasites, and in particular ripe segments of tape-worms, may remain a source of infection through flies for as long as two weeks.

The eggs of the following parasitic worms have been shown experimentally to be capable of being carried by *Musca domestica* :— *Taenia solium*, *Taenia serrata*, *Taenia marginata*, *Hymenolepis nana*, *Dipylidium caninum*, *Dibothriocephalus latus* (?), *Oxyuris vermicularis*, *Trichuris* (*Trichocephalus*) *trichiurus*, both internally and externally ; *Necator americanus*, *Ankylostoma caninum*, *Sclerostomum equinum*, *Ascaris megalocephala*, *Toxascaris limbata* (=*Ascaris canis* e.p.), *Hymenolepis diminuta* externally only. No Trematode parasites have as yet been experimented with.

The observations of Stiles that the larval fly can ingest Ascarid eggs and pass them on to the adult fly lacks confirmation.

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4.—Further Observations on the Ways in which Artificially Infected Flies (*Musca domestica* and *Calliphora erythrocephala*) carry and distribute Pathogenic and other Bacteria. By G. S. Graham-Smith, M.D., University Lecturer in Hygiene, Cambridge.

Contents.

The experiments described in this paper, which were carried on during the year 1910, were designed with the purpose of further investigating certain problems in relation to the distribution of bacteria by flies, to which but little attention was paid in the work described in the last report.* In that report (p. 25) are described certain experiments made with the object of ascertaining whether artificially infected flies can contaminate fluids on which they feed. The experiments were few in number and inconclusive, but seemed to show that flies (*M. domestica*) do not usually contaminate fluids on which they feed. This subject has been re-investigated, and, as will be seen in the following pages, it has been shown that both house-flies (*M. domestica*) and blow-flies (*C. erythrocephala*) can infect fluid substances for several days after they have been allowed to feed on emulsions of cultures.† Two other problems have also received attention; the development of infected blow-flies from artificially infected larvæ, and the anatomy and function of the oral discs of the proboscis of the blow-fly. The investigations on the latter subject will be described in a separate paper.

Experiments designed to ascertain whether Flies can Infect Fluids on which they Feed.

Experiments were carried out both with house-flies (*M. domestica*) which had been caught in traps, and with blow-flies (*C. erythrocephala*) which had been bred in captivity from eggs. The fluids used for the experiments were syrup and sterilised milk.

I.—*Experiments with House-flies* (*M. domestica*).

Experiment 1.—Flies (*M. domestica*), confined in three different cages, were allowed to feed for 30 minutes on syrup infected with

* Reports to the Local Government Board on Public Health and Medical Subjects. Further Reports (No. 3) on Flies as Carriers of Infection.

† The signs made use of in the previous paper (for explanation *see* p. 18) are retained for indicating the results of cultures from infected flies, etc.

B. prodigiosus, *B. pyocyaneus*, and a *coccus* forming pink colonies respectively. At various intervals, after feeding, two flies were removed from each cage and placed in a watch-glass containing sterile milk. Owing to the difficulty of obtaining a foothold on the sloping sides of the watch-glass the flies were unable to climb out, and remained struggling on the surface of the milk. In case occasional flies should succeed in climbing out, a second clean watch-glass was inverted over the first to prevent their escape. Cultures were made on agar from the milk samples after the flies had remained in them for about 30 minutes ; and, in most experiments, at least one of the flies was removed from the milk after 30 minutes, killed with chloroform, and, after immersion in alcohol, singed in the flame. Cultures were then made from the contents of its crop and intestine, to ascertain the degree of infection of the flies.

The results of the cultures made from the milk in which the flies were immersed are given in Table I., and the results of the cultures made from the crop and intestinal contents of the immersed flies in Table II.

TABLE I.—*Showing the results of cultures made from milk contained in watch-glasses in which flies previously infected with the organisms mentioned below had been immersed for* 30 *minutes.*

Time after flies were infected.	*B. prodigiosus.*	*B. pyocyaneus*	*Pink coccus.*
2 hours	+++	+++	+++
4 "	+++	+++	++
6 "	+++	+++	+
25 "	+ (few)	++	+
74 "	+	+	+

TABLE II.—*Showing the results of cultures made from the combined crop and intestinal contents of the flies which had been immersed in the milks mentioned above.*

Time after flies were infected.	No. of fly.	*B. prodigiosus.*	*B. pyocyaneus.*	*Pink coccus.*
2 hours ...	1	+	+	++
	2	++	0	+
	3	+++	+	+
	4	+++	+	0
25 "	5	+	+	+
74 "	6	+	+	0

These experiments show that flies infected with the non-spore-bearing micro-organisms mentioned can contaminate milk in which they are placed for as long a period as 74 hours, and that the micro-organisms are usually to be found in the crop or intestinal contents of the flies during that period.

Experiment 2.—Six flies (*M. domestica*) were carefully fed on an emulsion of *B. prodigiosus* which was placed on the floor of their cage in single drops. During the process of feeding the flies did not fall into the syrup or soil their wings. Their proboscides and feet were the only organs which could become infected by direct contact with the contaminated syrup. They were then transferred to a clean cage. Sterile milk was poured into a watch-glass and the latter was placed in the incubator until some of the milk had evaporated and a rim of partially dried milk was left round the fluid portion. Three hours after the flies had been fed on the infected emulsion, a watch-glass of sterile milk was placed in the cage, and left there for some hours. It was noticed that the flies fed on the dried parts as well as on the fluid milk. On examining the watch-glass after it had been removed from the cage, there were noticed on the areas covered with the partially dried milk, not only numerous proboscis marks, but also several red marks produced by vomit composed of an emulsion of *B. prodigiosus*. Cultures were made from (*a*) the fluid milk, (*b*) the dried parts (not obviously contaminated with vomit), and (*c*) vomit marks. Similar experiments were carried out 24, 26, and 28 hours after the infection of the flies, with positive results as shown in Table III.

