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Regurgitation by the Face Fly, *Musca autumnalis* DeGeer

Russell E. Coleman
University of Tennessee, Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Russell E. Coleman entitled "Regurgitation by the Face Fly, *Musca autumnalis* DeGeer." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Reid R. Gerhardt, Major Professor

We have read this thesis and recommend its acceptance:

Charles D. Pless, M. L. Pan

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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We have read this thesis
and recommend its acceptance:

Charles L. Blau

Accepted for the Council:

Lee Minkel

The Graduate School

REGURGITATION BY THE FACE FLY

MUSCA AUTUMNALIS

(DeGeer)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Russell E. Coleman

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ABSTRACT

Face flies normally began regurgitating within 10 minutes after feeding, and continued for periods of up to 240 minutes. Almost 100 percent of flies fed a concentrated (4.8%) solution of trypticase-soy broth were observed regurgitating. The deposition of droplets onto a substrate was almost never observed, indicating that the regurgitation is not analagous to vomiting. The droplet exuded contained from five to ten percent of the food consumed, with the crop the source of the fluid. The crop is also the most likely destination of the regurgitated material. The purpose of this phenomenon is still not clear. However, the fluid in the regurgitation droplet was almost constantly circulating, possibly increasing the concentration of the liquid through the evaporation of excess water. This theory is supported by results from tests on the osmolality of the regurgitated droplets. The osmolality of the regurgitation droplets was more than two times greater than that of the ingested food, indicating some change had taken place.

The effect of various factors on regurgitation was also determined. The concentration and amount and type of food all significantly affected regurgitation. Solid foods did not result in regurgitation, and large amounts and high concentrations of liquids increased the

frequency and duration of the process. Low relative humidity levels significantly increased the occurrance of regurgitation, and a temperature of 29°C promoted regurgitation. Significant differences were found when interactions between these factors were examined.

Since regurgitation droplets were rarely deposited onto a substrate, various factors were examined which would result in this deposition. Increasing the number of flies within a given area increased the number of drops deposited, however, the actual number of drops deposited per fly did not increase. More important was the effect of adding unfed flies to flies already regurgitating. Flies which were regurgitating normally remained motionless, with no interactions occurring between regurgitating flies. Unfed flies actively searched for a meal, and during the search often disturbed regurgitating flies. This resulted in the deposition of droplets onto the substrate. The presence of a cow also increased this deposition of droplets. This was due to activity of the cow, and may also have been a result of the microclimate produced by the cow. These results prove that face flies frequently regurgitate and may in fact be ideal vectors of pathogens.

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CHAPTER 1

INTRODUCTION

1 - The Face Fly

The face fly, Musca autumnalis (DeGeer), was first reported in Nova Scotia in 1952 (Vockeroth, 1953), and is now found throughout southern Canada (Depner, 1969) and the continental United States, with the exception of Alaska, Arizona, Florida, New Mexico, and Texas (USDA, 1966, 1967, 1968, 1969, 1975; Poorbaugh and Smith, 1968; Pickens and Miller, 1980). Wang (1964) reported the life history of M. autumnalis in the laboratory, while Pickens and Miller (1980), Tesky (1960, 1969), Ode and Matthysse (1967), and Killough et al. (1965) reviewed the biology and field behavior of the fly in North America.

Musca autumnalis has been classified by West (1951) as a "facultative haematophagous" species which feeds upon blood when it can obtain it, but will utilize other food, such as manure, afterbirth, and oral and nasal secretions, in its absence. The presence of blood has been shown to stimulate egg production (Wang, 1964), as have eye and muzzle secretions (Hammer, 1942; Matthew, 1960; Tesky, 1960; Treece, 1960; Turner and Hair, 1967). Musca autumnalis females are most often found

clustered around the faces of cattle, feeding on the oral and nasal secretions, although horses, humans, swine, sheep, and dogs may also be affected (Hammer, 1942). This habit leads to M. autumnalis's common name, the face fly. Between 80% (Dobson and Matthew, 1960; Tesky, 1967) and 97.5% (Hower and Cheng, 1972) of the flies on cattle at a given time are female. Of the female flies that are on cattle, 93% have immature oocytes (Miller, 1967), i.e. at ages three and eight days. Five-day old female face flies are 12 times as abundant on cattle as one-day old female flies (Hower and Cheng, 1972), and only 4% of the face fly population are on cattle at any one time (Miller and Treece, 1968). Oral and nasal secretions from cattle presumably are the primary source of protein for ovarian development. Males two to four days old will feed upon cattle (Dobson and Matthew, 1960), but thereafter feed primarily upon nectar (Wang, 1964).

In Europe, where it is indigenous, M. autumnalis is only a moderate pest of livestock (Hammer, 1942). It has become a major pest since its introduction into North America, with losses due to M. autumnalis estimated as approximately 80 million dollars per year (USDA, 1968).

There are several methods by which face flies cause damage to cattle. The presence of face flies around the

eyes and muzzle have been reported to cause cattle to spend less time feeding and more time "fighting" flies (Benson and Wingo, 1963; Hansens and Valiela, 1967; Ode and Matthysse, 1967; Tesky, 1969; Arends, 1981), although Schmidtman et al. (1981) found little difference in grazing times of cattle heavily infested with face flies and those lightly infested. Peterson and Boreherding (1962) and Hansens (1963) reported decreases in weight gain and milk production due to face flies, although Arends (1981) found no evidence of this. Despite the USDA's (1968) report on losses due to face flies, there is no evidence as to an actual economic threshold (Steelman, 1976).

The feeding activity of the face fly can also cause direct damage to the cornea of the eye (Brown and Adkins, 1972; Shugart et al., 1979). Shugart et al. (1979) proposed that one face fly/eye for 33 days could cause injury to an eye. Broce and Elzinga (1984) found that the prestomal teeth of face flies have jagged terminal points which project beyond the pseudotrachae when the labellae are in the scraping position, resulting in mechanical damage to the eye.

The most economically significant damage caused by the face fly can be attributed to its role as a vector of disease causing organisms. It is a biological vector of

four species of Thelazia eyeworms: T. rhodesia, T. gulosa, T. skrjabini, and T. lacrymalis (Krecob, 1949; Stoffolano, 1970, 1971; Weinmann et al., 1974; Wetzel, 1974; Geden and Stoffolano, 1977; Pickens and Miller, 1980), and a mechanical vector of infectious bovine rhinotracheitis virus, Branhamella catarrhallis, Corynebacterium pyogenes and Moraxella bovis (Formston, 1954; Steve and Lilly, 1965; Cheng, 1967; Wetzel, 1974; Gerhardt et al., 1976, 1982; Pickens and Miller, 1980). A dramatic increase in eye disease in cattle has been reported since the introduction of the face fly to North America in 1952 (Dobson and Matthew, 1960; Treece, 1960; Decker, 1961; Benson and Wingo, 1963).

Infectious bovine keratoconjunctivitis, or pinkeye, is the most frequent eye disorder of cattle (Pugh and Hughes, 1975). Manifestations of the disease consist of conjunctivitis, photophobia, ecchymosis, blephrospasm, and edema of the lids in the earlier stages, followed by ulceration and the formation of lesions (Glass, 1983). Killinger et al. (1976) developed a system of staging for pinkeye based on the degree of corneal involvement, while Glass and Gerhardt (1983) included both conjunctival manifestations and the type and size of corneal lesions. In its most serious stage the disease consists of a lesion with complete corneal vascularization. In some

instances the eye may rupture. The lesion eventually disappears, leaving only a faint, milky white area (nebula) which does not completely fade out (Bruner, 1973; Blood, 1979; Blogg, 1980).

Jones and Little (1923) isolated a bacterium from the eyes of cattle with pinkeye which was later identified as Hemophilus bovis Hauduroy, presently known as Moraxella bovis Hauduroy (Barner, 1952; Breed et al., 1957). Moraxella bovis remains the most likely cause of the disease, as it is the only organism which satisfies all of Koch's postulates for infectious bovine keratoconjunctivitis (Henson and Grumbles, 1960; Hughes et al., 1965). Other suspected causes of pinkeye include infectious bovine tracheitis virus, and various other viruses, bacteria, rickettsia, and psitticosis agents (Pugh et al., 1970; Farley et al., 1950; Siegmund, 1961; Sweat, 1963). None of these organisms by itself is normally sufficient to produce pinkeye with all of its clinical symptoms. Apparantly, the eye must be irritated prior to infection, with ultraviolet radiation, dust, and mechanical irritation by flies all possible predisposing factors (Hughes et al., 1965; USDA, 1965; Wilcox, 1968; Pugh and Hughes, 1975; Baptista, 1979).

Musca autumnalis has been suspected of transmitting M. bovis to cattle (Steve and Lilly, 1965; Cheng, 1967;

Brown and Adkins, 1972; Gerhardt et al., 1976, 1982; Arends, 1981; Berkebile et el., 1981). Cheng (1967) and Gerhardt et al. (1976, 1982) found an increase in the number of face flies corresponded to an increase in the number of cases of pinkeye, while M. bovis has been recovered from flies that had been fed on both asymptomatic (Gerhardt et al., 1982) and symptomatic (Berkebile et al., 1981; Gerhardt et al., 1982) cattle. Steve and Lilly (1965) recovered the bacteria from the external surfaces of M. autumnalis for up to 3 days, but not from the digestive tract. Burton (1966) confirmed Steve and Lilly's finding that M. bovis does not survive in the digestive tract of the face fly, and suggested that nearly 100% of the bacteria were dead within two hours of ingestion. The pH of the crop was not the factor resulting in the mortality of M. bovis (Burton, 1966). Simpson (1981) and Burton (1966) could not substantiate Steve and Lilly's (1965) finding of external survival on the face fly of up to three days. Glass et al. (1982) found that M. bovis survived in the crop for up to 48 hours after ingestion, but not 72 hours. 37% of the midguts examined contained M. bovis, while none of the hindguts did. Glass and Gerhardt (1984) suggested that regurgitation of M. bovis from the crop of face

flies onto the eyes of cattle was the most likely means of transmission of the bacteria.

ii - Regurgitation

Regurgitation is a common phenomenon in many species of arthropods. It is known to occur in many Homoptera, Hemiptera, Coleoptera, Hymenoptera, Siphonaptera, Diptera, and Ixodidae (Klott et. al., 1980). Klott et al. (1980) suggested that regurgitation most likely occurs in many more arthropods than is currently known and plays a significant role as a mechanism of transmission of pathogens to both plants and animals.

Regurgitation typically consists of the egestion of some material, normally food. The purpose of regurgitation varies widely, ranging from a defense mechanism in larvae of the European Spruce Sawfly, Gilipinia hercyniae (Entwistle et al., 1983), to a means of reducing the water content of honey by bees (Townsend, 1974). In some instances the egested material can contain disease producing organisms, with the insect thus serving as a vector, as with Musca autumnalis and M. domestica transmitting Moraxella bovis.

Apion vorax (Coleoptera: Curculionidae) is one of the primary vectors of both Broad Bean Stain Virus and Echtes Ackerbohnenmosaik Virus to field beans (Vicea faba

minor). Cockbain et al. (1975) suggested that transmission of these viruses could occur by regurgitation, although they only observed A. vorax regurgitate when anaesthetized. Many viruses are also believed to be transmitted by Crysomelid beetles in this fashion, although this also has been shown to occur only when the beetles are anaesthetized (Markham and Smith, 1949; Dale, 1953; Freitag, 1956; Walters, 1969).

Plant sucking insects, such as aphids, coccids, leafhoppers and planthoppers, piesmids, whiteflies, and thrips, along with the mites and nematodes, are the most important vectors of plant pathogens (Kloft et al., 1980; Garret, 1973; Harris and Bath, 1973). In experiments using radioisotopes (^{32}P) as tracers, Garret (1973) showed that a large proportion of the tracer, and in some cases all of it, could be transmitted to plants by regurgitation. Studies using Tritium (^{3}H) showed that salivation could be ruled out as the mechanism of transfer, and that the high activity of deposition was due to regurgitation alone (Kloft, 1977).

In aphids, feeding is preceded by an exploratory phase during which the aphid tests the suitability of a plant for food with superficial probes. Uptake of a virus normally occurs during these superficial probes (Wensler, 1962). Electron microscopy and electronic

recording indicated that aphids both ingested food and regurgitated during these brief probes (Sylvestor, 1954; Simons, 1956), and that regurgitation was identical to ingestion in every way except for direction of flow (Auclair and Cartier, 1963; Mittler and Dadd, 1963; Srivastava and Auclair, 1971). Harris (1977) suggested that regurgitation during phloem feeding might serve to clear clogged food and salivary sheath canals. During feeding, the injured sieve tubes of the plant become rapidly plugged with depositions that can block the valve between the esophagous and the midgut in the aphid. Contrary to earlier reports that showed that viruses could only be transmitted externally on the terminal portion of the stylet (Bradley, 1964; Pirone, 1969), it now appears that viruses carried internally in the alimentary canal of aphids can be innoculated into plants by regurgitation (Harris, 1973). Aphid saliva is known to inactivate viruses upon contact, however, during feeding the tip of the aphid's stylet is not always enclosed by saliva. This is an important feature of virus transmission as neither ingested nor regurgitated material comes into contact with the saliva (Harris, 1977).

Of those arthropods that are pests of animals, the Hemiptera, Siphonaptera, Diptera, and Ixodidae (hard

ticks) contain species which are known to regurgitate. Many of these arthropods are vectors of various diseases, with regurgitation often playing a role in transmission (Kloft et al., 1980).

