

SACBROOD: A VIRUS DISEASE OF THE HONEYBEE

by

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ABSTRACT

Sacbrood virus causes changes in honeybees which may be summarized as follows:

A. Larvae

1. Fewer protein granules than usual form in the cytoplasm of the fat body cells of growing larvae.

2. The protein reserves that form in the fat body cells, and the cells themselves, remain intact when the larvae become mature, whereas in healthy larvae they are broken down.

3. The endocuticle of mature larvae remains undigested, when the last larval skin is being shed, and so prevents ecdysis.

B. Adults

1. The protein granules in the cytoplasm of the fat body cells and the proteinaceous secretory globules in the cytoplasm of the hypopharyngeal glands disappear sooner than usual.

2. The lives of bees are decreased to about three weeks, the same length of life as of bees deprived of protein (pollen).

3. Worker bees are more susceptible to chilling than usual, probably because of their decreased metabolism, an effect also caused when healthy bees are deprived of protein.

4. Young worker bees fail to eat pollen, to attend the queen or drones, and to rear brood.

5. Young worker bees and drones try to fly and worker bees start to forage sooner than usual.

6. Foraging worker bees do not collect pollen, or collect very little.

7. The eggs of unmated queens mature sooner than usual.

8. The mitochondria in the brain of drones appear to be damaged.

The changes caused by sacbrood virus in adults summarized in (4), (5), (6) and (7) above, are the same as those that follow when healthy individuals are anaesthetized with carbon dioxide, and are equivalent to changes that occur in senile individuals, possibly activated by accumulated acid metabolites. Most of these changes are probably important in preventing rapid spread of sacbrood in nature.

CONTENTS

	<u>Page</u>
Title	1
Abstract	2
Contents	4
Acknowledgments	7
Part 1. <u>GENERAL INTRODUCTION</u>	8
Part 2. <u>MATERIALS AND METHODS</u>	13
2.1. Preparation of virus suspensions	13
2.2. Infecting larvae	13
2.3. Infecting adult bees by feeding	14
2.4. Infecting young drones by feeding	17
2.5. Infecting adult bees by injection	17
2.6. Laboratory experiments	17
2.7. Infectivity tests	20
a. Bioassay: 1. With larvae	20
2. With drones	20
b. Serological tests.	21
2.8. Histological methods:	
a. with light microscope	23
b. with electron microscope.	24
Part 3. <u>EFFECT OF SACBROOD VIRUS ON HONEYBEE LARVAE</u>	25
3.1.a. Methods	26
1. Infectivity tests	27
2. Histology	27
3.1.b. Results	27
3.1.c. Discussion	43
Part 4. <u>EFFECT OF SACBROOD VIRUS ON ADULT BEES</u>	45
4.1. <u>Introduction</u>	45
4.1.a. Methods	46
1. Length of life of adults	
2. Pollen consumption of infected adults in colonies.	47

	Page
4.1.b. Results	48
1. Length of life	48
2. Pollen consumption	48
4.1.c. Discussion	51
4.2. <u>Behaviour in the colony</u>	52
4.2.a. Methods	53
1. Behaviour of worker bees	53
2. Behaviour of drones	56
3. Length of life and infectivity checks	56
4.2.b. Results	56
4.2.c. Discussion	68
1. Queen attendance	68
2. Clustering	69
3. Departure from colonies	69
4.3. <u>Protein metabolism</u>	69
4.3.a. <u>Hypopharyngeal glands</u>	69
1. Methods	72
2. Results	72
3. Discussion	79
4.3.b. <u>Fat-bodies</u>	80
1. Methods and Results	81
2. Discussion	85
4.4. <u>Clustering</u>	85
a. Methods	86
b. Results and Discussion	87
4.5. <u>Sugar consumption</u>	90
a. Methods	90
b. Results	91
c. Discussion	99
4.6. <u>Behaviour in the field</u>	100
a. Methods	101
b. Results	103
c. Discussion	116

	<u>Page</u>
4.7. <u>Effect of sacbrood virus on the brains</u> <u>of adult honeybees</u>	118
a. Methods	118
b. Discussion	121
4.8. <u>Effect on the ovaries of the queen honeybee</u>	123
a. Methods	124
b. Results and Discussion	125
4.9. <u>Some comparisons of the effects of</u> <u>sacbrood virus with those of acute bee</u> <u>paralysis virus.</u>	131
a. Methods	132
b. Results	133
c. Discussion	141
Part 5. <u>GENERAL DISCUSSION AND CONCLUSIONS</u>	143
<u>REFERENCES</u>	148

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Part 1.

GENERAL INTRODUCTION

There are at least three known viruses that can cause diseases of the honeybee but that commonly persist without causing apparent symptoms, especially in adults. One of these is sacbrood, the insect disease whose viral nature has longest been established. The other two viruses are chronic bee paralysis virus and acute bee paralysis virus.

The first reports of a disease that probably was sacbrood were those of Langstroth (1857); subsequently several authors (Doolittle, 1881; Jones, 1883; Simmins, 1887; Cook, 1904) also observed a "foulbrood-like" disease among honeybee larvae, in which no bacteria could be detected, even after their death. However, no name was given to this disorder till White (1913; 1917) called it "sacbrood". He concluded that it was a virus disease because he successfully infected honeybee larvae in colonies with bacteria free filtrates of larvae that had died of sacbrood. He observed that when infected larvae were sealed in their brood-comb cells they failed to shed their final skin, which became a translucent sac, accumulating much ecdysial fluid around the pupal integument, and then died within two days. Unless adult bees detected and ejected them, the dead larvae soon dried up to form flat, brown scales in their cells. White also showed that sacbrood had an incubation period of approximately six days and that diseased larvae lost their infectivity about three

weeks after death. However, he found that adult honeybees were not susceptible to the disease.