TABLE III.—*Showing the results of cultures made from fluid and partially dried milk on which flies infected with B. prodigiosus had been allowed to feed.*

Time after flies were infected.	Fluid milk.	Partially dried milk.	Vomit.
3 hours	++	+++	+++
24 "	+++	+++	—
26 "	+++	++	—
28 "	+++	+++	—

Experiment 3.—In this series of experiments three sets of flies were carefully infected by feeding on syrup containing in suspension (1) *B. prodigiosus*, (2) *B. pyocyaneus*, and (3) a spore-bearing culture of *B. anthracis*, respectively. The flies were then transferred to clean cages. Two and a-half hours after feeding, a watch-glass containing sterile milk was placed in each cage and removed after the flies had fed on it. Cultures were then made from the specimens of milk. The flies were fed daily in this way for several days, and after each feeding removed to clean cages. It will be seen from the following Table (IV.) that the milk on which they fed was infected by them for several days. Specimens of the flies, which were dissected at various times, showed that the organisms were present on their limbs for at least two days. This may to some extent have been due to their occasionally falling into the milk which had already been infected by feeding, and so re-infecting their limbs. Infection of the limbs probably also occurred during the attempts of the flies to clean themselves (*see* p. 41).

TABLE IV.—*Showing the results of cultures from samples of milk on which flies, infected with B. prodigiosus, B. pyocyaneus and B. anthracis, had been allowed to feed.*

Time after flies were infected.	*B. prodigiosus.*	*B pyocyaneus.*	*B. anthracis.*
2½ hours	+++	+++	+++
1 day	+++	+++	+++
2 days { dry milk ...	+++	+++	+++
2 days { fluid „ ...	+++	+++	+++
3 „ { dry milk ...	+++	+ (3 colonies)	+++
3 „ { fluid „ ...	+++	+ (3 „)	+++
4 „	++	0	+++
5 „	—	+ (1 colony)	++
6 „	+	0	+ (1 colony)
7 „	+	0	+ (3 colonies)
8 „	+	0	+
9 „	+	0	0
10 „	+	0	0
11 „	+ (3 colonies)	0	+ (1 colony)

All these experiments show that artificially infected flies, kept in captivity, may contaminate milk on which they feed for several days. As in the last series of experiments (3) many more flies were employed for the experiment with *B. prodigiosus* than were used in the experiments with other organisms, the relative infecting capacity of the organisms cannot be inferred from the results obtained.

The results of the observations recorded in the following table (V) show that though the limbs of the flies infected with *B. prodigiosus* and *B. pyocyaneus* were heavily contaminated on the second day, neither of the organisms could be cultivated from them on the eleventh day. *B. anthracis* was, however, cultivated from the legs and wings even on the eleventh day, a result which is in accordance with previously published experiments (3rd report, p. 32, Table 19).

TABLE V.—*Showing the results of cultures made from the limbs and intestinal contents of the flies which had infected the milk samples recorded in Table IV.*

Time after flies were infected.	No. of fly.	Cultures from										—
		Legs.						Wings.		Head.	Intestines.	
		1.	2.	3.	4.	5.	6.	1.	2.			
1 day ...	1	+	+	+	+	+	+	+	+	+	+++	*B. prodigiosus.*
	2	+	+	0	0	0	0	+	0	—	+++	
2 days ...	1	+	+	+	0	0	0	+	0	—	+++	
	2	+	+	0	0	0	0	+	0	—	+++	
	3	+	+	+	0	0	0	0	0	+	+++	
11 „ ...	1	0	0	0	0	0	0	0	0	+	+++	
	2	0	0	0	0	0	0	0	0	+	+++	

Time after flies were infected.	No. of fly.	Cultures from										—
		Legs.						Wings.		Head.	Intestines.	
		1.	2.	3.	4.	5.	6.	1.	2.			
1 day ...	1	+	+	+	+	0	0	0	0	+	+++	*B. pyocyaneus.*
	2	+	+	+	+	+	0	+	+	+	+++	
2 days ...	1	+	+	+	+	0	0	+	0	—	+++	
	2	+	+	+	+	+	0	+	0	—	+++	
11 " ...	1	0	0	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	0	0	
2 " ...	1	+	+	+	+	+	+	+	+	+	+++	*B. anthracis.*
	2	+	+	+	+	+	0	+	+	+	+++	
11 " ...	1	+	+	+	+	0	0	+	0	0	+	
	2	+	+	+	+	+	0	+	+	+	+++	
	3	+	+	+	0	0	0	+	+	+	+++	

II.—*Experiments with blow-flies* (*Calliphora erythrocephala*).

In order to ascertain how long blow-flies are capable of infecting milk on which they feed, the following experiments were carried out :—

Experiment I.—A number of blow-flies bred in captivity and fed on syrup were infected by being allowed to feed on an emulsion of *B. prodigiosus* in syrup. After infection some of the flies were placed in a clean cage and fed daily on drops of syrup, and the rest were placed in a second clean cage and fed on sterile milk contained in watch-glasses. Those which were fed on syrup were used for dissection, and cultures were made from the remains of the milk on which the others had fed. The cages were changed daily. Flies infected with *B. pyocyaneus* were treated in the same manner.

TABLE VI.—*Showing the results of cultures made from the remains of milk on which the blow-flies infected with B. prodigiosus and B. pyocyaneus had fed.*

Time after flies were infected.	Milk on which *prodigiosus* infected flies had fed.	Milk on which *pyocyaneus* infected flies had fed.
1 day	+++	+++
2 days	+++	+++
4 "	++	++
6 "	++	+++
7 "	++	+++
8 "	0	+++
9 "	—	++
10 "	0	+
13 "	0	0
14 "	—	0

These experiments show that blow-flies fed on an emulsion of a culture of *B. prodigiosus* in syrup can infect milk to which they have access for at least seven days, and that those fed on a similar emulsion of *B. pyocyaneus* can infect the milk for ten days. The flies occasionally fell into the milk and in some instances possibly produced in this way a greater degree of infection of the milk than would otherwise have been the case, and may, on the other hand, on some occasions have reinfected limbs which were previously clean.