Fleas (Order: Siphonaptera) are vectors of the bacterium Yersinia pestis, the organism that causes plague. Yersinia pestis can be transmitted via feces, although the normal mechanism of infection is by regurgitation (Bacot and Martin, 1914). Masses of Y. pestis collect among the spines of the proventriculus and block the entrance to the stomach. Back pressure caused by the elastic recoil of the esophageal wall causes the infectious blood to be regurgitated into the feeding site (Pollitzer, 1954). The same mechanism of regurgitation occurs in the sandflies (Diptera: Psycodidae), with various Leishmania species being transmitted (Smith et al., 1940).

Regurgitation in the Diptera has been recorded since the early 1900's, when Graham-Smith (1914) found that Salmonella marcescens and Bacillus anthracis spores could be recovered from the vomit of Musca domestica. Since then regurgitation has been found to play a part in the transmission of a number of additional pathogens, including Salmonella enteritidis (Ostrolenk and Welch, 1942), Salmonella paratyphi B (Gross and Preuss, 1951),

Moraxella bovis (Glass and Gerhardt, 1984), Treponema pertenue (Kumm, 1935; Lamborn, 1936, 1937), Entamoeba histolytica (Sieyro, 1942), Ascaris lumbricoides (Dipeolu, 1977, 1982), and various Sarcosystis (Markus, 1980) and Leishmania (Smith et al., 1940; Kloft et al., 1980) species. Regurgitation will undoubtedly be found to play a much more important role in the transmission of pathogens by the Diptera than is presently known.

Most reports of regurgitation in the order Diptera concern the suborder Cyclorrhapha. Hippelates flavipes (Diptera: Chloropidae), Musca domestica, M. autumnalis, M. spectanda, M. sorbens, Fannia canicularis, Stomoxys calcitrans (Diptera: Muscidae), Calliphora vicana and Phormia regina (Diptera: Calliphoridae) are all known to regurgitate, with the liquid frequently containing pathogens (Graham-Smith, 1914, 1934; Wenyon and O'Connor, 1917; Thomson and Lamborn, 1934; Kumm, 1935; Ostrolenk and Welch, 1942; Sieyro, 1942; Zimin, 1944; Gross and Preuss, 1951; Greenberg, 1973; Thomson, 1975; Butler et. al., 1977; Kloft et al., 1980; Markus, 1980; Glass and Gerhardt, 1984).

Dipeolu (1977, 1982) found that Musca vicana and Musca domestica could regurgitate viable helminth eggs for up to 2 hours after ingestion. Viable hookworm larvae could be recovered up to 8 hours after ingestion,

while Ascaris lumbricoides larvae were retained indefinately, presumably due to the protective shell enclosing the larvae. Markus (1980) suggested that regurgitation could also result in the transmission of Sarcocystis sporocytes. This finding is contrary to Jausion and Deckester's (1923), who reported that protozoan cysts and helminth eggs were filtered out by the pseudotrachae during regurgitation, while trypanosomes and bacteria were not.

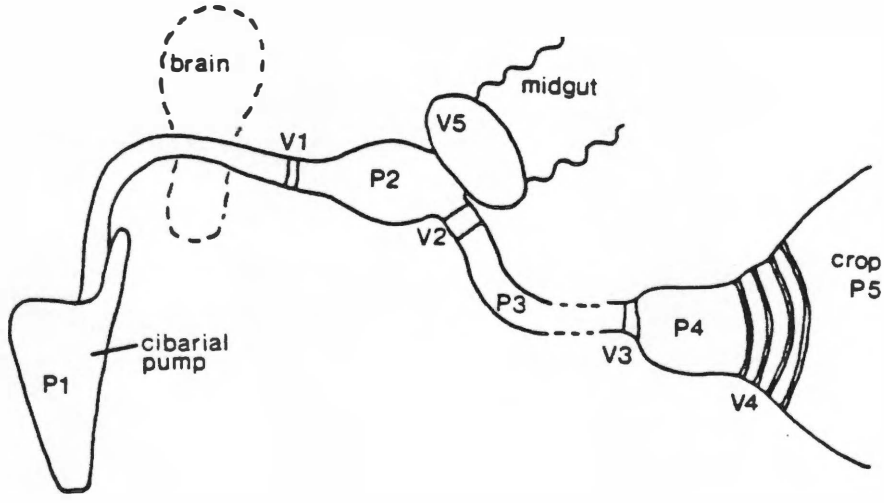
The potential of pathogen transmission by regurgitation, compared to transmission through the hindgut and feces, is still not fully realized. Kumm (1935) reported that during passage through the digestive tract the numbers of Treponema pertenue were drastically reduced, yet regurgitation droplets still contained viable organisms. Glass and Gerhardt (1983) found that Moraxella bovis survived better in the crop, from where it could be regurgitated, than in either the midgut (lower survival), hindgut (no survival) or haemolymph (no occurrence). Estimates of the numbers of regurgitation droplets deposited on a substrate by a single fly ranged from 30 over a four hour period (Kumm, 1935) to 80-240 per day (Zimin, 1944). Zimin (1944) calculated that seven billion drops were deposited on food, counters. . . by fecal and vomit drops each day in one market in

Tadzhikistan, which gives some idea of the potential transmission rates via regurgitation. Kloft et al. (1980) and Butler et al. (1977) found that in the stable fly, Stomoxys calcitrans, up to 1/10 of the food ingested was regurgitated, while in the tsetse fly, Glossina morsitans, the amount regurgitated ranged from 1/10 to 3/10 of the total ingested.

The mechanism by which regurgitation occurs was included in Graham-Smith's (1934) work with Calliphora vicana and Thomson's (1975) work with Phormia regina. Other than these studies, little work has involved the mechanism of regurgitation by the Diptera, except for its role as a means of pathogen transmission.

Graham-Smith (1934) described four sphincters in the foregut of Calliphora vicana: 1) an anterior esophageal sphincter where the esophagus arrises, 2) a posterior esophageal sphincter at the level of the bifurcation of the crop duct, 3) a crop-duct sphincter, and 4) a proventricular sphincter. Regurgitation occurs when sphincters 1, 2, and 3 are open, and sphincter 4 is closed. Contractions of the crop can then force fluid out the proboscis.

The foregut of Phormia regina (Figure 1) has been described as including: valves 1 through 5 (V1, V2, V3, V4, and V5) and pumps 1 through 5 (P1, P2, P3, P4, and



V1 - In the post-ganglionic region of the oesophagous.
V2 - The sphincter at the mouth of the crop duct.
V3 - The anterior limit of the coarse muscle bands of the crop duct.
V4 - Circular muscles in the crop wall.
V5 - The proventricular valve.

P1 - The cibarial pump.
P2 - The post-ganglionic region of the oesophagous.
P3 - The crop duct.
P4 - Coarse muscle fibers in the expanded end of the crop duct.
P5 - The crop.

Figure 1. Diagram of the foregut of P. regina showing the position of the valves and pumps.

Source: Alan J. Thomson, "Syncronization of Function in the Foregut of the Blowfly Phormia regina (Diptera: Calliphoridae) during the Crop-Emptying Process." Can. Entomol. 107:1193-1198 (1975).

P5). Regurgitation occurs when the crop is greatly distended, valves 3 and 4 are forced open, and P3 is distended (peristalsis is damped). The pressure from the crop forces V2 to leak, with fluid entering P2. If V2 opens while P2 is partially filled, P2 will contain an abnormally high volume of liquid. When this occurs the fluid will be forced through either V1, resulting in regurgitation, or V2, with the fluid returning to P3 (Thomson, 1975). According to this theory, regurgitation should only be an occasional occurrence. Thomson and Holling (1975) also found that an increase in the osmotic blood pressure would reduce the rate of crop emptying, and presumably the incidence of regurgitation.

iii - Purpose of this Study

Preliminary observations indicated that three forms of regurgitation commonly occur in Musca autumnalis: 1) regurgitated fluid is used to dissolve a solid substance, i.e. powdered milk, 2) small amounts of liquid may be deposited onto the substrate during tarsal probing, and 3) drops of liquid may be extruded from the tip of the proboscis (Figure 2). This latter form of regurgitation frequently occurs following feeding and appears to serve no logical purpose. Although this phenomenon has been noted before (Graham-Smith, 1914), there have been no

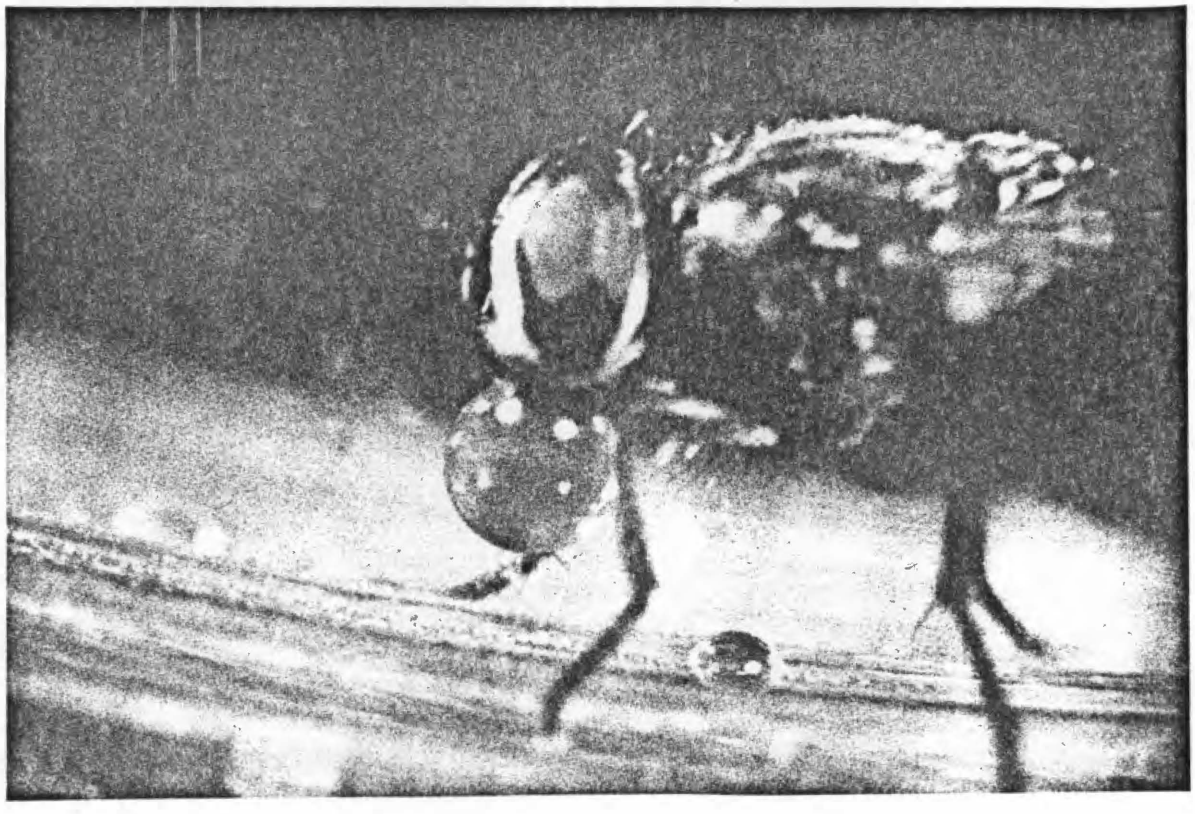


Figure 2. A Regurgitation Droplet Extruded from the Proboscis of the Face fly, Musca autumnalis.

detailed observations or experiments attempting to define this process of regurgitation. Other than the fact that its occurrance has been noted, it remains an unknown process. This study was designed to provide such an understanding by 1) analyzing the mechanism of regurgitation, 2) determining the effect of various factors (type of food, amount and concentration of food, temperature, relative humidity. . .) on regurgitation, and 3) determining if regurgitation does occur onto the eyes of cattle and might have the potential to play a role in the transmission of pathogens by the face fly.

CHAPTER II

ANALYSIS OF THE MECHANISM OF REGURGITATION BY THE FACE FLY

i - Introduction

Regurgitation is known to occur in a number of Dipteran species, including the face fly, Musca autumnalis DeGeer (Graham-Smith, 1914, 1934; Kloft et al., 1980; Glass and Gerhardt, 1984), and has been implicated as a possible mechanism of pathogen transmission (Kumm, 1935; Zimin, 1944; Dipeolu, 1977, 1982; Glass and Gerhardt, 1984). Graham-Smith (1934) described the mechanism by which regurgitation occurs in Calliphora vicana, and Thomson (1975) examined regurgitation by the blowfly, Phormia regina. Thomson concluded that regurgitation occurs when the crop is greatly distended, valves 3 and 4 (Figure 1, page 15) are forced open by the liquid, and P3 is distended. If V2 opens while P2 is partially filled, P2 will contain an abnormally high volume of liquid, which will be forced through either V1, resulting in regurgitation, or through V2, with the fluid returning to the crop duct.

No other research has been conducted on the mechanism of this potentially significant method of

disease transmission by the Diptera. The objective of this study was to examine some of the basic features of regurgitation, such as the path the liquid follows during regurgitation and the duration and frequency of this phenomenon.

ii - Materials and Methods

Fly Standardization Procedures

Face fly pupae were placed, 24 hours prior to emergence, into screened one-pint ice-cream containers. The containers held sterilized water and solid sucrose. Six-day old female flies were deprived of sucrose for the 24 hours preceding any experiment in order to obtain standardized flies (6SFFF). Water was available in all cases except where noted. The majority of the studies were conducted within a 91 x 41 x 41 cm environmental chamber made of plexiglass (Figure 3). 76% relative humidity (RH) was maintained using a solution of glycerol in water and the temperature was 24°C. The light regime was 16:8 (L:D). The pint ice-cream containers holding pupae were placed in the environmental chamber prior to emergence of the flies.