Nothing further was discovered about the virus till Morgenthaler (1947) confirmed that filtrates of diseased larvae were infective, and that no inclusion bodies resembling those of the wellknown insect virus diseases, the polyhedroses and granuloses were in their tissues. In the first attempt to classify insect viruses, Holmes (1948) created the genus Morator to distinguish this type of virus from other insect viruses which formed inclusion bodies. Using electron microscopy, Steinhaus (1949) noted spherical particles about 60 nm. in diameter, and Brcaak, Svoboda and Kralik (1963) and Brcaak & Kralik (1965) found icosohedral particles 30 ± 2 nm. in diameter in extracts of larvae with sacbrood, whereas they found no such particles in extracts of healthy larvae. However, these authors did not test whether these particles would cause sacbrood. Bailey, Gibbs & Woods (1964) also obtained isometric particles 28 m in diameter from diseased larvae and showed that purified preparations of these particles caused sacbrood when fed to healthy larvae. Two day-old larvae were the most susceptible; the median lethal dose by mouth was between 10^7 and 10^8 particles per larva. About 10^{13} virus particles can be extracted from a single infected larva (Bailey, 1969). Similar particles have been recognized in ultra-thin sections of larval tissue (Lee & Furgala, 1965a). Using

Dische diphenylamine and orcinol reactions on a cold water-phenol saturated solution of the virus extract, Lee & Furgala (1965b) showed the nucleic acid is of the ribose type.

Recently, Bailey (1968; 1970) has shown that sacbrood virus multiplies in adult bees and found that the median infective dose by mouth for adults younger than 8 days was between 10^8 and 10^9 particles; by injection it was between 10^3 and 10^4 particles for worker bees and between 10^5 and 10^6 particles for drones. The infected individuals showed no apparent symptoms.

Bailey, Gibbs & Woods (1964) stated that sacbrood virus resembles acute bee paralysis virus having the same size and shape of particles and a similar sedimentation rate (SW,20) of 160, but that serological and cross infectivity tests showed no relationship between them.

Smirnova (1966) trying to maintain infectivity of sacbrood virus under various conditions, such as refrigeration, desiccation, suspension in a 50% glycerine solution at 4°C ., or in honey, or stored pollen, stated that she obtained best results by using virus cultures in chick allantoic fluid and frozen for 10 months, or with virus desiccated at room temperature. Smirnova also observed cytopathogenic effects in chick fibroblast cell cultures but not in HeLa and C-18 cell cultures, when they were inoculated with sacbrood virus. Bailey (1968) has stated that the infectivity of sacbrood virus decreases rapidly in dead larvae, and none remains

after three weeks.

Occurrence: Sacbrood was first recorded in U.S.A. and was thought to be rare, at least in Britain. Tarr (1937) thought that most disease resembling sacbrood was a non-infectious hereditary fault, called "addled brood", because it did not spread when combs containing such diseased larvae were placed in healthy honeybee colonies. However, as sacbrood does not spread readily this way (Hitchcock, 1966), and as the descriptions given by Tarr clearly resemble those of larvae known to have sacbrood, and as recent surveys in England and Wales have shown that over 80 percent of diseased larvae free from micro-organisms have sacbrood (Bailey, 1967, 1971b), Tarr was probably dealing with this disease. Severe outbreaks occur during spring (White, 1917), and Bailey (1971b) has found up to 6 percent of larvae from apparently healthy colonies in August with sacbrood.

Multiplication and spread: Although the ecdysial fluid in the sac that surrounds the pupal integument of dying larvae is rich in sacbrood virus, little is known of where the virus multiplies within the body of the infected larva. Lee and Furgala (1967) saw particles of sacbrood virus in the cytoplasm of fat, muscle, and tracheal-end cells of artificially infected laboratory reared larvae that became "sick" or died three days after infection when they were about 4 days old. Bailey (1971b) considered that this was atypical of sacbrood as infected 4 day-old larvae still appear healthy in colonies;

the early death of the laboratory reared larvae may have been because they had ingested overwhelming amounts of virus. Fyfe (1962) considered that sacbrood virus upsets the endocrine mechanism of the infected larva, and Bailey (1970) showed that much sacbrood virus multiplies in the brains of drones even though they showed no symptoms when it is injected into their haemolymph. This suggested that the virus was neurotropic.

Although infected worker bees show no apparent symptoms, they eat little or no pollen after infection and much virus accumulates in their hypopharyngeal glands, more than in other regions of the body (Bailey, 1969). As hypopharyngeal gland secretions are a constituent of food for worker and drone larvae and the "royal jelly" for queen larvae, it seems likely that larvae would be infected by nurse bees that are infected with sacbrood virus. Queen bees are as susceptible to virus infection as workers and drones but do not transmit sacbrood to their offspring (Bailey, 1970).

This summarizes the present knowledge of sacbrood. My investigations contribute further knowledge on the pathology of honeybee larvae and adults infected with the virus.

Part 2. MATERIALS AND METHODS

The following sections describe the general methods used throughout the study. Special techniques are described in the relevant sections.