It was at first thought that some information might be obtained as to whether the infection of the milk was mainly brought about by vomit, or by the insertion into it of contaminated limbs from the control cage of infected blow-flies fed on drops of syrup, and consequently some of these flies were killed and dissected at intervals. Subsequent experiments, however, showed that the period of infection of syrup-fed blow-flies may be longer than that of milk-fed blow-flies, and that the period of infection of the limbs is dependent to some extent on the consistency of the syrup. The results of the cultures obtained from the infected flies are, however, of interest, and are recorded in Table VII.

TABLE VII.—*Showing the results of cultures made from the limbs and organs of blow-flies infected with B. prodigiosus and fed on* (a) *syrup, and on* (b) *milk.*

Time after flies were infected.	No. of fly.	Cultures from the limbs and organs of blow flies fed on Syrup.										No. of fly.	Cultures from the limbs and organs of blow-flies fed on Milk.									
		Legs. 1,	2	3.	4.	5.	6.	Wings. 1.	2.	Head.	Gut.		Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Gut.
3 days ...	1	+	+	+	+	+	+	+	+	+	++											
	2	+	+	+	+	+	+	+	+	+	++											
	3	+	+	+	0	0	0	+	+	+	++											
	4	0	0	0	0	0	0	+	0	0	+											
	5	0	0	0	0	0	0	0	0	0	0											
4 " ...	1	+	+	+	+	0	0	+	+	+	++											
	2	+	+	+	+	0	0	+	+	+	++											
	3	+	0	0	0	0	0	+	0	+	++											
6 " ...	1	+	+	+	+	+	+	+	+	+	0											
	2	+	+	+	+	0	0	+	+	+	++											
	3	+	+	+	+	0	0	+	+	+	++											
7 " ...	1	+	+	+	+	+	+	+	+	+	++											
	2	+	+	+	+	+	+	+	+	+	++											
	3	0	0	0	0	0	0	0	0	0	0											
	4	0	0	0	0	0	0	0	0	0	0											
10 " ...												1	0	0	0	0	0	0	0	0	0	0
												2	0	0	0	0	0	0	0	0	0	0
	1	+	+	+	+	+	0	+	+	+	++	3	0	0	0	0	0	0	0	0	0	0
	2	+	+	+	+	+	0	+	+	+	++	4	0	0	0	0	0	0	0	0	0	0
												5	0	0	0	0	0	0	0	0	0	0
13 " ...	1	+	+	+	+	+	+	+	+	+	++											
	2	+	+	+	+	+	+	+	+	—	—											
14 " ...	...	...	...	...	...	...	...	...	...	...	...	1	0	0	0	0	0	0	0	0	0	0
												2	0	0	0	0	0	0	0	0	0	0

TABLE VIII.—*Showing the results of cultures made from the limbs and organs of blow-flies infected with B. pyocyaneus and fed on* (a) *syrup, and on* (b) *milk.*

Time after flies were infected.	No. of fly.	Cultures from limbs and organs of blow-flies fed on Syrup. Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Gut.	No. of fly.	Cultures from limbs and organs of blow-flies fed on Milk. Legs. 1.	2.	3.	4.	5.	6.	Wings. 1.	2.	Head.	Gut.
2 days ...	1	+	+	+	0	0	0	+	+	+	+++											
	2	+	0	0	0	0	0	0	0	0	+											
	3	0	0	0	0	0	0	0	0	+	++											
4 " ...	1	+	+	0	0	0	0	+	0	0	++											
	2	+	+	+	+	+	+	+	+	+	++											
	3	+	+	+	0	0	0	+	+	+	++											
6 " ...	1	0	0	0	0	0	0	0	0	0	0											
	2	0	0	0	0	0	0	0	0	0	++											
	3	0	0	0	0	0	0	0	0	0	++											
7 " ...	1	0	0	0	0	0	0	0	0	+	++											
	2	0	0	0	0	0	0	0	0	0	++											
	3	0	0	0	0	0	0	0	0	0	++											
8 " ...	1	0	0	0	0	0	0	0	0	+	++	1	0	0	0	0	0	0	0	0	+	++
	2	0	0	0	0	0	0	0	0	0	++	2	+	+	+	+	+	+	+	+	+	+*
10 " ...	1	+	+	+	0	0	0	+	+	+	++	1	0	0	0	0	0	0	0	0	0	+
	2	+	+	+	+	+	+	+	+	+	++	2	0	0	0	0	0	0	0	0	+	++
	3	0	0	0	0	0	0	0	0	+	++											
13 " ...	1	0	0	0	0	0	0	0	0	0	++	1	0	0	0	0	0	0	0	0	0	++
	2	0	0	0	0	0	0	0	0	0	—	2	0	0	0	0	0	0	0	0	+	++
14 " ...	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	+
	2	0	0	0	0	0	0	0	0	+	++	2	0	0	0	0	0	0	0	0	+	++
												3	0	0	0	0	0	0	0	0	+	++
16 " ...	1	0	0	0	0	0	0	0	0	0	+											
	2	0	0	0	0	0	0	0	0	0	++											
	3	0	0	0	0	0	0	0	0	0	0											

* Fell into milk.

These experiments indicate that syrup-fed blow-flies may show large numbers of *B. prodigiosus* on their limbs and in their intestinal contents for at least 13 days. Such a long period of continued infection of the limbs has not previously been observed in the case of a non-spore-bearing micro-organism. It seems not unlikely that this result was owing to the fact that the syrup on which the flies were allowed to feed were very concentrated, and that in consequence they reinfected their limbs in their endeavours to clean their proboscides (*see* p. 41). *B. prodigiosus* was on the other hand absent from cultures made from milk-fed specimens on the 10th and 14th days.