General Features of Regurgitation

It was necessary to obtain an overview of the processes both preceding and during regurgitation.

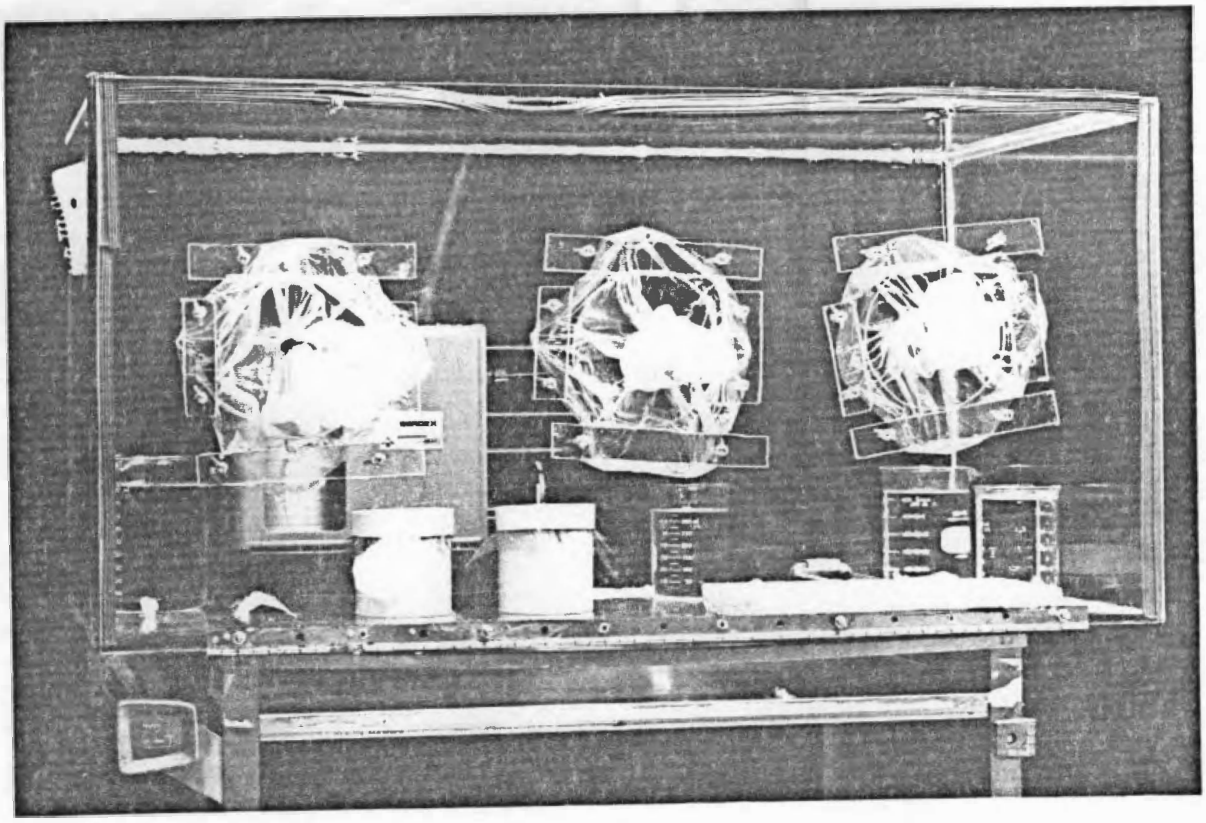


Figure 3. The Environmental Chamber used for the Majority of Experiments.

Six-day old standardized female face flies (6SFFF) were placed in 9 cm diameter petri dishes containing 5 ml of 4.8% (weight/volume) trypticase soy broth (TSB) in water. This amount was sufficient to ensure that all flies put in a petri dish could feed to repletion. The behavior of individual flies and groups of flies was examined following feeding and during regurgitation.

The choice of 6-day old female flies as experimental animals was based on an experiment which examined the effects of the age and sex of the flies on regurgitation. Flies of certain ages are known to preferentially feed upon certain foods (Hower and Cheng, 1972), and proteins are necessary for the development of the ovaries in many insects (Wang, 1964; Turner and Hair, 1967). In order to determine if these factors affect regurgitation, groups of 10 standardized face flies of each sex were fed 10 ml of 4.8% TSB. Each of these groups of 10 flies consisted of flies of a known age, ranging from one to six days old. All flies were examined for the extrusion of regurgitation droplets at the tip of the proboscis for periods of two minutes duration. These periods commenced 10 minutes after feeding began and continued every 10 minutes until no flies were observed regurgitating during three consecutive 10 minute observation periods. The number of periods during which regurgitation occurred and

the highest percentage of flies regurgitating during a single period were the criteria used to make comparisons between flies of both sexes at various ages.

Video Analysis of Regurgitation

Female face flies (6SFFF) were placed in petri dishes and allowed to feed to repletion on a 4.8% solution of TSB containing small amounts of fluorescein isothiocyanate or carborundum. Feeding, post-feeding activity and regurgitation by the flies was recorded using a video camera attached to a dissecting microscope.

Analysis of the Path of Fluid During Regurgitation

In order to determine the source of the regurgitated fluid, 6SFFF were fed a 4.8% solution of TSB containing phenyl red (an indicator dye that turns yellow at a pH of less than 6.8). The color of the regurgitated fluid was observed in 100 flies, red indicating that the fluid had come from the crop and yellow indicating that it had come from the acidic midgut. Twenty-five flies which had been fed solutions containing phenyl-red were dissected to reveal the entire alimentary canal.

In order to determine the destination of the regurgitated fluid, 6SFFF were individually confined in 45 x 13 mm vials with screened ends. The flies were

offered 15 ul of 4.8% TSB, which was a light yellow color. Once regurgitation commenced, powdered nigrosin on the tip of a Hamilton microsyringe was touched to the regurgitation droplet. Flies which continued regurgitating for a minimum of 15 seconds after the nigrosin was innoculated into the regurgitation droplet were immediately frozen at -40°C. This period of time allowed the nigrosin to completely circulate with the regurgitated fluid. Flies which immediately ceased regurgitating or which only regurgitated for periods of less than 15 seconds were discarded. An additional group of flies was allowed to continue regurgitating for a period of at least 45 seconds in order to determine if the destination of the regurgitated fluid changed over time. This group of flies was also immediately frozen at -40°C at the end of the 45 second period. All flies which were frozen were dissected to reveal the alimentary canal from the bifurcation of the crop duct and the mid-gut back to the hindgut. Any areas containing nigrosin (purple color) were recorded.

Analysis of the Volume of the Regurgitation Droplet

The volume of the fluid extruded in a regurgitation droplet in relation to the total amount of fluid ingested was recorded using two separate techniques. 6SFFF were

offered 15 ul of 3.0 or 4.8% TSB (under ambient conditions). The diameter of the regurgitation droplets was measured (using a micrometer in a dissecting microscope) for 20 flies fed each concentration of TSB, and the volume of the drop calculated. The mean percent of ingested fluid contained in the regurgitation droplets was determined. In addition, a capillary tube with 1 ul intervals marked on it was touched to the regurgitation droplet of 15 flies fed each concentration of TSB. The average volume of the drops was determined.

Analysis of Changes in Ingested Material due to Regurgitation

In order to determine if the process of regurgitation resulted in any detectable changes in the ingested material (such as a reduction of water content), the osmolality of the ingested material (4.8% TSB) was measured and compared to the osmolality of regurgitation droplets. Regurgitation droplets were collected by touching a capillary tube to a droplet at the tip of a flies' proboscis. Collection of the regurgitation droplets began 30 minutes after a fly began regurgitating and continued for a 30 minute period.

Qualitative determinations were made by collecting regurgitation droplets and analyzing them using the technique of disc electrophoresis. 6SFF were fed a

solution containing 30% sucrose, 1% bovine albumin, and 69% water. Collection of regurgitation droplets began 30 minutes after each fly was observed regurgitating and continued for 30 additional minutes. The electrophoretic pattern of the regurgitated droplets was compared to the pattern of the ingested solution.

iii - Results and Discussion

General Observations

During our observations of feeding and regurgitation by several hundred flies a general pattern emerged. Standardized face flies introduced into a petri dish rapidly, and seemingly randomly, moved throughout the dish until they encountered the TSB with their tarsi. The proboscis was then extended and the fly would feed for several seconds. After this initial feeding period the flies would continue searching for food throughout the petri dish. When food was again encountered the proboscis would be extended, but in this case it would remain extended for only a fraction of a second. This is in agreement with observations by Dethier (1948), who found that flies fed to repletion on a given food source would only react to a more concentrated solution of the same substance.

After several minutes of continued searching activity, during which they were extremely mobile, the flies would become relatively motionless and commence to "groom" themselves (i.e. clean wings and legs) for periods ranging from 30 seconds up to several minutes. Following this "grooming" period the flies would normally remain stationary and begin regurgitating. The onset of regurgitation normally occurred within 5 to 10 minutes after feeding had ceased. Once flies were regurgitating they normally continued without interruption for periods lasting from several minutes up to an hour or more. If disturbed by movement of other flies or by other activity, such as movement by the viewer, the flies ceased regurgitating and normally moved rapidly throughout the petri dish in an agitated state. The flies soon resumed a stationary position and regurgitated again. The majority of flies within a petri dish began regurgitating within 10 minutes after feeding and continued to do so for at least 45 to 60 additional minutes.

The regurgitation droplet was normally held motionless at the tip of the proboscis, however, several variations were observed. In some instances the regurgitation droplet would slowly be sucked in and out, while at other times the proboscis itself was slowly

vibrated, resulting in the regurgitation droplet being moved back and forth. While not a common occurrance, this behavior occurred in approximately 10% of the flies observed regurgitating. Face flies apparently have precise control over movement of the regurgitation droplet.

Closer examination of a regurgitation droplet through a dissecting microscope revealed an almost constant circulation of the fluid. By innoculating several flakes of fluorescein isothiocyanate into the TSB before feeding it was possible to demonstrate the apparrent constancy and rapidity of circulation within the droplet. In most cases, circulation of the liquid began with the extrusion of the droplet and continued until the drop was sucked in by the fly. The means by which this circulation occurred was more closely studied by using a videotape of regurgitation to examine the process.

Table 1 summarizes the variations in feeding and regurgitation in male and female flies aged one - six days old. While the age and the sex of the flies apparently did affect regurgitation, the results were more a reflection of variations in feeding rather than actual differences in the levels of regurgitation. Female flies aged three to six days, however, did differ

Table 1. Effect of Age and Sex of Flies on Regurgitation.

Age (Days)	Sex	Approximate Amount (ul) of Food Consumed	Average Percentage of Flies Regurgitating in a 10 min. Period	No. of Periods With Flies Regurgitating
1	Male	0	0.0	0/5
	Female	0	0.0	0/5
2	Male	0	0.0	0/7
	Female	0	0.0	0/7
3	Male	3	1.3	1/8
	Female	10	3.8	3/8
4	Male	3	0.7	1/14
	Female	10	2.9	2/14
5	Male	5	2.2	2/9
	Female	10	30.0	14/19
6	Male	5	3.3	3/15
	Female	10	38.3	20/22

in the amounts of time spent regurgitating. These differences could not be explained by variations in feeding alone, and were due solely to the age of the fly. With increasing age female flies were observed regurgitating in more 10 minute observation periods than their younger cohorts. On this basis six-day old female flies were chosen as experimental animals for the remainder of the studies.

Video Analysis of Regurgitation

The videotape of regurgitation (in pocket) is a detailed examination of the events outlined in the previous section. It is intended to provide an in depth view of the events occurring both preceding and during regurgitation. Feeding and post-feeding activity normally precede regurgitation and play an integral role in the process. They are included in the videotape as a clarification of previously discussed observations.

When face flies were fed a solution of TSB it was impossible to determine if the fluid within a regurgitation droplet was circulating. The addition of fluorescein isothiocyanate or carborundum to the TSB resulted in a solution containing visible particles. These particles were readily detected within regurgitation droplets under certain lighting conditions.

Regurgitation normally commenced with the proboscis retracted. On some occasions, however, the proboscis was extended and retracted several times as the regurgitation droplet was extruded. Regurgitation in all cases began with the closed labellae being spread open, revealing the pseudotrachae. As the labellae were spread liquid began exuding from the food canal. From the time of the initiation of regurgitation until the time the drop was sucked in again the liquid was normally circulating. Under certain lighting conditions this circulation was not apparant; however, when the lighting was modified it became evident.

Although Thomson (1975) postulated that regurgitation by Phormia regina was the result of accidental overfilling of P2 (Figure 1, p. 15), this does not appear to be the case with M. autumnalis. If regurgitation in face flies was the result of accidental leakage due to an overfilling of P2 it should happen only rarely and no circulation of the liquid should occur. Observations of face flies regurgitating demonstrated that the process continued for up to four hours and that circulation of the fluid occurred in almost 100% of the cases viewed. This would preclude accidental leakage of fluid through V1 (Figure 1, p. 15) as the means by which regurgitation occurs.