2.1. Preparation of virus suspensions

For infecting: Sacbrood virus was extracted from larvae that had been preserved at -20°C . immediately after they had died or were about to die of the disease when they were 8-9 days old. Extracts were prepared by macerating the larvae in a glass tube or in a mortar with distilled water with $\frac{1}{4}$ volume of carbon tetrachloride. The resulting emulsion was coarsely filtered through non-absorbent cotton wool and the filtrate clarified by centrifuging at 8000 g. for 10-15 minutes. The supernatant containing the virus was then either used without further treatment or after dilution with sterile distilled water. The clarified extract of one larva killed by sacbrood usually contains about 10^{13} virus particles (Bailey, 1969) and will be referred to below as one "unit".

Extracts for control treatments were prepared similarly from healthy larvae.

For testing: Sacbrood virus was extracted from infected adult bees by macerating heads of individuals in 0.1 ml. distilled water together with one drop of diethyl ether followed by one drop of carbon tetrachloride in a conical tube. The homogenate was clarified by centrifuging at 4000 g. for 10 minutes and the supernatant was used for tests.

2.2. Infecting larvae

Larvae from healthy colonies were infected when they

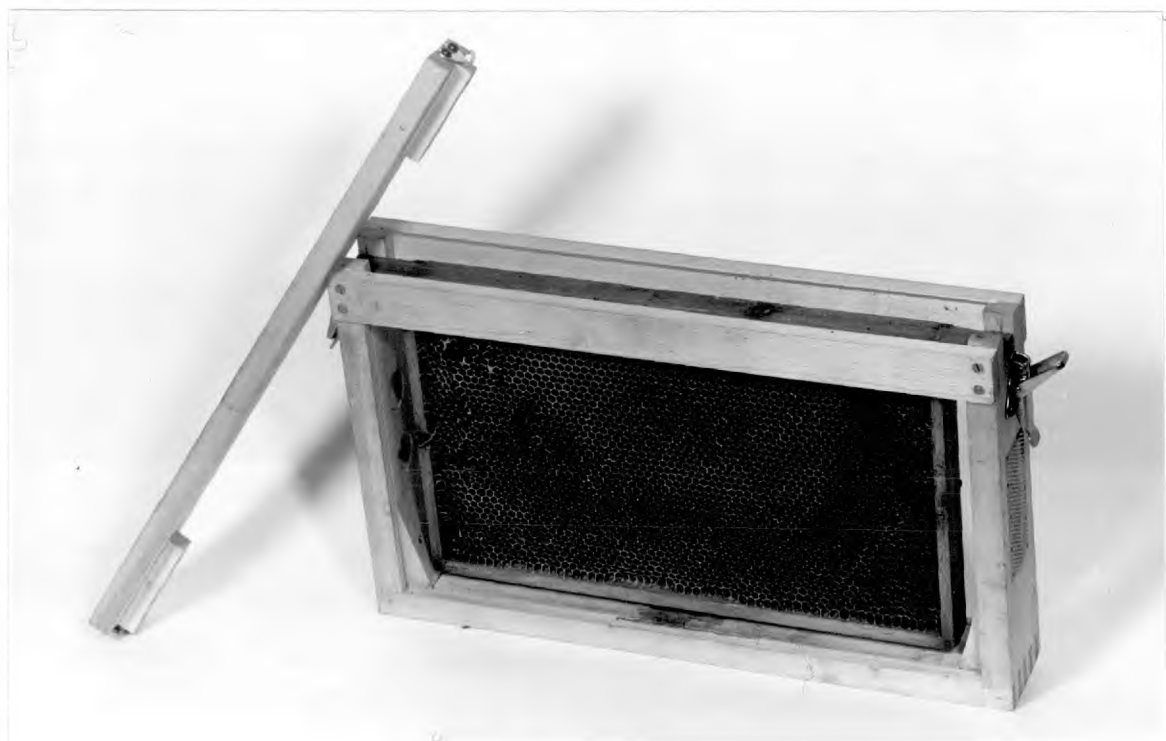
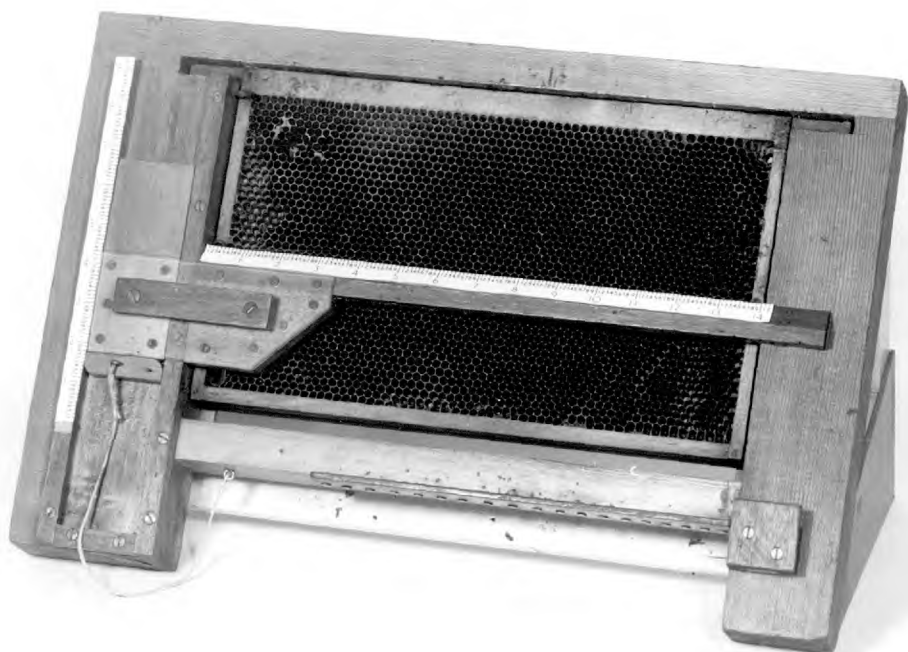
were one to two days old, when they are most susceptible (Bailey, 1969). The age of the larva was known from the date when eggs were laid in an empty comb placed in the colony, or it was estimated by comparing the size of the larvae with preserved specimens. Between 70 to 150 such larvae, individually identified in their combs by means of a calibrated frame (Fig.1), were used. A suspension containing one unit of virus per ml. was prepared and $1\mu\text{l}$ (10^{-3} units) was placed into the food surrounding each larva by means of an 'Agla' micrometer syringe. The combs were returned at once to the colony till the larvae were sealed in their cells, about three or four days later. The combs were then removed from the colony and incubated, without adhering bees, at 35°C . in thermostatically controlled constant temperature cabinets and examined four or five days later when the healthy larvae should have pupated.