The results of the cultures made from the blow-flies infected with *B. pyocyaneus* were very different. The syrup-fed specimens were fed on very dilute syrup, and the organisms had disappeared from their legs as early as the 6th day. Only on one occasion were they again found on the legs, namely on the 10th day, when the flies were fed on concentrated syrup. They were still present, however, in the intestinal contents on the 16th day. Although *B. pyocyaneus* was constantly present in the intestinal contents of milk-fed specimens up to the 14th day, it was only found on the limbs of a single specimen on the 8th day. This specimen had fallen into the milk which was subsequently proved by cultures to be infected (*see* Table VII.).

After making cultures from the milk on which the blow-flies infected with *B. pyocyaneus* had been fed on the 10th day, four blow-flies, about five days old, were allowed to feed on the remainder of the milk. A few hours later they were dissected and *B. pyocyaneus* was found in the intestinal contents of two of them, as shown in Table IX.

TABLE IX.—*Showing the results of cultures made from the limbs and intestinal contents of clean blow-flies which were allowed to feed on milk contaminated by blow-flies infected* 10 *days previously with B. pyocyaneus.*

No. of flies.	Legs.						Wings.		Head.	Intestinal contents.
	1.	2.	3.	4.	5.	6.	1.	2.		
1	0	0	0	0	0	0	0	0	0	+
2	0	0	0	0	0	0	0	0	0	+
3	0	0	0	0	0	0	0	0	—	0
4	0	0	0	0	0	0	0	0	0	0

In the experiment just quoted the blow-flies not infrequently fell into the milk, and in this manner perhaps infected it, at least for the first few days, by means of the organisms still present on their limbs. Moreover, in attempting to clean themselves they may have reinfected their limbs from their crop contents, since they frequently applied their proboscides to their legs.

Although, under natural conditions, it is a common occurence for flies to fall into fluids on which they are feeding, especially when the fluids are contained in vessels with smooth, vertical walls, it was thought desirable to attempt a series of experiments so arranged that the flies could feed on the fluids, but could not fall into them. For this purpose the following experiment was made :—

Experiment 2.—About 20 blow-flies were confined in each of four cages, the flies in two of the cages being infected by feeding on an emulsion of *B. prodigiosus* in syrup, and those in the other two cages being similarly infected by feeding on an emulsion of *B. pyocyaneus.*

In each instance the emulsion was placed in a watch-glass, and a short distance above it was fixed a piece of zinc pierced by round perforations about three-sixteenths of an inch in diameter. Through these perforations the flies put their proboscides and drank the fluid, and occasionally, though rarely, soiled their legs by putting them through. It was, however, impossible for them to fall into the fluid. It was noticed that, after feeding, they not infrequently deposited vomit on the zinc, and no doubt sometimes infected their feet and wings by walking and falling into it. They also infected their limbs by contact with their proboscides in cleaning them (p. 41). The two sets of flies infected with *B. prodigiosus* and the two sets infected with *B. pyocyaneus* were fed daily through sterilised perforated zinc trays on syrup and sterilised milk respectively, and cultures were made from the remains of these fluids. Flies were also dissected at intervals and cultures made from their limbs and intestinal contents. The results of the feeding experiments are given in Table X.

TABLE X.—*Showing the results of cultures made from the remains of syrup and milk on which flies infected with B. prodigiosus and B. pyocyaneus had been allowed to feed through perforated zinc trays.*

Time after flies were infected.	Infection with *B. pyocyaneus*.		Infected with *B. prodigiosus*.	
	Syrup.	Milk.	Syrup.	Milk.
1 day	—	+++	—	+++
2 days	+++	+++	+++	+++
3 "	+++	+++	+++	+++
4 "	+++	+++	+++	+++
5 "	+++	+	++	++
7 "	+++	+	++	+
8 "	++	+	++	0
9 "	++	+	++	+
10 "	+	+	++	+
11 "	+ (2 colonies)	+	++	+
13 "	0	+	++	+
14 "	+	+	++	0
15 "	+ (1 colony)	+	+	0
16 "	+	+	+ (1 colony)	0
17 "	0	+	+	+ (1 colony)
18 "	+	0	+	0
20 "	0	0	+	+ (1 colony)
21 "	0	0	+	+ (2 colonies)
22 "	0	0	+	0
23 "	0	0	+	0
24 "	+	0	+ (1 colony)	0
25 "	0	0	+	0
26 "	0	+ (1 colony)	+	+ (2 colonies)
27 "	0	0	+ (1 colony)	0
28 "	+ (1 colony)	0	+	0
29 "	0	0	+	0
30 "	0	0	0	0
31 "	0	0	+ (1 colony)	0
33 "	0	0	0	0

These experiments show that blow-flies infected with non-spore-bearing micro-organisms are capable of seriously contaminating both syrup and milk for at least a week by feeding on them. Blow-flies originally fed on an emulsion of *B. pyocyaneus* constantly produced some degree of infection in both syrup and milk for 16 days, and at even later periods occasional colonies of *B. pyocyaneus* could be cultivated from their food. Milk was apparently not infected after the 26th day, or syrup after the 28th day. Blow-flies which had been fed on *B. prodigiosus* constantly produced infection in syrup up to the 29th day, though the degree of infection was small after the 14th day. Milk was infected to a smaller extent, and only occasional colonies were cultivated from it after the 8th day.

It is of course likely that the table shows a smaller degree of infection than really existed since only a small proportion of the milk was cultivated on each occasion.