One phenomenon regarding circulation of the fluid within the drop was noted. The sucking mouthparts of the Diptera contain both a food and a salivary canal (Snodgrass, 1944). We have frequently observed regurgitation droplets in face flies which maintain a uniform size despite the rapid circulation of liquid. This is a counterintuitive result, as a constant circulation of fluid within a drop should result in either an increase or a decrease in the size of the drop, given the single food canal. A fully satisfactory explanation of this phenomenon has not been developed, although a tentative hypothesis is suggested. A bidirectional, or counter-current, flow of liquid through the food canal would result in an equal amount of food entering the droplet as was leaving it. The size of the droplet would not necessarily vary given this situation. A further evaluation of the events occurring during regurgitation is needed before the exact mechanism by which it occurs is understood.

Analysis of the Path of Fluid During Regurgitation

The main question which arrose from our observations of the videotape of regurgitation was where was the source of the circulating fluid and what was its eventual destination?

Of the 100 flies which fed on TSB containing phenyl red, 100 extruded red regurgitation droplets, indicating the liquid had come from the crop. The midguts and the hindguts of all 25 flies which were dissected were yellow, while the crops were all red, proving conclusively that the red TSB had come from the crop, and not the acidic midgut. This is in agreement with Thomson (1975) who showed that accidental overfilling of P2 (Figure 1, page 15) from the crop of Phormia regina resulted in leakage of liquid through V1 and led to regurgitation.

Of 75 attempts to innoculate nigrosin into regurgitation droplets only 13 were successful (Table 2). This low success rate can be attributed to the typical response of a fly when confronted with the syringe containing Nigrosin. In at least 50% of the attempts to inject Nigrosin into the droplet the flies would move away and immediately cease regurgitating. In most instances, even if nigrosin was successfully innoculated into the droplet, regurgitation would normally cease before the nigrosin had a chance to fully circulate with the liquid.

The crops of all eight of the flies which continued to regurgitate for 15 seconds after nigrosin was innoculated into the regurgitation droplet appeared

Table 2. The Destination of Regurgitated Fluid Containing Nigrosin.

Length of Time Regurgitating After the Innoculation of Nigrosin into the Regurgitation Drop	No. of Flies	Areas of the Alimentary Canal Containing Nigrosin		
		Crop	Midgut	Hindgut
15 secs.	8	Yes	No	No
45 secs.	5	Yes	Yes	Yes

purple. None of the midguts or hindguts contained nigrosin. This indicates that the immediate destination of the regurgitated fluid was the crop.

All five flies which regurgitated for a minimum of 45 seconds contained at least traces of nigrosin in the mid and hindguts in addition to the relatively greater amounts of nigrosin in the crop. While these results are tentative, they support the theory that the crop is the immediate destination of fluid and the mid and hindguts are only a secondary destination.

While the video analysis of the regurgitation droplets clearly demonstrated the constancy of fluid circulation, it did not distinguish between possible sources or destinations of the liquid. This experiment clearly demonstrates that the source of the fluid found in regurgitation droplets must be the crop and that this is also the most likely destination of fluid returning to the alimentary canal.

Analysis of the Volume of the Regurgitation Droplet

The two techniques used to calculate the volume of regurgitation droplets resulted in similar data for flies fed 3.0% TSB. The results for flies fed 4.8% TSB, however, were inconclusive (Table 3).

Table 3. The Volume of Regurgitation Droplets Collected from Flies Fed 3.0% and 4.8% Solutions of Trypticase-soy broth.

Concentration of TSB (Percent)	Number of Drops Measured	Mean Amount of TSB (ul) Ingested	Mean Diameter (mm) of Droplet	Mean Volume (ul) of Droplet	Percent of Ingested Fluid
3.0 [a]	20	10.0	1.1	0.68	6.8
4.8 [a]	20	12.0	1.3	1.15	9.5
3.0 [b]	15	9.5	-----	0.60	6.3
4.8 [b]	16	12.0	-----	0.61	5.1

[a] - Diameter of the droplets measured using a micrometer.

[b] - Volume of the droplets measured using a capillary tube with 1 ul increments marked.

By using a micrometer to measure the size of a droplet it was not necessary to disturb the fly, except in moving the petri dish. However, the micrometer was calibrated for the surface of the petri dish, so the position of the fly may have affected the accuracy of measurements. We attempted to make all measurements from flies which were upside-down on the top surface of the petri dish in order to obtain standard results.

The collection of droplets using capillary tubes disturbed the flies, possibly causing them to begin sucking the drop in before capillary action took effect and the complete drop was collected. This may have affected the accuracy of these measurements.

The regurgitation droplets of flies fed 3.0% TSB contained approximately 6.5% of the total fluid ingested by the flies. The percentage regurgitated by flies fed 4.8% TSB ranged from 5.08% to 9.5% (Mean - 7.29%). The mean percent of fluid regurgitated was significantly greater ($p < .05\%$) for those flies fed 4.8% TSB when compared to those fed 3.0% TSB when measured using the micrometer (Table 3). The results obtained from the collection of regurgitation droplets are presumably less accurate, as previously explained

These amounts of material regurgitated are comparable to the amounts regurgitated by Stomoxys

calcitrans. Stable flies fed blood were found to deposit up to 1/10 of the ingested material on a substrate during regurgitation (Kloft et al., 1980). Our results, calculated after measurements with a micrometer, indicate that higher concentrations of food result in increasing amounts of material regurgitated. It is likely that flies fed on either a blood meal or on secretions from a cow with visible oral lacrimation would regurgitate amounts of food comparable those witnessed by Kloft et al. (1980). These results do indicate that a relatively large portion of a meal may be regurgitated at any one time. This is corroborated by the videotape of regurgitation (in pocket), where in some instances the size of the regurgitation droplet is almost equal to the size of the flies' head.

Analysis of Changes in Ingested Material due to Regurgitation

The osmolality of the ingested and the regurgitated fluids differed significantly ($p < .05$). The mean osmolality of the 4.8% TSB was 475.9 milliosmoles/kg ($n = 13$) while the osmolality of the fluid regurgitated 30 minutes after feeding averaged 1166.4 milliosmoles/kg ($n = 16$).

This data did indicate that a change had taken place in the interval between feeding and the period when the

regurgitation droplets were collected. Possible changes which might have ocurred include 1) a reduction of the water content of the ingested material, 2) the addition of enzymes to the fluid, and/or 3) the breakdown of the protein molecules present in TSB.

Simple evaporation of water from the regurgitated fluid may have resulted in an increase in osmolality. To control for this we placed 10 ul drops of 4.8% TSB (n=10) on the surface of petri dishes. These dishes were exposed to ambient conditions. After 1 hour the osmolality of these drops had increased to a mean of 539.8 milliosmoles/kg. This increase by itself was not sufficient to explain the increase in the osmolality of the regurgitated fluid. The regurgitated fluid, however, is constantly circulating, which would increase the rates of evaporation. Evaporation should not be ruled out as the mechanism which resulted in the increase in the osmolality.

The results of the disc electrophoresis were not conclusive. While the regurgitated fluid was found to still contain proteins, qualitative determinations were not possible. Several additional protein bands were evident in the regurgitated fluid which were not seen in the electrophoretic pattern of the ingested fluid. This suggests that the proteins may have been partially broken

down, or enzymes added to the liquid. Protein determinations comparing the ingested fluid to regurgitation droplets collected over a period of time are needed to solve this problem.

iv - Summary

This form of regurgitation is a common phenomenon in face flies, as indicated by its frequency. The deposition of regurgitation droplets onto the substrate was almost never witnessed, and it is believed that this deposition was accidental when it did occur. This indicates that regurgitation most likely has some function other than a removal of excess liquid. The regurgitated material is changed during the interval between ingestion of the food and the time when the droplet was collected. It is possible that the constant circulation of the material could have accounted for this change. A great deal of additional research is needed to completely define the mechanism of this process.

CHAPTER III

THE EFFECT OF VARIATION IN INGESTED FOOD AND ENVIRONMENTAL FACTORS ON REGURGITATION

i - Introduction

The face fly, Musca autumnalis (DeGeer), is a major pest of livestock which occurs throughout most of the continental United States (Poorbaugh and Smith, 1968; Pickens and Miller, 1980). The most significant damage caused by the face fly is its role vectoring of the bacterium Moraxella bovis Hauduroy, the primary causative agent of pinkeye in cattle (Gerhardt et al., 1976, 1982; Pickens and Miller, 1980; Hall, R.D., 1984). Glass and Gerhardt (1984) suggested that regurgitation of ingested lacrimal secretions containing the bacteria was the most likely mechanism of pathogen transmission.

Various environmental factors, such as relative humidity and temperature, are known to affect the behavior of various species of flies belonging to the family Muscidae. Roberts and Pitts (1971) and Dakshinamurty (1948) demonstrated that the face fly and the house fly, M. domestica, preferred low relative humidities over high relative humidities when offered a choice. Face flies also preferred a temperature of 30°C

over both lower and higher temperatures (Dakshinamurty, 1948). The effect of these environmental factors upon regurgitation has not been determined.

Various aspects of ingested food, such as amount and concentration of material, are known to affect feeding behavior of flies (Dethier, 1948). The acceptance threshold for feeding varies with the age and sex of the fly, its nutritional needs, and the quality (concentration) and quantity of the food. The precise role of these factors in relation to regurgitation is not known, although Thomson and Holling (1975) found that the osmotic blood pressure affected the rate of crop emptying. This may also affect the rate of regurgitation.

The effects of temperature, relative humidity and dessication, as well as the effects of various amounts and concentrations of food on regurgitation were determined.

ii - Materials and Methods

Effects of the Type of Food on Regurgitation

Groups of 10 six-day old female face flies (6SFFF) were confined individually within 45 x 13 mm vials and offered either solid sucrose, powdered milk, 10 ul 3.0% Trypticase-soy broth (TSB), 10 ul 4.8% TSB, 10 ul 4.8% sucrose (in water), or 10 ul 25% sucrose. All flies were

examined during two minute periods for the extrusion of regurgitation droplets at the tip of the proboscis. These observation periods began 10 minutes after feeding had started and continued every 10 minutes until no flies were observed regurgitating during three consecutive periods. The percentage of flies regurgitating during each observation period was calculated. The number of periods during which regurgitation occurred and the average percentage of flies regurgitating during the 10 minute periods were the criteria used to make comparisons between the effects of various food types on regurgitation.

Effects of Amount of Food, Food Concentration, Relative Humidity and Temperature on Regurgitation

This section was designed as two separate experiments. The first examined the effects of four relative humidity levels (32%, 54%, 74%, and 90%), and three concentrations (1.2%, 3.0%, and 4.8%), and amounts (5 ul, 10 ul, and 15 ul) of TSB on regurgitation. The interactions between these variables were also examined (the temperature was maintained at 24°C). The second experiment substituted four temperatures (18°C, 24°C, 29°C, and 34°C) for the four relative humidity (RH) levels, with the concentrations and amounts of TSB the same as in the first experiment (the RH was maintained at

75%). The design of each experiment consisted of a 4 x 3 x 3 factorial.

For each temperature or relative humidity under which the experiment occurred, nine groups of nine 6SFFF were observed for the extrusion of regurgitation droplets at the tip of the proboscis. One fly in each group was offered one of the following combinations of TSB: 5 ul, 1.2%; 5 ul, 3.0%; 5 ul, 4.8%; 10 ul, 1.2%; 10 ul, 3.0%; 10 ul, 4.8%; 15 ul, 1.2%; 15 ul, 3.0%; 15 ul, 4.8%. In this way, 81 observations (or sets of observations) were made for each relative humidity level or temperature tested. 108 observations were made for each amount or concentration of TSB tested in each experiment.

The face flies were reared under the conditions they were tested in (i.e. at 34°C and 76% RH), and standarized as with previous experimental flies. The flies being tested were individually placed in 45 x 13 mm vials (under test conditions) and the predetermined amount and concentration of TSB was placed into the vial using a Hamilton microsyringe.

Each fly was examined every 30 seconds for the presence or absence of a regurgitation droplet at the tip of its proboscis. These observations commenced two minutes after the TSB had been injected into the vial, and continued for 30 minutes. The presence or absence of

a regurgitation droplet during each 30 second observation period was noted with a plus (+) or a minus (-) for each fly. The total number of plus observations of regurgitation for each fly was determined and converted to minutes spent regurgitating during a 30 minute period (each positive observation equals 1/2 minute). The mean number of drops deposited was calculated for each main factor and for the interactions. The data were tabulated for the main factors and the two-way interactions and the effects of the main factors graphed.

A 4 x 3 x 3 factorial was used to determine if significant differences existed amongst the main factors as well as among the interactions between factors for each separate experiment. The total number of (+) interactions/30 minute period was calculated and used to test for significant differences, and the Student-Newman-Keul Test used to separate means.

The effect of the concentration and amount of TSB on the duration of regurgitation was also determined. 6SFFF were placed in 45 x 13 mm vials (24°C and 75%RH) and fed 10 ul of 1.2%, 3.0%, or 4.8% TSB, or offered 5, 10, or 15 ul of 4.8% TSB. Ten flies in each group were examined for regurgitation beginning 10 minutes after feeding had commenced and continuing every 10 minutes until no flies were observed regurgitating during three consecutive

observation periods. The percentage of flies regurgitating during each observation period was calculated and used to graph the effect of each concentration and amount of TSB.