2.3. Infecting adult bees by feeding

Young bees become infected when they ingest a concentrated extract of sacbrood virus whereas bees older than about eight days do not (Bailey, 1969). The youngest bees are the most susceptible, so bees were obtained by incubating combs containing mature pupae (Fig.2) from healthy colonies at 35°C . Within 24 hours of emergence from their cells they were kept in wire-gauze cages measuring $10 \times 7.5 \times 4.5$ cm. (Fig.3). A virus extract containing 5 units in 10 ml. of 60 per cent sucrose was prepared and 5 ml. of this suspension were fed to

Fig.1. Calibrated frame for identifying
individual honeybee larvae in their
combs.

Fig.2. Cage for incubating combs containing mature
honeybee pupae.



100 worker bees by dropping a little at a time with a pipette over them in their cage. The bees cleaned each other, as they usually do, by ingesting the sucrose solution, and in this way soon consumed it all. The bees were kept at 30°C. as they consume sucrose faster at this temperature than at 35°C. A similar preparation of an extract of healthy larvae was used for control treatments.

2.4. Infecting young drones by feeding

Drones, unlike worker bees, do not readily feed themselves, but are usually fed and tended by worker bees. Therefore each young drone was fed with 5×10^{-2} units by placing 1 μ l. of a suspension directly between its mandibles with an 'Agla' micrometer syringe, while it was held by its wings. Drones used as controls were fed similarly with an equivalent extract of healthy larvae. They ingested the small drop of fluid within a few seconds.

2.5. Infecting adult bees by injection

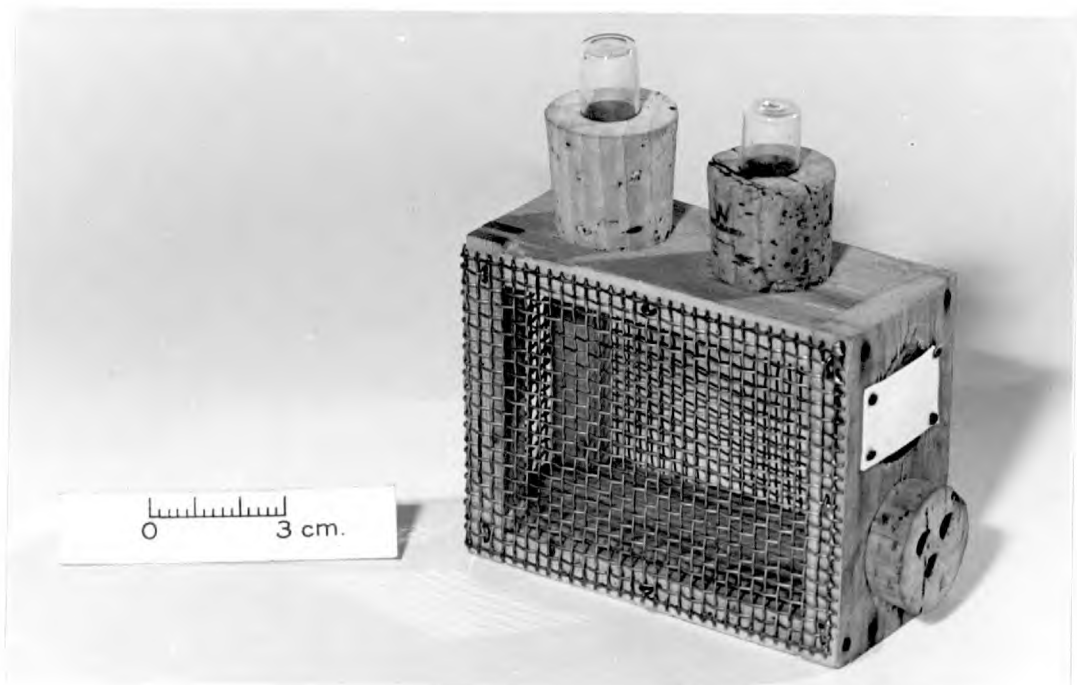
10^{-6} units were injected into the haemocoel of each bee with a sterile 'Agla' micrometer syringe through the second dorsal abdominal intersegmental membrane. Unless indicated otherwise, bees were anaesthetized before injection either with carbon dioxide or by chilling at 4°C.

2.6. Laboratory experiments

Adult bees were kept in wire-gauze cages measuring 11 x 2.5 x 2.5 cm. (Fig.4). Twenty worker bees, or ten drones with fifteen young attendant worker bees, were in each

Fig.3. Cage for containing newly emerged worker bees for infection by feeding them with sacbrood virus or extracts of healthy larvae.