TABLE XI.—*Showing the results of cultures made from the limbs and intestinal contents of blow-flies infected with B. pyocyaneus and subsequently fed either on syrup or on milk.*

Time after flies were infected.	Syrup-fed flies: No. of fly.	Legs 1.	Legs 2.	Legs 3.	Legs 4.	Legs 5.	Legs 6.	Wings 1.	Wings 2	Head.	Gut.	Milk-fed flies: No. of fly.	Legs 1.	Legs 2.	Legs 3	Legs 4	Legs 5.	Legs 6.	Wings 1.	Wings 2.	Head.	Gut.
2 days ...	...	+	+	+	+	0	0	+	+	+	+++	...	+	+	+	+	+	+	+	+	+	+++
4 " ...	...	+	+	+	+	+	+	+	+	+	+++	...	+	+	+	+	+	+	+	+	+	++
6 " ...	...	+	+	+	+	+	0	+	+	+	++	...	0	0	0	0	0	0	0	0	+	++
7 " ...	...	+	+	+	+	+	+	+	+	+	++	...	0	0	0	0	0	0	+	0	0	++
8 " ...	...	+	+	0	0	—	—	+	0	+	++	...	+	0	0	0	0	0	+	+	0	++
9 " ...	...	+	+	0	0	0	0	+	0	0	++	...	0	0	0	0	0	0	0	0	0	+
10 " ...	...	+	0	0	0	0	0	+	+	+	++	...	0	0	0	0	0	0	+	+	+	+
11 " ...	...	+	0	0	0	0	0	0	0	+	++	...	0	0	0	0	0	0	+	0	+	++
13 " ...	...	+	+	+	0	0	0	0	0	+	++	...	+	+	0	0	0	—	0	0	+	++
32 "	1	0	0	0	0	0	0	0	0	0	+	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	+	+	2	0	0	0	0	0	0	0	0	0	0
												3	0	0	0	0	0	0	0	0	0	0
												4	0	0	0	0	0	0	0	0	0	0
												5	0	0	0	0	0	0	0	0	0	0

This table shows that *B. pyocyaneus* was isolated from the intestinal contents of blow-flies fed on somewhat concentrated syrup up to the 32nd day (when the experiment ceased), though by that time none could be isolated from their limbs. In milk-fed flies the legs were only occasionally found infected after the 4th day, though the bacilli were always present in the intestinal contents up to the 13th day. On the 32nd day cultures were made from the limbs and intestinal contents of five flies, but not a single colony of *B. pyocyaneus* was found. The whole experiment indicates that flies fed on syrup remain infected longer than flies fed on milk.

TABLE XII.—*Showing the results of cultures made from the limbs and intestinal contents of blow-flies infected with B. prodigiosus and subsequently fed either on syrup or on milk.*

Time after flies were infected.	No. of fly.	Syrup-fed flies.										No. of fly.	Milk-fed flies.									
		Legs.						Wings.		Head.	Gut.		Legs.						Wings.		Head.	Gut.
		1.	2.	3.	4.	5.	6.	1.	2				1.	2.	3.	4	5.	6.	1.	2.		
2 days ...		+	+	+	+	+	+	+	+	+	+++	...	+	+	+	+	+	0	+	+	+	+++
4 " ...		+	+	+	+	+	+	+	+	+	+++	...	+	+	+	+	+	+	+	+	+	+++
6 " ...		0	0	0	0	0	0	+	+	+	++	...	+	0	0	0	0	0	—	—	—	—
7 " ...		+	0	0	0	—	—	+	0	0	++	...	+	0	0	0	0	0	+	0	0	0
8 " ...		+	+	0	0	0	0	+	+	+	++	...	+	+	+	0	0	0	+	0	+	++
9 " ...		0	0	0	0	0	0	0	0	0	++	...	+	+	0	0	0	0	+	0	0	+
10 " ...		+	+	0	0	0	0	+	0	+	++	...	+	0	0	0	0	0	+	+	+	+
11 " ...		0	0	0	0	0	0	+	0	+	++	...	0	0	0	0	0	0	0	0	+	0
12 " ...		+	+	+	+	0	0	+	0	+	++	...	+	0	0	0	0	0	+	0	0	0
32 "	1	0	0	0	0	0	0	0	0	0	+											
	2	0	0	0	0	0	0	0	0	+	++	1	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	++	2	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0											

This table shows that little difference may exist between the degree of infection of the limbs of blow-flies originally infected with *B. prodigiosus* and, later, fed on milk or on dilute syrup. The organism may be found on the legs or wings up to the 12th day, but had entirely disappeared on the 32nd day. The intestinal contents of syrup-fed flies however show a much higher degree of infection than those of milk-fed flies, and while, in the latter, not a single colony of *B. prodigiosus* was found on the 32nd day, the intestinal contents of three out of the four syrup-fed flies showed several colonies. This corresponds with the results of the cultures made from their food.

Observations on the habits of blow-flies and their bearing on feeding experiments.

Blow-flies spend a large proportion of their time, when in captivity, in cleaning their limbs and proboscides in the following manner :— The wings are usually cleaned with the posterior pair of legs, which are passed over and under each wing. Often two legs are simultaneously applied to a wing which is drawn between them. Subsequently the legs which have been used are rubbed together. The posterior pair of legs are also used for cleaning the abdomen, and, in so doing, are passed over the anus. Probably they are frequently infected in this way and, later, infect the wings. For cleaning the proboscis and head the anterior pair of legs are made use of. During this process the proboscis is usually extended to its fullest extent, and the legs are passed along it from its proximal to its distal extremity. Occasionally the oral discs are applied to some part of a leg, probably in order to remove some irritating material. Flies have been seen to clean their proboscides immediately after feeding, and to leave infected fluid on their legs.

The efforts made to cleanse their limbs are often very prolonged when the flies have been allowed to feed on very sticky materials, such as concentrated syrup, and an observation which has been quoted (Table VIII., syrup-fed flies tenth day) appears to indicate that, under such circumstances, gross reinfection of the limbs may occur. On one occasion a fly sucked at some semi-fluid syrup mixed with carmine for a long time and moved away from the food with a large mass adhering to its proboscis. This mass was deliberately removed with the anterior pair of legs. The same thing was frequently noticed in subsequent similar experiments.