Effects of Changing the Concentration of Food on Regurgitation

Flies which feed to repletion on one concentration of food will increase their intake if offered a more concentrated source of food (Dethier, 1948). It is possible that this increase in food ingested could result in an increase in the amount of regurgitation. Twenty groups of ten 6SFFF were placed in petri dishes and offered 10 ml of 1.2% TSB and allowed to feed to repletion. The number of flies regurgitating was recorded during the 30 minute period following the introduction of the TSB into the petri dish, and the percentage of flies regurgitating was calculated. After this 30 minute period had ended, 10 ml of 4.8% TSB was added to the petri dish, and the number of flies regurgitating during the next 30 minutes was calculated.

Effects of Dessication on Regurgitation

Groups of 10 female face flies were deprived of sucrose (the only food source) for 24 hours, and water for either 0, 3, 6, 12, 18, 24, or 48 hours prior to the

placement of TSB in the petri-dish. Regurgitation was recorded (as per the experiment on the long term effect of the concentration and amount of TSB on regurgitation) for all seven groups of flies, and the mean amount of time spent regurgitating was calculated and graphed for each group. The correlation coefficient between the amount of time without water and the amount of regurgitation was calculated.

iii - Results and Discussion

Effects of the Type of Food on Regurgitation

Effects of various food types on regurgitation are summarized in Table 4. Solid sucrose and powdered milk were readily ingested, although the period needed to feed to satiation was longer than that generally needed for flies fed liquids. The amounts consumed were not quantitatively determined. In no instance was a fly which had fed upon a solid substance observed regurgitating. Because the completion of feeding took much longer than observed for flies fed liquid substances, these flies were examined for regurgitation droplets over a three hour period. The delay in feeding to satiation could have adversly affected regurgitation and delayed its initiation. Regurgitation was not observed in any fly during this three hour period.

Table 4. Effect of Type of Food Ingested on Regurgitation.

Type of Food	Amount (ul) of Food	Mean Amount (ul) Eaten	Average Percentage of Flies Regurgitating in a 10 min. Per.	No. of Periods With Flies Regurgitating
Solid Sucrose	----	Unknown	0	0/5
Powdered Milk	----	Unknown	0	0/5
3.0% TSB	10	8	19	7/10
4.8% TSB	10	10	38	20/23
4.8% Sucrose	10	2	0	0/5
25% Sucrose	10	10	51	35/40

Liquid TSB and sucrose solutions were readily ingested. A higher concentration of sucrose, however, was required to stimulate feeding. All of the flies used for this experiment were 6SFFF which had been deprived of protein until the experiment began. For the first five days of their lives sucrose was freely available. This difference in feeding on carbohydrates and proteins during the first five days of their lives possibly resulted in a lowering of the initiation threshold required for feeding upon proteins. Female flies require a protein source for ovarian development so TSB was readily consumed after five days of protein deprivation. This may explain the difference between the concentrations of TSB and sucrose required to initiate feeding. If these differences are taken into account the incidence of both feeding and regurgitation may be adequately explained.

Regurgitation occurred in the majority of the flies fed the higher concentration of TSB or sucrose. Slightly higher percentages of flies which had fed upon 25% sucrose regurgitated, and they did so for a longer time period than did those flies fed 4.8% TSB. Regurgitation occurred much more frequently in the flies fed the higher concentrations of both substances than in flies fed either of the lower concentrations.

It appears that regurgitation will only occur when face flies feed upon liquids. The effects of carbohydrates and proteins were comparable (in terms of both the numbers of flies regurgitating and the duration of regurgitation) once the feeding history was taken into account. The concentration of the food source apparantly affects regurgitation.

Effects of the Amount of Food, Food Concentration Relative Humidity and Temperature on Regurgitation

The amount of food, food concentration, relative humidity level and temperature were all found to significantly affect regurgitation. The analysis of variation (ANOVA) for the effect of the relative humidity level and the amount and concentration of TSB on regurgitation is summarized in Table 5. The effects of temperature as well as the amount and concentration of TSB are presented in Table 6. All observations for regurgitation were transformed. The transformation consisted of taking the square root of each observation period (the number of plus observations per 30 minute period) plus one. Many values of zero were recorded during the experiment due to the technique used for measuring regurgitation. The transformation was intended to account for this.

Table 5. ANOVA: Effects of Relative Humidity, Amount of TSB, and Concentration of TSB on Regurgitation (Transformed).

Source	DF	Sum Squares	F Value	PR > F
Hum	3	35.64747	5.56	0.0001
Conc	2	813.11263	190.09	0.0001
Hum*Conc	6	28.07126	2.19	0.0443
Amt	2	360.64989	84.31	0.0001
Hum*Amt	6	27.88005	2.17	0.0457
Conc*Amt	4	109.46768	12.80	0.0001
Hum*Conc*Amt	12	29.71034	1.16	0.3137

Table 6. ANOVA: Effects of Temperature, Amount of TSB, and Concentration of TSB on Regurgitation (Transformed values).

Source	DF	Sum Squares	F Value	Pr > F
Temp	3	181.47941	25.87	0.0001
Conc	2	412.94612	88.30	0.0001
Temp*Conc	6	36.54797	2.60	0.0179
Amt	2	221.03031	47.26	0.0001
Temp*Amt	6	66.60625	4.75	0.0001
Conc*Amt	4	29.55777	3.16	0.0145
Temp*Conc*Amt	12	56.15183	2.00	0.0241

Effect of Main Factors. Effects of relative humidity, temperature, concentration of TSB and amount of TSB on regurgitation appear in Figures 4, 5, 6, and 7, respectively.

Significant differences ($P < .05$) were found among the four relative humidity levels tested. Table 7 summarizes these differences. Low relative humidity levels (32% and 54%) resulted in higher amounts of regurgitation by the face flies then did high levels (72% and 90%). In no instance were significant differences noted between the most similar relative humidity levels (32% and 54%, 54% and 72%, or 72% and 90%).

Increasing the amount or concentration of TSB significantly ($P < .05$) increased the incidence of regurgitation (Figures 6 and 7). The effect of these factors had already been noted and discussed in a previous section. The data presented in these figures confirms those findings.

Regurgitation was significantly ($P < .05$) different at each temperature examined. The two extreme temperatures examined (35°C and 18°C) resulted in lower levels of regurgitation than did temperatures of either 29°C (maximum regurgitation) or 24°C (intermediate amount of regurgitation). These results are not suprising, as the

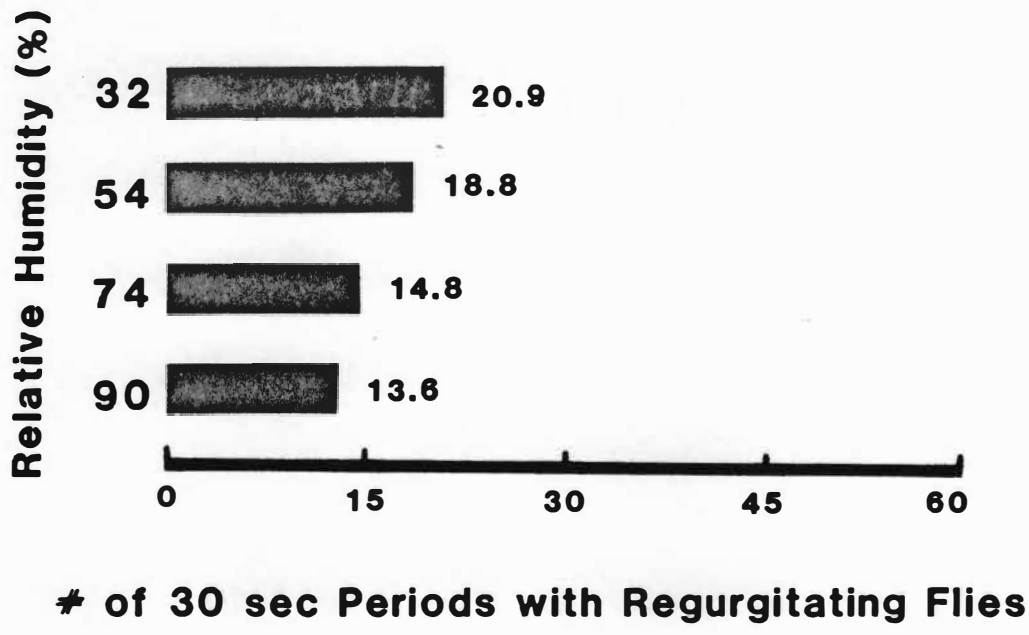


Figure 4. Effect of Relative Humidity on Regurgitation.

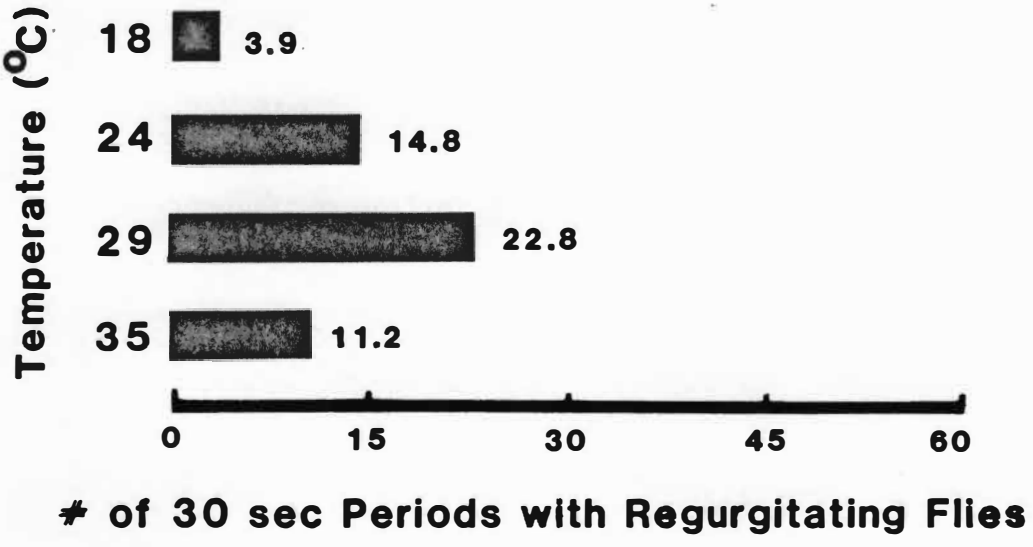


Figure 5. Effect of Temperature on Regurgitation.

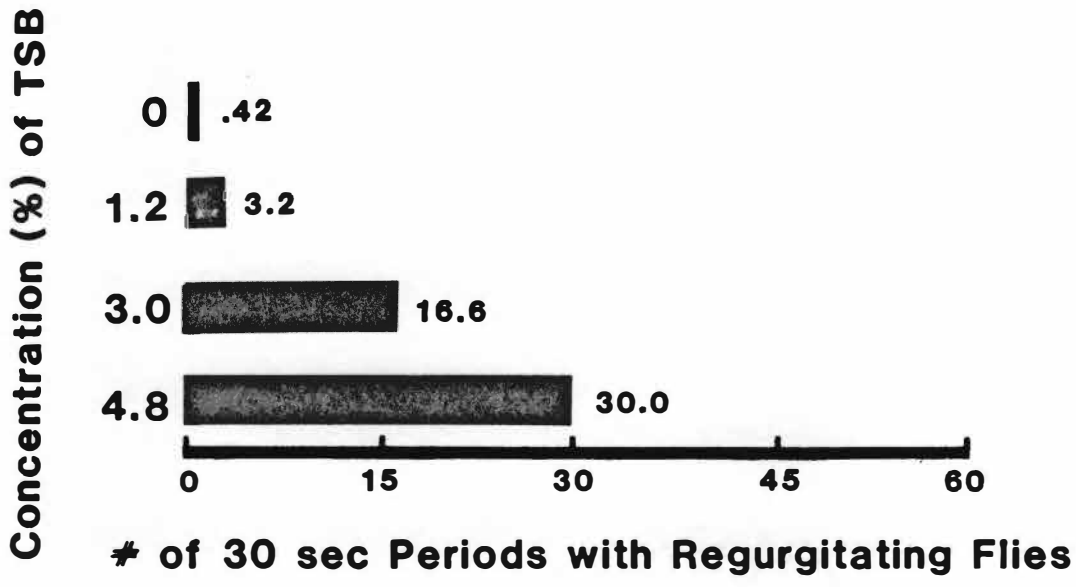


Figure 6. Effect of Concentration of TSB on Regurgitation.

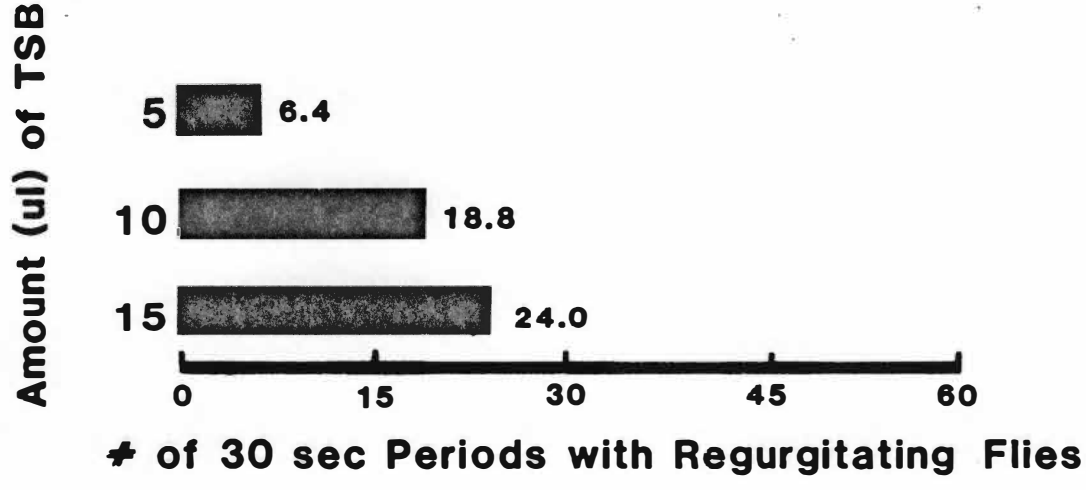


Figure 7. Effect of Amount of TSB on Regurgitation.