Fig.4. Cage for keeping treated worker bees or drones in constant temperature cabinets for laboratory experiments.



cage and were supplied with 60 per cent sucrose solution and water in gravity feeders. Unless stated otherwise, newly emerged bees were also supplied with pollen, taken from honey-bee combs, packed into vials (5 x 11 mm.) and inserted into corks that fitted in the cages (Fig.4). Newly emerged worker bees eat this pollen mostly during their first five days. After treatment all cages containing bees were incubated either at 30°C. or 35°C. in thermostatically controlled constant temperature cabinets.

2.7. Infectivity tests

To establish the presence of virus in treated individuals the infectivities of extracts were tested either (a) by bioassay or (b) serologically.

(a) Bioassay:

(1) With larvae: Each virus-containing or control suspension was tested on one-day-old larvae by adding 1 μ l. of the suspension to the food surrounding each larva as described above (section 2.2). Treatments were interspersed over the comb surface to minimise differences in susceptibility of larvae because of their location within the colony. The infectivity of the extracts was measured by comparing the number of treated larvae that failed to pupate with the number of control larvae that succeeded.

(2) With drones: Sacbrood virus multiplies sufficiently within three days of injection in the heads of drones, to be detected serologically (Bailey, 1970), and

drones were sometimes used conveniently as follows:

A test or control suspension, e.g. of individual heads or brains of worker bees in 0.1 ml. distilled water, or 10 heads in 10 ml. distilled water, was prepared as described in section 2.7~~9~~ for infectivity tests. $1\mu\text{l}$ of this suspension was injected with a sterile 'Agla' micrometer syringe into each drone as described for worker bees (section 2.5). After incubating the treated drones in cages at 35°C . for five days, individual heads were ground in a conical tube in a drop of diethyl ether and 0.05 ml. of 0.85% saline, and tested serologically as described in the next section.

(b) Serological tests:

A modified method by micro-immunoprecipitation-in-gel described by Mansi (1958) using anti-serum prepared from rabbits as described by Bailey, Gibbs & Woods (1964) was used.

The antigen and antibody diffuse in the agar and when they are in optimum proportions, they form a precipitate where they meet, after about twenty-four hours, as a sharp line (Fig. 4).

A 1% solution of Ionagar was made in 0.85% saline and 0.20 M buffer expressed as H_3BO_3 , and adjusted to pH7. The molten agar was spread evenly into a thin layer approximately 2 mm. thick on clean glass plates 5 cm. x 5 cm. square and allowed to solidify in petri dishes containing filter paper soaked in 0.5% phenol. Before use, a central reservoir 6 mm. diameter surrounded by six others of the same diameter and

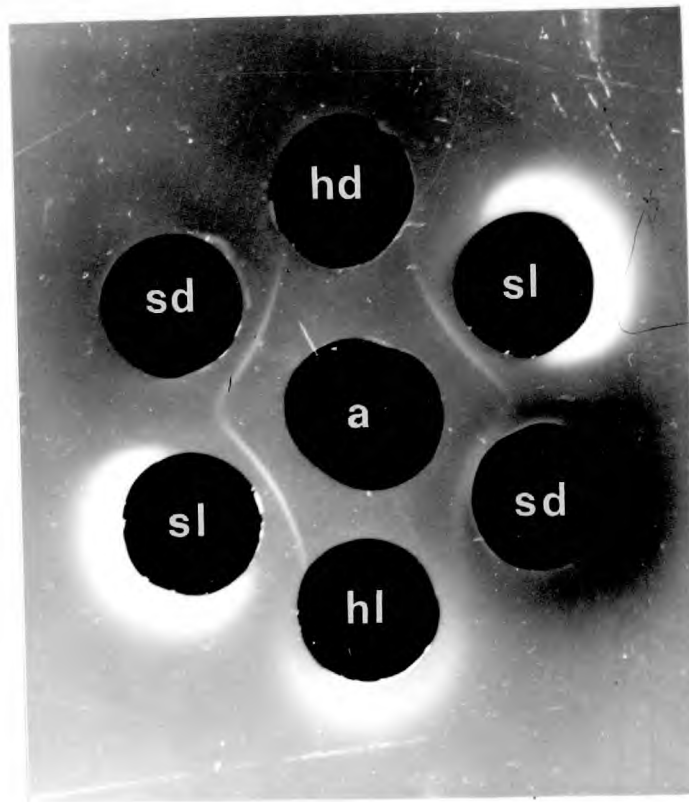


Fig.5. Gel-diffusion serological test showing precipitin lines.
sl = extract of larva with sacbrood;
sd = extract of head of drone infected with sacbrood virus;
hl = extract of healthy larva;
hd = extract of head of healthy drone;
a = sacbrood virus antiserum.

9 mm. apart, centre to centre, were cut in the agar with a cork-borer using a template.

Sacbrood virus antiserum was introduced into the central reservoir and the extracts (antigens) into the six surrounding reservoirs. The plates were examined 24-30 hours later and the presence of any precipitin lines was considered as positive.

2.8. Histological methods

Histopathology was done by light microscopy and electron microscopy.

(a) For observations with the light microscope, specimens were fixed for 24 hours in aqueous Bouin's fixative or for three hours in Carnoy's fixative (Pantin, 1964). After fixation, the specimens were dehydrated in a dilution series of tertiary-Butyl-alcohol and embedded in Paraplast (m.p. 58°C.). Sections were cut at 6 μ . with a Cambridge rocking microtome and fixed on slides with albumen adhesive (Pantin, 1964). Infected tissue was compared with healthy tissue prepared simultaneously.