From all the observations which have been made it seems highly probable that blow-flies frequently reinfect their limbs when cleaning themselves.

Attempts were made to ascertain experimentally to what extent infection or re-infection of the limbs may occur owing to the methods of cleansing themselves which blow-flies adopt. The results however were not satisfactory, since it was found practically impossible to kill recently fed blow-flies without large quantities of fluid being discharged from their proboscides. Whatever method is adopted for quickly killing them the flies exude fluid from their proboscides, and in their struggles soil their limbs. The cultures made from the limbs of recently fed blow-flies do not therefore necessarily show their degree of infection during life. The presence of the micro-organisms on the limbs shows either that they had been in that situation from the commencement of the experiment or that the limbs had been reinfected with vomit or faeces. If the cultures give negative results it may be assumed either that the limbs had never been contaminated, or that the micro-organisms had disappeared after infection or re-infection.

Measurements which were made of the contents of the crops of recently fed blow-flies showed that more than 0·02 c.c. of fluid might be contained in them. On one occasion a blow-fly which had recently fed on coloured syrup was held by the wing and the material which it vomited collected and measured. It was found that 0·01 c.c. of coloured syrup had been deposited. Subsequently the fly was killed and dissected and 0·01 c.c. of fluid obtained from its crop.

All the experiments which were made on this subject show that blow-flies take up large quantities of fluid in feeding, and are capable of vomiting at least half of the contents of their crops.

If flies are allowed to feed on fairly large volumes of milk contained in watch-glasses they often fall into the milk. After emerging they leave long trails as they crawl up the sides of the watch-glass. Frequently also they soil their limbs while feeding, and in walking away leave trails like those just mentioned. On several occasions these trails have been proved to be infected. Other flies approaching the milk usually stop and suck at the half-dried trails and drops left by previous flies, and probably infect their feet and take up micro-organisms into their crops.

The experiments and observations on the feeding habits of blow-flies all show that the individual blow-fly (*C. erythrocephala*) is capable of distributing greater numbers of bacteria, and over a longer period of time, than the individual house-fly (*M. domestica*). House-flies, however, are probably a greater source of danger owing to their greater prevalence and to their more frequent occurrence in situations where food can be easily contaminated.

Experiments with blow-flies (C. erythrocephala) bred from artificially infected larvæ.

Faichnie's observations on flies (*M. domestica*) bred from infected larvæ have been quoted in these reports (Third Report p. 15), and the experiments about to be described have been conducted in order if possible to verify and extend his observations.

Experiment 1.—Blow-flies were collected and allowed to deposit eggs on pieces of meat and dead animals. The larvæ which emerged developed under natural conditions until they were seven days old. Then considerable numbers were placed in each of five round tin boxes without lids, containing slightly moist earth. Glass cages covered with gauge, like those previously described and figured (Third Report p. 3, Plate I., fig. 1), were placed over the open tops of the tins.

The larvæ in the five tins were allowed to feed on meat infected with (*a*) a spore-bearing culture of *B. anthracis*, (*b*) *B. typhosus*, (*c*) *B. enteritidis* (*Gaertner*), (*d*) *B. prodigiosus*, and (*e*) *V. cholerae*, derived from cultures respectively. After seven days the remains of the meat were removed, and the larvæ fed on fresh meat. By the 12th day after infection all the larvæ had ceased to feed and the majority had disappeared under the earth. The flies all emerged between the 18th and 25th days after infection.

Cultures from the larvæ.—At various times specimens of the larvæ were removed from the cages and placed in sterile water. Cultures from the water in which the larvæ, fed on meat infected with anthrax bacilli, had been immersed, showed numerous colonies of *B. anthracis*, but none of the other micro-organisms were isolated from the cultures which were made. Other larvæ were placed in alcohol for 15 minutes, and subsequently taken out and passed through the flame till the alcohol had burnt away in order to sterilise their surfaces. They were then dissected and cultures made from their internal organs. *B. anthracis* was obtained, but none of the other organisms.

Cultures from the flies.—Each morning the cages were examined and the flies which had emerged removed. Some were killed and dissected within a few hours of emerging, and others were placed in glass cages and kept alive for various periods of time, on syrup.

(A.) *B. anthracis.*—Altogether about 70 flies emerged from the larvæ fed on meat infected with *B. anthracis*. Of these 17 were dissected and cultures made from their organs within a few hours of emerging. From four specimens *B. anthracis* was not cultivated, but from the other 13 cultures were obtained. It was present in the intestinal contents of 10 ; on one or both wings of 8 ; on one or more legs of 12 ; and on the heads of 8.

Three specimens (18, 19, 20) were dissected after living two days in a cage. From the legs, wings, crop and intestinal contents of all, *B. anthracis* was obtained. One specimen (21), three days old, was dissected, and *B. anthracis* was obtained from one wing. *B. anthracis* was cultivated from a leg and from the head of a specimen six days old (22). Three specimens (23, 24, 25) were dissected after living ten days in a cage. *B. anthracis* was obtained from one leg of No. 23, and from one leg and one wing of No. 24, but was not found in the cultures made from No. 25. Six specimens (26, 27, 28, 29, 30, 31), eleven days old, were dissected. *B. anthracis* was obtained from one leg and both wings of No. 26, from one leg and the intestinal contents of No. 27, and from one leg of No. 28, but was not obtained from any of the cultures made from the other three flies (29, 30, 31). Three flies (32, 33, 34), fifteen days old, were dissected and *B. anthracis* cultivated from two of them ; from one leg and the head of No. 33, and from one leg and one wing of No. 34. *B. anthracis* was only obtained from one leg of one (35) of the three flies (35, 36, 37), nineteen days old, which were dissected.

Two flies (38, 39) twenty-two days old, three (40, 41, 42) twenty-three days old, two (43, 44) twenty-nine days old, three (45, 46, 47) thirty days old, and four (48, 49, 50, 51) thirty-three days old were dissected and cultures made from their limbs and organs, but *B. anthracis* was not found in any instance.