Table 7. Mean Separation for the Effects of Relative Humidity Levels on Regurgitation.

Grouping [a]	Mean [b]	N	Humidity (%)
A	13.73	81	32
A			
B A	12.84	81	54
B			
B C	9.74	81	72
C			
C	8.11	81	90

[a] Means with the same letter are not not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

optimum temperature for face flies has been found to be 30°C (Dakshinamurty, 1948).

Effects of Two-Way Interactions. Interactions between humidity level and amount of TSB, humidity level and concentration of TSB, amount and concentration of TSB, temperature and amount of TSB, and temperature and concentration of TSB all significantly affected regurgitation. The results of these interactions are summarized in Tables 8, 9, 10, 11, and 12, respectively.

The relative humidity level had a significant effect upon regurgitation when face flies consumed large amounts of TSB (15 ul). When fed lower amounts of TSB (5 or 10 ul) the relative humidity level had no effect (Table 8). The amount of TSB had a significant effect upon regurgitation over all relative humidity levels.

The results for the interaction between relative humidity level and the concentration of the TSB (Table 9) are similar to those for RH and amount of TSB. The concentration of the food affected regurgitation more than did relative humidity. At high concentrations of TSB, however, low humidity levels (32% and 54%) resulted in significantly greater amounts of regurgitation ($P < .05$) than did lower humidities.

Flies which had fed on large amounts (15 ul) of highly concentrated (4.8%) TSB regurgitated much more

Table 8. Mean Separation for the Interactions Between Relative Humidity Level and Amount of TSB.

Grouping [a]	Mean [b]	N	Hum (%)	Amt (ul)
A	27.28	27	32	15
A B A	21.40	27	54	15
B B	16.91	27	72	15
B B	16.22	27	32	10
B B	14.78	27	90	10
B B	13.58	27	54	10
B B	12.41	27	90	15
B B	12.22	27	72	10
C	5.82	27	54	5
C D C	3.20	27	32	5
D C D C	2.85	27	72	5
D D	1.02	27	90	5

[a] Means with the same letter are not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

Table 9. Mean Separation for the Interactions Between Relative Humidity and Concentration of TSB.

Grouping [a]	Mean [b]	N	Hum (%)	Conc (%)
A	35.39	27	32	4.8
A				
A	32.00	27	54	4.8
B	22.53	27	72	4.8
B				
C B	17.86	27	90	4.8
C B				
C B	16.71	27	32	3.0
C B				
C B	14.65	27	54	3.0
C				
C	10.77	27	90	3.0
C				
C	10.70	27	72	3.0
D	1.43	27	72	1.2
D				
D	1.14	27	54	1.2
D				
D	0.65	27	90	1.2
D				
D	0.63	27	32	1.2

[a] Means with the same letter are not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

Table 10. Mean Separation for the Interactions Between Amount of TSB and Concentration of TSB.

Grouping [a]	Mean [b]	N	Amt (ul)	Conc (%)
A	27.78	36	15	4.8
B	19.39	36	10	4.8
B B				
B	16.11	36	15	3.0
C	7.94	36	10	3.0
C C				
C	7.37	36	5	4.8
D	3.03	36	15	1.2
D				
E D	1.48	36	5	3.0
E D				
E D	1.16	36	10	1.2
E				
E	0.00	36	5	1.2

[a] Means with the same letter are not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

Table 11. Mean Separation for the Interactions Between Temperature and Amount of TSB.

Grouping [a]	Mean [b]	N	Temp (°C)	Amt (ul)
A	28.25	27	29	15
B	16.91	27	24	15
B C B	13.16	27	29	10
C B C B	12.68	27	35	15
C B C B C	12.22	27	24	10
C D D	7.36	27	35	10
E D E D	4.11	27	29	5
E D E D	3.03	27	18	15
E D E	2.85	27	24	5
E	1.96	27	18	5
E E	1.81	27	18	10
E E	0.81	27	35	5

[a] Means with the same letter are not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

Table 12. Mean Separation for the Interactions Between Temperature and Concentration of TSB.

Grouping [a]	Mean [b]	N	Temp (°C)	Amt (ul)
A	31.74	27	29	4.8
B	22.53	27	24	4.8
C	13.53	27	35	4.8
C				
C	12.84	27	29	3.0
C				
D C	10.70	27	24	3.0
D				
D E	6.29	27	35	3.0
D E				
D E	6.00	27	18	4.8
E				
F E	2.96	27	29	1.2
F E				
F E	2.10	27	18	3.0
F E				
F E	1.43	27	24	1.2
F				
F	1.02	27	35	1.2
F				
F	0.00	27	18	1.2

[a] Means with the same letter are not significantly different ($P < .05$).

[b] Mean number of 30 sec observation periods (out of 60 periods) with regurgitation observed.

frequently than did flies fed upon lower amounts of less concentrated TSB (Table 10). It appears that the effect of large amounts of food corresponds to the effect of high concentrations of food. The amount of regurgitation in flies that had fed upon small amounts of highly concentrated TSB was similar to that found in flies which had fed upon large amounts of diluted TSB. These results again demonstrate the importance of these two factors on regurgitation. The concentration of the TSB is significant as it affects the amount of food which will be consumed.

As with the relative humidity level, the temperature had a significant effect ($P < .05$) on regurgitation when flies were fed certain amounts of TSB (Table 11). The effect of temperature on regurgitation, however, was more pronounced than the effect of relative humidity level. Temperature had a significant effect on regurgitation in flies which had fed on either 10 or 15 ul of TSB (not just 15 ul as for RH). The amount of TSB significantly affected regurgitation at all temperatures tested.

The concentration of TSB significantly affected regurgitation at all temperatures. As with the amount of TSB the temperature affected regurgitation at high concentrations of TSB but not at low concentrations (Table 12).

Effects of Three-Way Interactions. No significant differences ($P < .05$) were noted between the means of the interactions between relative humidity level, concentration of TSB and amount of TSB (Table 5). As discussed previously the effect of relative humidity on regurgitation is pronounced only at high concentrations and amounts of TSB.

Significant differences ($P < .02$) occurred in the interactions between temperature, amount of TSB and concentration of TSB (Table 5). All three main factors alone were previously found to have a pronounced affect on regurgitation. When two of the three factors were at optimum levels (for example 29°C and 4.8% TSB) the third factor (in this case amount of TSB) significantly affected regurgitation. When two of the factors were at sub-optimum levels (for example 18°C and 1.2% TSB) the amount of regurgitation was low and the third factor had no significant ($P < .05$) effect.

Effect of the Amount and Concentration of TSB on the Duration of Regurgitation. The effects of the amount and concentration of TSB on the duration of regurgitation are summarized in Figures 8 and 9, respectively. Higher concentrations and larger amounts of TSB resulted in regurgitation over a much longer period of time than did lower amounts or concentrations

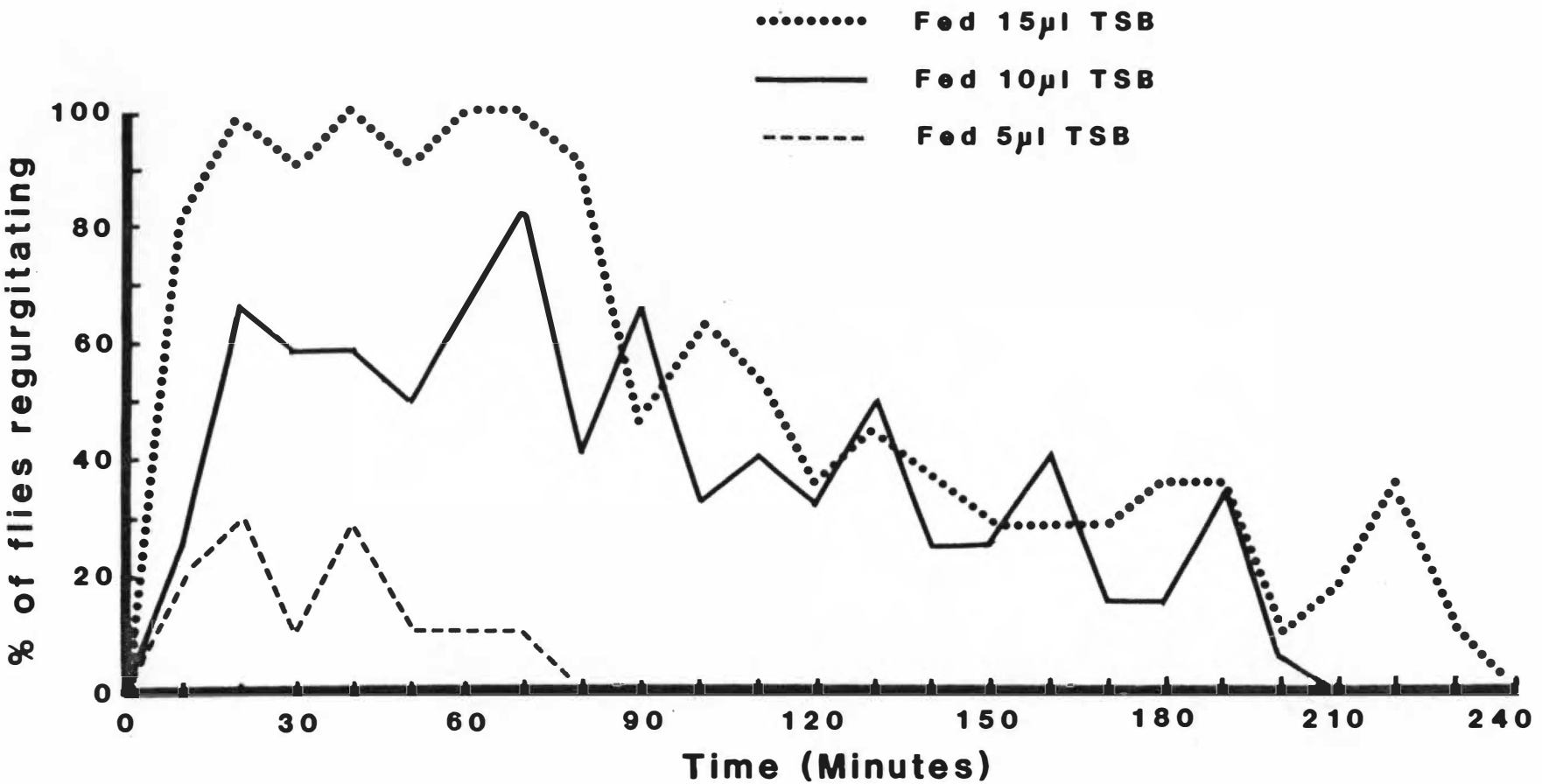


Figure 8. Effect of Amount of TSB on the Duration of Regurgitation.

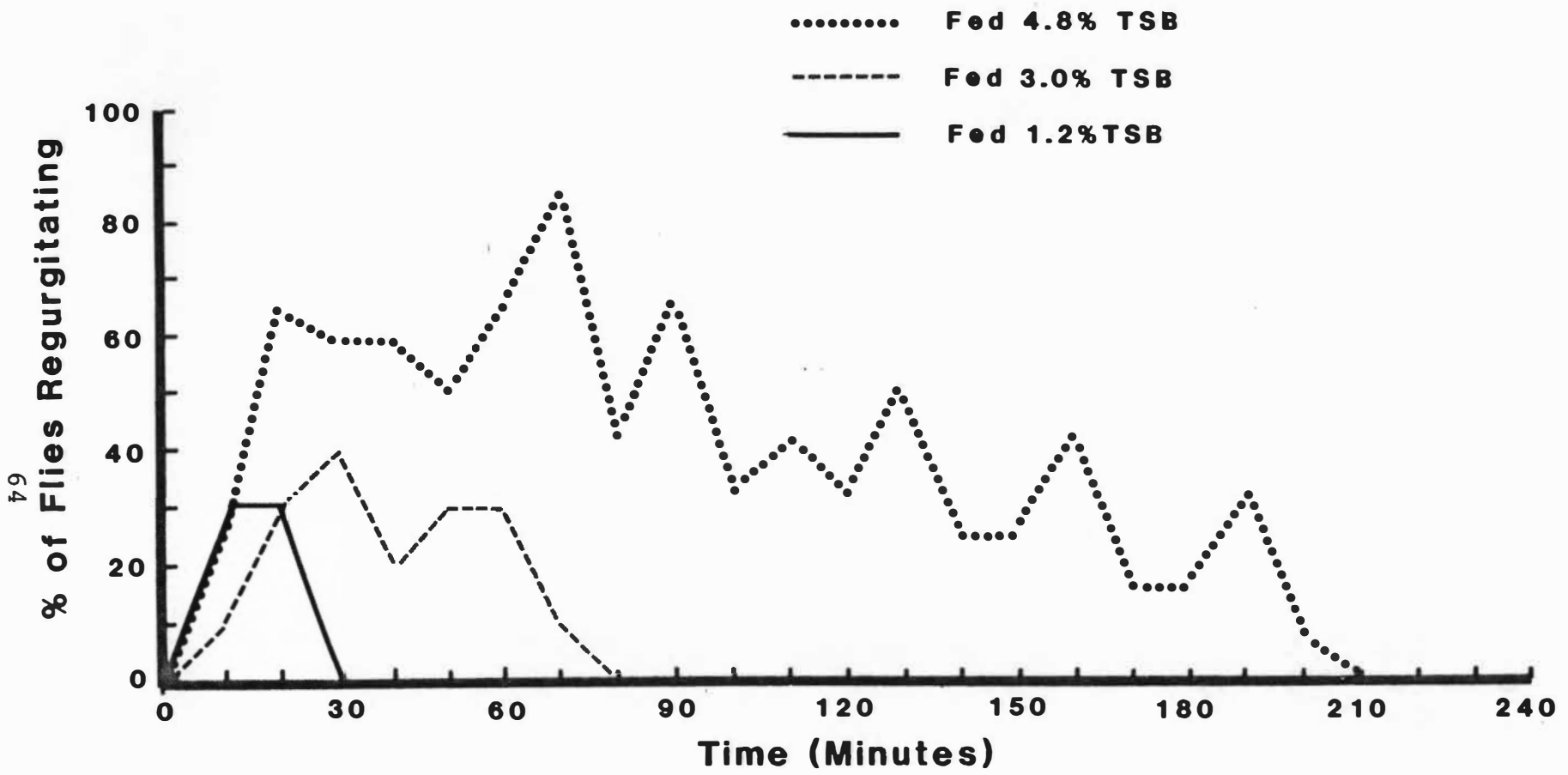


Figure 9. Effect of Concentration of TSB on the Duration of Regurgitation.