Heidenhein's iron-alum haematoxylin counter-stained with alcoholic eosin or Mallory's triple stain (Pearse, 1968) or a broad spectrum Azan stain (Hamm, 1966) were used to observe general morphological or cytological features.

Protein was detected by the mercurio-bromophenol blue method (after Bonhag, in Pearse, 1968), and glycogen was detected by Best's carmine method (Pearse, 1968). Fat was

detected by immersing entire portions of fresh fat body tissue in ethylene glycol saturated with Sudan IV (Gurr, 1956).

(b) For electron microscopy, brains were dissected from anaesthetized bees that were held in 0.85% saline. All tissues adhering to the brain, except the finest tracheae, were removed with a pair of fine forceps. They were fixed overnight at 4°C. in 10% glutaraldehyde in 0.025 M phosphate buffer at pH 6.8. After three washings in the same buffer, the tissue was transferred into cold 1% osmium tetroxide in the same phosphate buffer for four hours at 4°C. After rinsing in buffer and two washes of 10 minutes each in 50% acetone, the tissue was transferred to 70% acetone saturated with ~~uran~~anyl acetate, where they remained in the dark for three hours. Dehydration in 100% acetone was followed by embedding in Epon (Luft, 1961) and sectioning on a Reichert Om U2 ultramicrotome with glass knives. Sections were stained in lead citrate and examined in a Siemens Elmiskop I electron microscope. I am indebted to Mr. R. Turner, Plant Pathology Department, Rothamsted Experimental Station, for kindly sectioning the preparations and examining them in the electron microscope.

Part 3. EFFECT OF SACBROOD VIRUS ON HONEYBEE LARVAE

3.1. Pathology of the honeybee larva

White (1917) first described the histology of larvae killed by sacbrood. He observed that most of the body consisted of fat tissue, and the fat cells contained more or less spherical bodies of "varying sizes and staining black". He also described the clear watery-fluid contained in the "sac" or space between the outer "moult skin" and the inner cuticle lying next to the epidermis of larvae killed by sacbrood.

Subsequently, Fyg (1959, 1962) stated that disturbance of the moulting process accompanied by excessive secretion of ecdysial fluid under larval skin caused the sac to form. He observed that certain regions of the integument of diseased larvae had the colour, with sclerotization and setae, characteristic of the imago and considered that sacbrood virus upsets the production of endocrine secretions. He produced symptoms resembling those of sacbrood by ligatures that prevented secretions of the neurosecretory cells of the brain and corpora allata from diffusing with the body of the larva. Development of similar abnormal or "intermediary forms" as described by Fyg (1962) had also been shown on ligated healthy honeybee larvae by Schaller (1952) and Lukoschus (1955a, 1955b). Recently, Bailey (1970) has demonstrated by infectivity and serological tests that sacbrood virus multiplies in the brains of drones when it is

injected into their haemolymph. All these results suggest that the virus is neurotropic and, by damaging nervous tissue, may affect neurosecretory centres and sites known to control the production or secretion of juvenile and moulting hormones (Wigglesworth, 1953).

The histology of the healthy honeybee larva has been adequately described by several authors (Bishop, 1922; Nelson, 1924; Snodgrass, 1956; Fyg, 1965). However, White (1917) used honeybee larvae after they had been killed by sacbrood and Fyg (1962) appears to have used similar larvae, either dead or at a very late stage in the disease, for studies on histopathology. It seemed desirable, therefore, to study changes in infected larvae before they showed symptoms to obtain information about the development of the disease, especially by comparing them with changes occurring in healthy larvae of a similar age.

3.1a. Methods

Larvae for histological studies were obtained by feeding one-day-old honeybee larvae each with 10^{-3} units of sacbrood virus as described in section 2.2. Control larvae were fed with an equivalent extract of healthy larvae in a similar manner.

On the second and subsequent days after treatment till the healthy larvae had pupated, a few larvae from infected and healthy groups were removed from their cells, and fixed (section 2.8a). Three larvae from each group, at the age

of 5, 6 and 8 days, were bisected transversely, one half fixed for histological examination and the other used for infectivity tests, to see whether the virus had begun to multiply. Larvae younger than 5 days could not be tested this way as they were too damaged when bisected to be of use for histological studies.

1. Infectivity tests: The halves of the test larva were each macerated in 0.5 ml. distilled water and 1 μ l. of this extract was placed in the food surrounding each of thirty one-day-old larvae that were then treated as described in section 2.2.

2. Histology: Histological studies of the integument, alimentary tract, silk glands and brain of treated larvae were made in the manner described in section 2.8a.

3.1b. Results

Table 1 shows that the inoculum used to infect the honeybee larvae taken for histological examination was highly infective, and Table 2 shows that much virus was in the halves of the larvae that were actually examined. All the control larvae in the tests pupated eight days after treatment whereas almost all the larvae that were fed virus died of sacbrood.

According to the dilutions used each larva fed on the extracts from the half-larvae would have received 10^{-6} units of sacbrood virus from the original extract - approximately one fifth of a median lethal dose (Bailey, Gibbs & Woods,

Table 1. Results eight days after feeding one-day-old honeybee larvae with 10^{-3} units of sacbrood virus or with extracts of healthy larvae.