The results of these experiments are given in Table XIII.

TABLE XIII.—*Showing the results of cultures made from the limbs and intestinal contents of blow-flies which emerged from larvæ fed on meat infected with B. anthracis.*

No. of fly.	Legs.						Wings.		Head.	Crop.	Intestine.	Age.
	1.	2.	3.	4.	5.	6.	1.	2.				
1	0	0	0	0	0	0	0	0	0	...	0	
2	0	0	0	0	0	0	0	0	0	...	0	
3	0	0	0	0	0	0	0	0	0	...	0	
4	0	0	0	0	0	0	0	0	0	...	0	
5	0	0	0	0	0	0	0	0	0	...	++	
6	+*	0	0	0	0	0	0	0	0	...	+	
7	+	0	0	0	0	0	0	0	0	...	0	
8	+	+	0	0	0	0	0	0	0	...	+	
9	+	+	0	0	0	0	+*	0	0	...	+	Dissected within a few hours after emerging.
10	+	+	+	+	0	–	+	+	+	...	+	
11	+	+	+	+	+	0	+	+	+	...	+	
12	+	+	+	+	+	+	+	+	+	...	+	
13	+	+	+	+	+	+	+	+	+	...	+	
14	+*	+	+	+	+	+	+	0	+	...	+	
15	+	+	+	+	+	+	+	+	+	...	+	
16	+	+	+	+	+	+	+	+	+	...	0	
17	+	+	+	+	0	0	0	0	+	...	0	

* Cultures proved to be virulent.

No. of fly.	Legs. 1.	Legs. 2.	Legs. 3.	Legs. 4.	Legs. 5.	Legs. 6.	Wings. 1.	Wings. 2.	Head.	Crop.	Intestine.	Age.
18	+	+	+	+	0	0	+	+	0	+	+	
19	+	+	+	+	+	0	+	+	0	+	+	Two days old.
20	+°	+	+	+	+	+	+	+	+	+	+	
21	0	0	0	0	0	0	+	0	0	0	0	Three days old.
22	+	0	0	0	0	0	0	0	+	0	0	Six days old.
23	+	0	0	0	0	0	0	0	0	0	0	
24	+	0	0	0	0	0	+	0	0	0	0	Ten days old.
25	0	0	0	0	0	0	0	0	0	0	0	
26	+	0	0	0	0	0	+	+	0	0	0	
27	0	0	0	0	0	0	+	0	0	0	+	
28	+	0	0	0	0	0	0	—	0	0	0	Eleven days old.
29	0	0	0	0	0	0	0	0	0	0	0	
30	0	0	0	0	0	0	0	0	0	0	0	
31	0	0	0	0	0	0	0	0	0	0	0	
32	0	0	0	0	0	0	0	0	0	—	0	
33	+	0	0	0	0	0	0	0	+	0	0	Fifteen days old.
34	+	0	0	0	0	0	+	0	0	0	0	
35	+*	0	0	0	0	0	0	0	0	0	0	
36	0	0	0	0	0	0	0	0	0	0	0	Nineteen days old.
37	0	0	0	0	0	0	0	0	0	0	0	
38	0	0	0	0	0	0	0	0	0	0	0	Twenty-two days old.
39	0	0	0	0	0	0	0	0	0	0	0	
40	0	0	0	0	0	0	0	0	0	0	0	Twenty-three days old.
41	0	0	0	0	0	0	0	0	0	0	0	
42	0	0	0	0	0	0	0	0	0	0	0	
43	0	0	0	0	0	0	0	0	0	0	0	Twenty-nine days old.
44	0	0	0	0	0	0	0	0	0	0	0	
45	0	0	0	0	0	0	0	0	0	—	0	
46	0	0	0	0	0	0	0	0	0	—	0	Thirty days old.
47	0	0	0	0	0	0	0	0	0	0	0	
48	0	0	0	0	0	0	0	0	0	—	0	
49	0	0	0	0	0	0	0	0	0	—	0	Thirty-three days old.
50	0	0	0	0	0	0	0	0	0	—	0	
51	0	0	0	0	0	0	0	0	0	0	0	

* Cultures proved to be virulent.

It should be pointed out that organisms of the *B. subtilis* type were very commonly present in these cultures, and no doubt occasionally overgrew the anthrax bacilli.

A few other experiments were carried out with these flies. Four flies were allowed to walk over agar plates a few hours after emerging. Numerous colonies of *B. anthracis* developed on these plates.

Twelve flies a few hours old were kept in a glass cage and fed on syrup. Shortly after their first meal some of the remains of the syrup on which they had been feeding was smeared on the surface of agar plates. Numerous colonies of *B. anthracis* developed on these plates. Nearly every fly very shortly after emerging deposited a large quantity of whitish, semi-fluid material. Cultures made from this material were negative. The fæces deposited by flies two days old contained *B. anthracis* in considerable numbers, as also did the remains of syrup on which they had fed.

B. anthracis was not found in cultures made from the fæces of flies 22 and 23 days old, nor in those made from the remains of syrup on which they had been feeding, but a single colony of *B. anthracis* was obtained from the remains of syrup on which flies 21 days old had fed.

From all anthrax-like colonies found on the original plates pure cultures were prepared, and a positive result was not recorded unless the organism isolated corresponded with *B. anthracis* in all its principal cultural characteristics. Eight of the pure strains were tested and proved by animal inoculations to be virulent. The cultures tested for virulence were obtained from the legs of flies 6, 14, 20, and 35, the wing of fly 9, the plate over which flies had walked, the remains of the syrup on which young flies had fed, and from the fæces deposited by a two days-old fly.