of TSB. These results were expected based on the results of previous experiments. Thomson (1975) postulated that regurgitation was the result of accidental leakage of fluid from the crop. Accidental leakage should result in regurgitation only occasionally, however almost continuous regurgitation was observed for periods of up to 240 minutes.

Effect of Changing the Concentration of Food on Regurgitation

Both the concentration and the amount of TSB were previously found to significantly affect regurgitation. Groups of flies which had fed upon 3.0% TSB regurgitated much less frequently than did flies fed 4.8% TSB. This experiment was intended to determine if regurgitation by individual flies would increase significantly if a more concentrated food source was provided to flies which had already fed to repletion on a less concentrated food.

None of the flies offered 1.2% TSB were observed regurgitating during the 30 minute period following feeding. Relatively small amounts (approximately 5 ul) of the TSB offered were ingested. Active searching for food continued throughout the 30 minute period, indicating the flies were not satiated. With the introduction of 4.8% TSB into the petri dishes all the flies began feeding again.

Within 15 minutes 100% of the flies were observed regurgitating. This regurgitation typically continued for the duration of the 30 minute observation period.

This data provides additional evidence that there is some threshold which must be reached before regurgitation will commence. It is likely that this threshold is dependant upon the amount of food present within the crop. The concentration of the ingested material is relevant as it determines the amount of food which will be ingested.

Effect of Dessication on Regurgitation

The physiological state of most insects is highly dependant upon their water balance. Based upon the results of previous experiments (the effect of the amount of food on regurgitation) it was theorized that dessication would most likely decrease regurgitation by the flies. Figure 10 is a summary of our results.

The length of time without water was negatively correlated ($r = -.23$) to the amount of time spent regurgitating. No clear pattern of regurgitation was evident as the time without water increased, explaining the low r-value. However, a distinct drop in the amount of time spent regurgitating did occur between 18 and 48 hours without water. The low amount of time spent

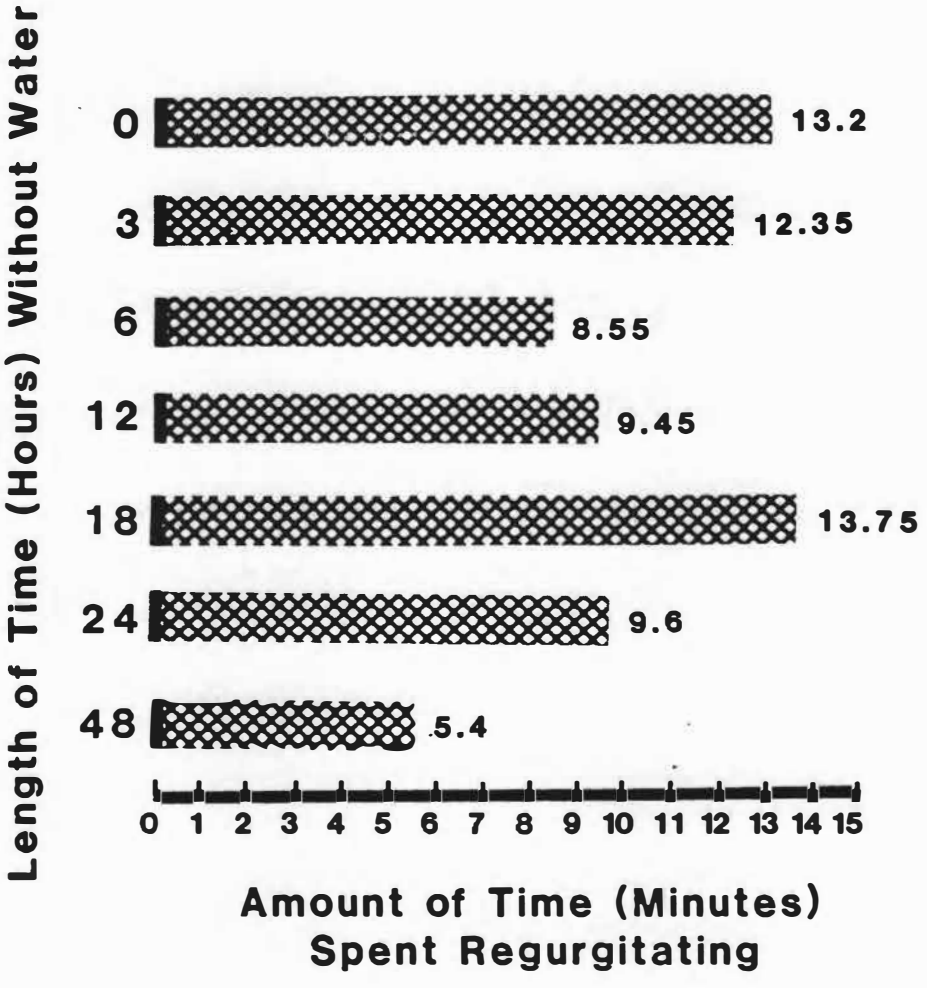


Figure 10. Effect of Dessication on Regurgitation.

regurgitating by flies deprived of water for 48 hours suggests that long periods of time without water will reduce the occurrance of regurgitation. This drop in the amount of time spent regurgitating occurred even though all flies had consumed 10 ul of 4.8% TSB

It has previously been suggested that regurgitation may result in the evaporation of excess water from the surface of a circulating regurgitation droplet (resulting in an increase in the concentration of the liquid). It would not be advantageous to regurgitate during periods of high water loss, as the requirement for water may override that for food. Given the fact that all flies consumed equal amounts of food, regurgitation was solely affected by the physiological state of the flies, supporting this idea. Whether this response is due to a direct effect upon regurgitation or to an indirect effect caused by some other physiological change (such as an increase in the osmotic blood pressure) has not been determined.

iv - Summary

The various factors examined all significantly affected regurgitation. The most dramatic effects on regurgitation were due to the concentration and amount of the food consumed. Temperature, relative humidity and

dessication all also effected regurgitation; however, the effect was not as pronounced. These findings correspond to those of Roberts and Pitts (1971) and Dakshinamurty (1948) who found that face flies preffered temperatures of 30°C and low relative humidity levels over other choices. These results indicate that the occurrance and duration of regurgitation responds to a wide range of factors.

CHAPTER IV

FACTORS AFFECTING REGURGITATION ONTO THE EYES OF CATTLE

i - Introduction

The bacterium Moraxella bovis Hauduroy is considered the primary causative agent of pinkeye in cattle. Moraxella bovis, at that time known as Hemophilus bovis, was first isolated from the eyes of cattle with pinkeye in 1923 (Jones and Little, 1923). Although other organisms have been suspected of causing pinkeye in cattle, M. bovis is the only organism which satisfies Koch's postulates for the disease (Henson and Grumbles, 1960; Hughes et al., 1965).

A dramatic increase in the incidence of eye disorders in cattle has been noted since the introduction of the face fly, Musca autumnalis, into North America (Dobson and Matthew, 1960; Treece, 1960; Decker, 1961; Benson and Wingo, 1963). Brown and Adkins (1972), Arends (1984) and Glass and Gerhardt (1984) showed that laboratory infected face flies could transmit M. bovis onto the eyes of susceptible calves, while Berkebile et al. (1981) and Gerhardt et al. (1982) isolated M. bovis from wild face flies aspirated from the heads of cattle.

Steve and Lilly (1965) reported survival of M. bovis on the external surface of face flies for up to three days, however, Simpson (1981) and Burton (1966) could not substantiate this finding.

Glass et al. (1982) recovered Moraxella bovis from the crops of face flies up to 48 hours after ingestion and demonstrated that the flies regurgitated into the eyes of cattle (Glass and Gerhardt, 1984). These results indicated that ingestion of the bacteria from infected cows' eyes and subsequent regurgitation into the eyes of susceptible cattle is the method of transmission of M. bovis.

The objectives of this study were to 1) determine the effect of the number of flies on the amounts of regurgitation droplets deposited, 2) to examine the importance of movement by the fly as a factor affecting the frequency of regurgitation droplet deposition, and 3) to quantitatively determine the frequency of regurgitation onto the eyes of cattle.

ii - Materials and Methods

Effect of the Number of Flies on the Number of Regurgitation Drops Deposited on a Substrate

Groups of 1, 2, 4, 8, 13, and 19 six-day old standardized female face flies (6SFFF) were placed in 9 cm

diameter petri dishes. The experiment was replicated 10 times. Each petri dish contained 20 ml of 4.8% trypticase soy broth (TSB), an amount that allowed all flies to feed to repletion. The experiments were conducted under standard testing conditions (25°C and 75% RH). During the 15 minutes following the onset of feeding the number of regurgitation droplets deposited upon the surface of the petri dishes was recorded for each group of flies. The mean number of droplets deposited by each size group was calculated, as was the mean number of drops deposited/fly/group. The correlation coefficient between the number of flies/petri dish and the number of regurgitation drops deposited was calculated.

Effect of Adding Unfed Flies to Flies Already Regurgitating

In an earlier experiment it had been noted that flies in the process of regurgitating were often disturbed by active, unfed flies. These interactions could possibly lead to an increase in the number of regurgitation droplets that would actually be deposited onto a substrate.

Ten groups of five 6SFFF were each placed in 9 cm petri dishes containing 10 ml of 4.8% TSB. The number of regurgitation droplets deposited upon the surface of the petri dishes was recorded for a 15 minute period,

beginning with the onset of feeding. Following this 15 minute period, five additional flies were added to each petri dish and the number of regurgitation droplets deposited during the next 15 minutes was also recorded. The total number of regurgitation droplets deposited during each 15 minute period for each group of flies was determined. The mean number of drops deposited/group was calculated, as were the mean number of drops deposited per fly per group in the two time periods.

Regurgitation by Artificially Fed Face Flies onto the Eyes of Cattle

Twenty groups of five 6SFFF were fed to satiation on 4.8% TSB. A small amount of fluorescein isothiocyanate was added to the TSB. Five minutes after the flies had fed they were placed in 6 x 9 cm wire cages attached over the eyes of a cow using the technique described by Glass and Gerhardt (1983). Every five minutes during the next 30 minutes the number of drops deposited onto either the skin around the eyes of the cow, the eye itself, or the surface of the wire cages was recorded. A hood was placed over the cages to darken them, and an ultraviolet lamp was used to locate any regurgitation droplets. This lamp caused the drops to fluoresce, making the drops readily visible. The mean number of drops deposited per group and deposited per fly per group was calculated.

As a control, eight groups of five 6SFFF were placed in 6 x 9 cm wire cages three meters from the cow. The same procedures as above were followed. The data for the control and test groups were analyzed by simple analysis of variance to determine if the presence of the cow significantly affected regurgitation.

iii - Results and Discussion

Effect of the Number of Flies on the Number of Regurgitation Drops Deposited on a Substrate

During earlier experiments utilizing individual flies the deposition of regurgitation droplets onto the surrounding substrate was almost never witnessed. In order for this form of regurgitation to be a viable mechanism of transmission of M. bovis, this deposition (onto the cow's eye) must occur. It was theorized that the increasing number of contacts which would occur with increasing numbers of flies in a given area should result in an interruption of regurgitation. This contact between flies might result in drops being deposited onto a substrate.