Inoculum	Number of larvae			
	Treated	Missing	Died of sacbrood	Pupated
Virus	75*	22	23	0
Extract of healthy larvae	75*	4	0	41

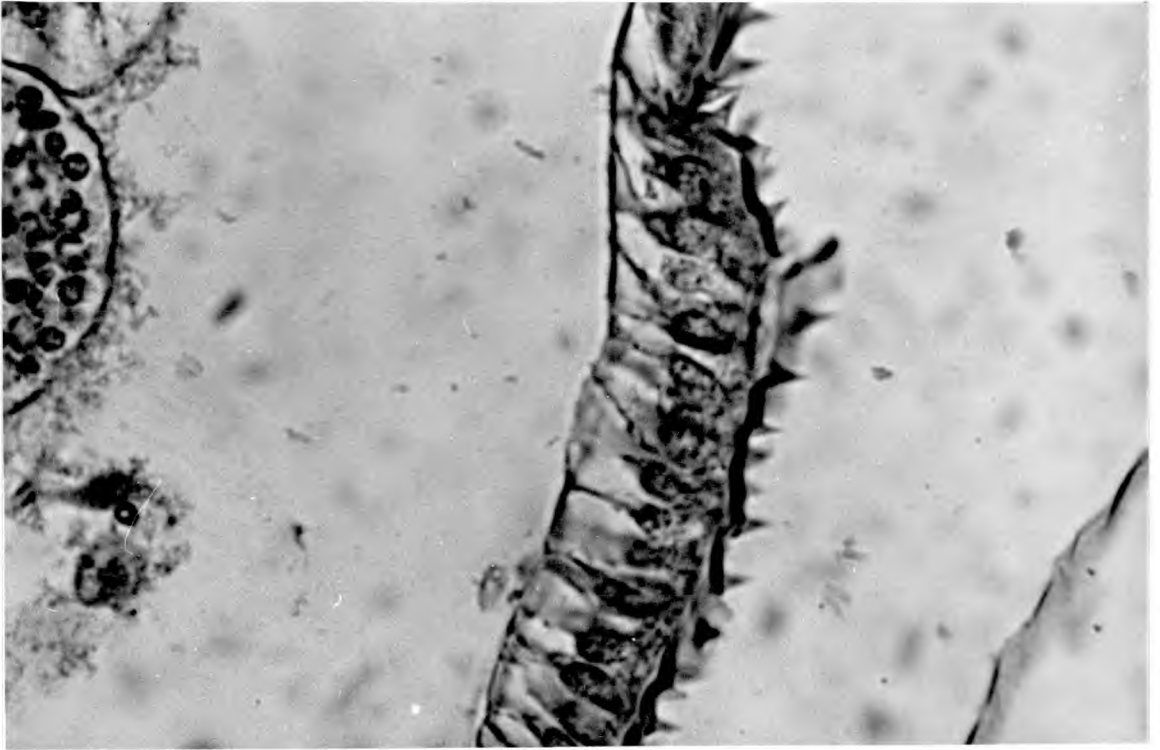
* A total of 30 larvae were removed when 3-8 days old for histological examination (see text) and infectivity tests (see Table 2).

Table 2. The number of larvae out of 30 killed by sacbrood when each was fed with the equivalent of $\frac{1}{2} \times 10^{-3}$ of an apparently healthy, 5, 6 or 8-day-old larvae that had previously been fed, when 1 day old, either with 10^{-3} units of sacbrood virus or with an equivalent preparation of healthy larvae.

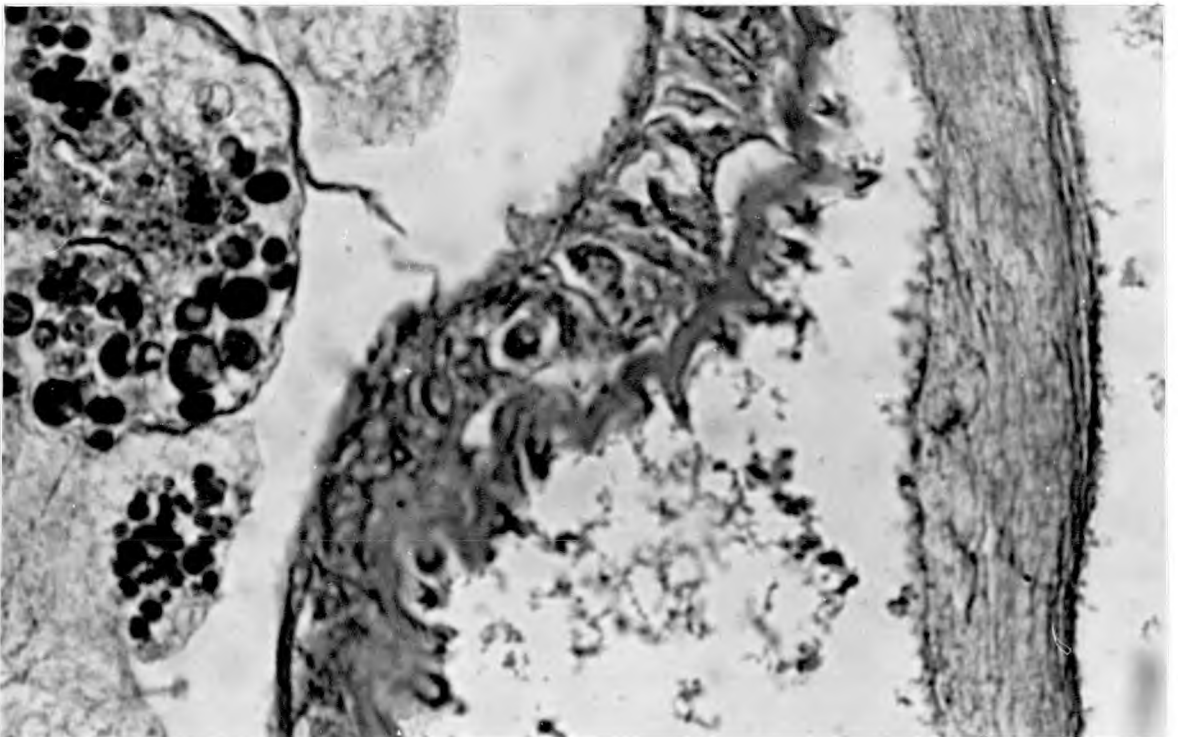
Age of larva (days) from which inoculum was prepared *	Test	Number of larvae					
		Fed extract of larvae previously fed on virus			Fed extract of healthy larvae		
		missing	pupated	died of sacbrood	missing	pupated	died of sacbrood
5	1	7	0	23	0	30	-
	2	5	0	25	1	29	-
	3	10	0	20	0	30	-
6	1	9	0	21	0	30	-
	2	8	0	22	2	28	-
	3	11	2	17	0	30	-
8	1	9	0	21	3	27	-
	2	14	0	16	2	28	-
	3	15	0	15	2	28	-