In the last report (p. 32, Table 19, fly C and Plate VII., fig. 19) it was shown that living and virulent anthrax bacilli may be isolated 155 days after death from the bodies of house-flies, which have died of infection with *E. muscæ*, after feeding on the spores of *B. anthracis*. Cultures were made recently, 428 days after death, from the body of a house-fly belonging to this batch, and numerous colonies of *B. anthracis* isolated. By animal inoculations these cultures were proved to be virulent.

Numerous control cultures were made from blow-flies both bred in captivity and caught in traps, and also from larvæ fed on non-infected meat. But in no case were bacilli cultivated which could not be easily differentiated from *B. anthracis* by their cultural characters.

This experiment shows that many of the blow-flies which emerged from larvæ fed on meat infected with the spores of *B. anthracis* were infected with *B. anthracis* and remained infected for 15 days or more. Immediately after emerging the flies are heavily infected, but, subsequently, only a few bacilli can be cultivated from each fly. After the 22nd day the flies appeared to have completely cleansed themselves. These experiments also show that the recently-emerged flies are capable of infecting materials over which they walk and on which they feed, and that the fæces deposited for at least two days are infected. Further experiments are needed to show for what periods of time they may deposit infected fæces or infect food substances. Incidentally the experiments also show that blow-flies may live for at least 33 days in captivity.

B. typhosus, B. enteritidis, B. prodigiosus, and V. choleræ. From the larvæ infected with *B. typhosus*, 45 flies emerged ; from those infected with *B. enteritidis*, 14 flies ; from those infected with *B. prodigiosus*, 25 flies, and from those infected with *V. choleræ*, 20 flies. These were treated in the same way as the flies which emerged from the anthrax-infected larvæ. No organisms resembling *B. prodigiosus* or *V. choleræ* were met with. Cultures were made on Drigalski-Conradi and other media from the flies which emerged from larvæ infected with *B. typhosus* and *B. enteritidis*, and, in many cases, suspicious colonies were found. Pure cultures were obtained from many of these, but in no case, with perhaps one exception, could it be definitely proved that either *B. typhosus* or *B. enteritidis* had been isolated. The exception mentioned was an organism, resembling *B. enteritidis* in all its cultural characters, obtained from the intestinal contents of a one-day-old fly, hatched from a larva fed on meat infected with *B. enteritidis*. Unfortunately the culture was accidentally destroyed before tests for agglutination or virulence could be made with it.

Experiment 2.—In this series of experiments larvæ which were almost full fed were placed in a number of cages like those previously described, and allowed to feed for 24 hours on meat infected with *B. typhosus*, *B. enteritidis*, and *B prodigiosus*. Subsequently the larvæ were removed to fresh cages in which they pupated. The flies which emerged were examined, with negative results, for the presence of the organisms mentioned, though many suspicious colonies were carefully studied.

Experiment 3.—A similar experiment to the above was carried out on flies which emerged from larvæ which had been allowed to feed on meat infected with *B typhosus*, *B. enteritidis*, *B. prodigiosus*, and a *coccus* forming pink colonies respectively. The larvæ fed and pupated in the same cages, and the flies emerged in them. In all, cultures were made from 30 flies which emerged from larvæ infected with *B. typhosus*, and from 29 flies which emerged from larvæ infected with *B. enteritidis*. Many suspicious colonies were subcultivated with negative results. Cultures were also made from 29 flies which had emerged from larvæ infected with *B. prodigiosus*, and 28 cultures from flies which emerged from larvæ infected with the *coccus* forming pink colonies. All were negative.

These experiments seem to indicate that, under the conditions described, none of the non-spore-bearing micro-organisms mentioned commonly survive sufficiently long to be found in the blow-flies which emerge from infected larvæ. Possibly, however, they might be found in flies which emerged from larvæ which had fed on the flesh of infected animals. Though these experiments appear to afford some evidence that blow-flies are not likely to be infected with non-spore-bearing organisms when they emerge, it must be admitted that they afford no evidence that infected house-flies (*M. domestica*) do not emerge from larvæ which have fed on excreta and similar materials contaminated with these organisms.

If possible the writer intends to carry out experiments with the larvæ of non-biting flies which ordinarily feed on excreta.

Summary.

The experiments and observations quoted in this paper show definitely that artificially infected flies, both house-flies (*M. domestica*) and blow-flies (*C. erythrocephala*) are capable of infecting fluids, such as milk and syrup, on which they feed and into which they fall. In the case of the house-fly, infected with certain micro-organisms (*B. prodigiosus* and *B. anthracis*) gross infection may be produced in milk for at least three days, and a smaller degree of infection for ten days or even longer. Blow-flies produce gross infection for six to nine days with non-spore-bearing micro-organisms (*B. prodigiosus* and *B. pyocyaneus*) and some degree of infection for three or four weeks. It is probable, at any rate in the later stages,

that infection is mainly due either to direct infection with the crop contents vomited through the proboscis, or to indirect infection by means of the limbs which have been reinfected with vomited material during the process of cleaning them.

Blow-flies bred under experimental conditions from larvæ fed on meat infected with the spores of *B. anthracis* are themselves heavily infected, and remain heavily infected for at least two days after emerging. In the single series of experiments undertaken *B. anthracis* could not be cultivated either from the limbs or intestinal contents of flies more than 15 or 19 days old. For the first two days after emerging flies are capable of infecting materials over which they walk and on which they feed, and deposit infected fæces. All the cultures which were tested proved to be virulent.

Positive results were not obtained in cultures made from the limbs and intestinal contents of blow-flies bred from larvæ which had been fed on meat artificially infected with non-spore-bearing bacteria, *B. typhosus*, *B. enteritidis*, *B. prodigiosus*, *V. choleræ*, and a *coccus* forming pink colonies. Possibly, however, positive results might be obtained if larvæ were fed on the bodies of diseased animals. These experiments, however, afford no information as to the extent to which house-flies (*M. domestica*) bred from larvæ fed on naturally infected excreta and similar materials are apt themselves to be infected.

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