The results are summarized in Figure 11. An increase in the actual number of drops deposited corresponds to the number of flies/petri dish, which would support this theory. A positive correlation

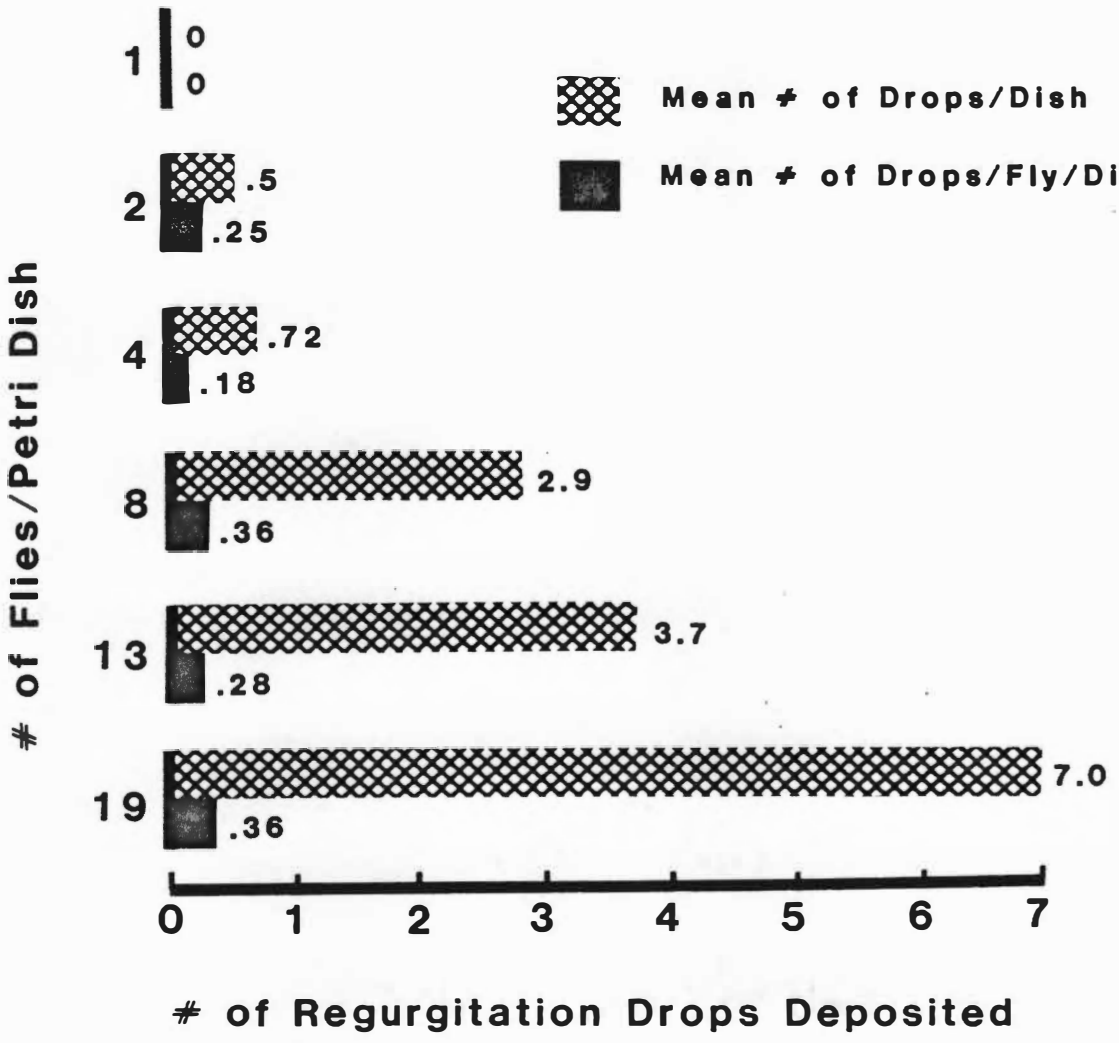


Figure 11. Effect of Number of Flies on Number of Regurgitation Droplets Deposited.

between the number of flies/petri dish and the number of regurgitation droplets deposited was noted (r = +.986). However, the actual number of drops deposited/fly/petri dish does not increase as significantly (r = +.14).

Flies placed in a petri dish all feed to satiation within a relatively short period of time. Also, all flies begin regurgitating at approximately the same time. During regurgitation face flies normally remain motionless unless disturbed. This reduces the number of interactions between flies, explaining the low number of regurgitation drops deposited/fly/dish.

Whether this relatively low number of depositions per fly could account for field transmission of M. bovis is unknown. However, other factors which can affect transmission do come into play.

The Effect of Adding Unfed Flies to Flies Already Regurgitating

In nature the number of flies on a cow and their hunger state vary. Unfed flies are constantly arriving on a cow and satiated flies are leaving. The previous experiment did not take this movement into account when considering the potential for transmission. This experiment was designed to overcome this shortcoming of the last procedure.

Figure 12 summarizes the increase in the deposition of regurgitation drops which results from adding unfed flies to flies in the process of regurgitating. Ten groups of five flies collectively deposited only two drops on the surface of the petri dishes (Mean/fly = 0.04). This low rate of deposition was explained in the last experiment. The addition of five unfed flies to each petri dish resulted in a total of 45 drops being deposited (Mean/fly = 0.45). Even taking into account the increase in the number of flies per dish, a 10-fold increase in drops deposited occurs.

It has been determined that starved flies added to a dish rapidly search for food. During this searching period the number of encounters between regurgitating flies and unfed flies was much higher than for a group of flies which all were regurgitating. This is likely much more analagous to a natural situation than one in which 100% of the flies are regurgitating at the same time. This suggests that flies may in fact frequently regurgitate into cows' eyes in nature.

Regurgitation by Artificially Fed Face Flies onto the Eyes of Cattle

The results of this experiment are summarized in Table 13. A significantly ($p < .01$) greater number of regurgitation droplets were deposited by flies on the cow

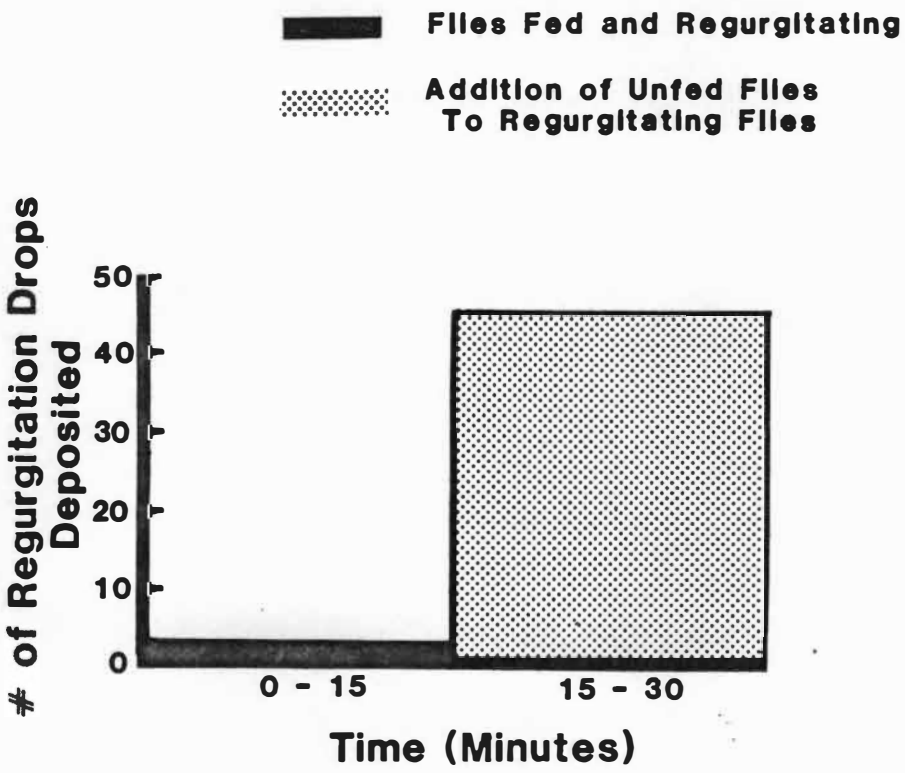


Figure 12. Effect of the Addition of Unfed Flies to Regurgitating Flies.

Table 13. Effect of the Presence of a Cow on the Number of Regurgitation Droplets Deposited.

Group	Mean No. of Drops Deposited Per Group	Mean No. of Drops Deposited Per Group/Eye (Socket Fluid)	Mean No. of Drops Deposited Per Fly/Group
On Cow	6.05	0.70	1.21
Control	1.37	---	0.27
Lab. Control	0.7 (4 Flies/Dish)	---	0.17
	2.9 (8 Flies/Dish)	---	0.36

than by the control group. The results for the control group were comparable to the results of our laboratory experiment examining the effect of the number of flies on regurgitation. The presence of the cow therefore did affect regurgitation.

There are several possibilities as to the cause of this difference. The cow provides a microclimate, with the temperature, RH, and CO_2 levels next to its body affected. The variation between this microclimate and the climate in which the control group was confined may have accounted for the differences in regurgitation.

The experiment examining the effect of adding active flies to flies already regurgitating demonstrated that a disruption of regurgitating flies resulted in an increase in the number of regurgitation droplets deposited. The cow on which the flies were caged was extremely active. Constant shaking of the head and twitching of the facial muscles may have disrupted regurgitation and resulted in the deposition of droplets onto the substrate.

The number of drops deposited per fly onto an eye was extremely low (0.14 drops deposited/fly). Whether these low levels of regurgitation could result in field transmission of M. bovis is unknown.

iv - Summary

These results indicate that the deposition of regurgitation droplets is increased by certain conditions. These conditions can be considered analagous to those found in the field, such as the constant arrival of unfed or partially fed flies and the movements made by a cow in an attempt to remove flies. This indisputably demonstrates that Musca autumnalis has the potential to transmit pathogens via regurgitation. Taken in conjunction with the work by Glass et al. (1982) and Glass and Gerhardt (1984) they incriminate this form of transmission as a likely means of transmission of the bacterium Moraxella bovis.

CHAPTER V

CONCLUSIONS

Regurgitation is known to play an important role in the transmission of a number of pathogens to humans and livestock. Regurgitation also occurs in a wide range of arthropods, including Homoptera, Siphonaptera, Coleoptera, Lepidoptera, Ixodidae and Diptera (Kloft et al., 1980). The mechanism of regurgitation has been documented for the Homoptera (Sylvestor, 1954; Srivastava and Auclair, 1971) and Siphonaptera (Bacot and Martin, 1914). The only research on regurgitation by Dipteran species consists of 1) acknowledgement of its occurrance in relation to pathogen transmission (Graham-Smith, 1914; Greenberg, 1973; Dipeolu, 1977, 1982), 2) recognition of the process in relation to other physiological functions (Thomson, 1975), and 3) a study by Glass and Gerhardt (1984) which examined regurgitation in relation to transmission of Moraxella bovis.

This study is the first thorough research conducted on the process of regurgitation by a Dipteran species. The mechanism of regurgitation, the effects of various environmental factors and food types on regurgitation, and the effects of factors which could influence transmission of pathogens were examined.

Regurgitation was a common occurrance in flies fed large amounts or high concentrations of liquids (trypticase soy broth or sucrose). Flies fed solid foods did not regurgitate. When flies were fed 15 ul of 4.8% trypticase soy broth (TSB) regurgitation occurred in the majority of flies examined. The duration of regurgitation in flies fed these amounts and concentrations of TSB ranged up to 240 minutes. Flies fed less concentrated food or smaller amounts did not regurgitate as frequently or for as long a period. These results differ from those of Thomson (1975), who exmined the crop function in the blowfly, Phormia regina. Regurgitation by the blowfly is a result of accidental leakage of fluid from the crop. This does not appear to be the case with face flies, based on the duration and frequency of regurgitation.

The mechanism by which regurgitation occurs, and the purpose of this process are still unknown. Fluid in the droplet arrived from the crop and not the mid or hindgut. Its eventual destination appeared to also be the crop, although additional research is needed to confirm this. The process which allowed the liquid within the droplet to circulate is unknown. Circulation of fluid was constant in the majority of regurgitation droplets observed, yet the diameter of the droplet did not appear

to vary. A bidirectional current of fluid within the proboscis could possibly account for the circulation of liquid and the constant size of the droplet. Preliminary results indicated that a change in fluid within the regurgitation droplet occurred during the interval between the consumption of fluid and the period the regurgitation droplets were collected (30 minutes following the onset of regurgitation). Three possible explanations could account for this change, 1) the addition of enzymes to the fluid, 2) the breakdown of molecules in the fluid, or 3) a concentration of the TSB through the removal of excess water by evaporation. Additonal research is needed to determine which of these factors resulted in this change in the fluid, although the circulation of fluid suggests that evaporation of excess water may be occurring.

Temperature, relative humidity and dessication all significantly effected regurgitation. Face flies preferred lower relative humidity levels over higher ones when offered a choice (Dakshinamurty, 1948), and experiments have shown that 30°C is the optimum temperature for activity (Dakshinamurty, 1948; Roberts and Pitts, 1971). Our data confirmed that these factors had a similar effect upon regurgitation. Most insects are extremely susceptible to dessication. Face flies,

however, normally feed upon liquids and are therefore not usually subjected to extreme dessication. Long periods without water (48 hours) resulted in a decrease in the amount of time spent regurgitating.

The work by Glass and Gerhardt (1984) implicated regurgitation as a means of pathogen transmission. Based on studies on 1) the effects of the numbers of flies on regurgitation, 2) the effect of the addition of unfed flies to regurgitating flies, and 3) the effect of the cow on the deposition of droplets, it appears that face flies could serve as vectors of pathogens to livestock.

Regurgitation is apparently an important process in face flies, and the potential it plays in terms of pathogen transmission make it even more important. This study has defined many of the basic features of regurgitation, however, additional research is needed to further examine many other features of this process.

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