* See footnote to Table 1.

Fig.6. Section through the integument of
(A) a healthy 8-day-old honeybee larva
and (B) a larva of the same age infected
with sacbrood virus when it was one day
old. ~~2400~~



A



B



Fig.7. 8 9-day old honeybee larvae.
Top row: infected with sacbrood virus.
Bottom row: healthy.
x3.5

1964; Bailey, 1969) - assuming that none of the original inoculum was lost or had lost infectivity. However, as almost all the bioassay larvae were killed, much virus clearly had multiplied even in the youngest larvae that were examined histologically.

However, although much sacbrood virus had multiplied in infected larvae by the time they were five days old (Table 2) no striking abnormalities were seen till they were about six days old and reaching the prepupal stage. Their integument was then obviously affected. At this stage the endocuticle in healthy individuals appears to be completely digested, the unshed portion consisting of the thin fragile exocuticle. The pupal cuticle which begins to form during this process of digestion becomes separated from the old larval cuticle by the exuvial space (Fig. 6A) and the larva is then ready to moult. In larvae infected with sacbrood virus the endocuticle of the last larval integument became separated from the pupal cuticle, as usual, but it then remained undigested and attached to the exocuticle. It is the space between the undigested larval endocuticle and the pupal cuticle that forms the sac containing the ecdysial fluid (Fig. 6B and Fig. 7). However, the pupal cuticle with rudiments of setae formed as in the healthy larvae.

The only other histological effects of sacbrood virus appeared in the fat-body tissue. The appearance of fat-body cells from the second day after infection were as

follows:

(a) 3-4 day-old larva: The cells of the fat-body of healthy larvae consisted of a compact mass of polygonal cells with a very basophilic nucleus and a homogeneous cytoplasm surrounded by a distinct cell membrane. Fat cells in larvae infected with sacbrood virus appeared to be the same as in healthy larvae at this stage.

(b) 5 day-old larva: The fat body cells of healthy larvae, that were now being sealed within their brood-comb cells, had enlarged and numerous vacuoles in the cytoplasm (Fig.8A). At the same time small, distinctly basophilic granules appeared in the cytoplasm close to the nucleus. The same changes occurred in the cells of infected larvae (Fig.8B and Table 3).

(c) 6 day-old larva: The fat-body cells of healthy larvae had enlarged further. The basophilic granules had become more numerous, and were distributed more towards the peripheral region of the cells (Fig.9A). There were also a few acidophilic granules among the basophilic ones. The fat-body cells of larvae infected with sacbrood virus were larger (Table 3) than those of the healthy individuals, but the basophilic granules, although more numerous than before, were now significantly ($P < 0.001$) fewer per cell than in healthy larvae (Table 4) and were still concentrated close to the nucleus (Fig.9B). There were a few acidophilic granules, but, again, fewer ($P < 0.05$) than in healthy

Table 3. Length (l) and breadth (b) (μ) of fat cells from five worker honeybee larvae fed when 1-day-old with either 10^{-3} units of sacbrood virus or with an equivalent preparation of healthy larvae.

Age of inoculated larva (days)	Inoculum											
	Sacbrood virus						Extract of healthy larvae					
	5	6	8				5	6	8			
No. of cells measured	50	50	50				50	50	50			
Mean	l.	b.	l.	b.	l.	b.	l.	b.	l.	b.	l.	b.
	36.0	27.9	45.2	34.9	47.1	36.2	35.2	28.0	37.3	30.7	39.7	31.3
	± 0.31	± 0.40	± 0.95	± 0.40	± 0.40	± 0.32	± 0.68	± 0.90	± 0.40	± 0.54	± 1.20	± 0.40
Significance of difference between means (P)	N.S.						<0.001					

Table 4. Number and types of cytoplasmic protein granules per section of fat cell in worker honeybee larvae 5 and 7 days after they were fed when one day old with either sacbrood virus or with an equivalent preparation of healthy larvae.

Inoculum	Number examined		Age of larvae (days) when examined			
			6		8	
	larvae	cells	Number and type of granule			
			Basophilic Acidophilic		Basophilic Acidophilic	
			mean	mean	mean	mean
Sacbrood virus	5	50	18.6 ± 1.0	0.8 ± 0.5	29.4 ± 2.4	0
Healthy larval extract	5	50	42.8 ± 4.2	5.2 ± 1.7	4.0 ± 2.2	62.6 ± 8.8
Significance of difference between means (P)			<0.001	<0.05	<0.001	<0.001

Fig.8. Sections of fat-body cells of (A) a healthy 5-day-old larva and (B) a larva of the same age infected with sacbrood virus when it was one day old.
X400

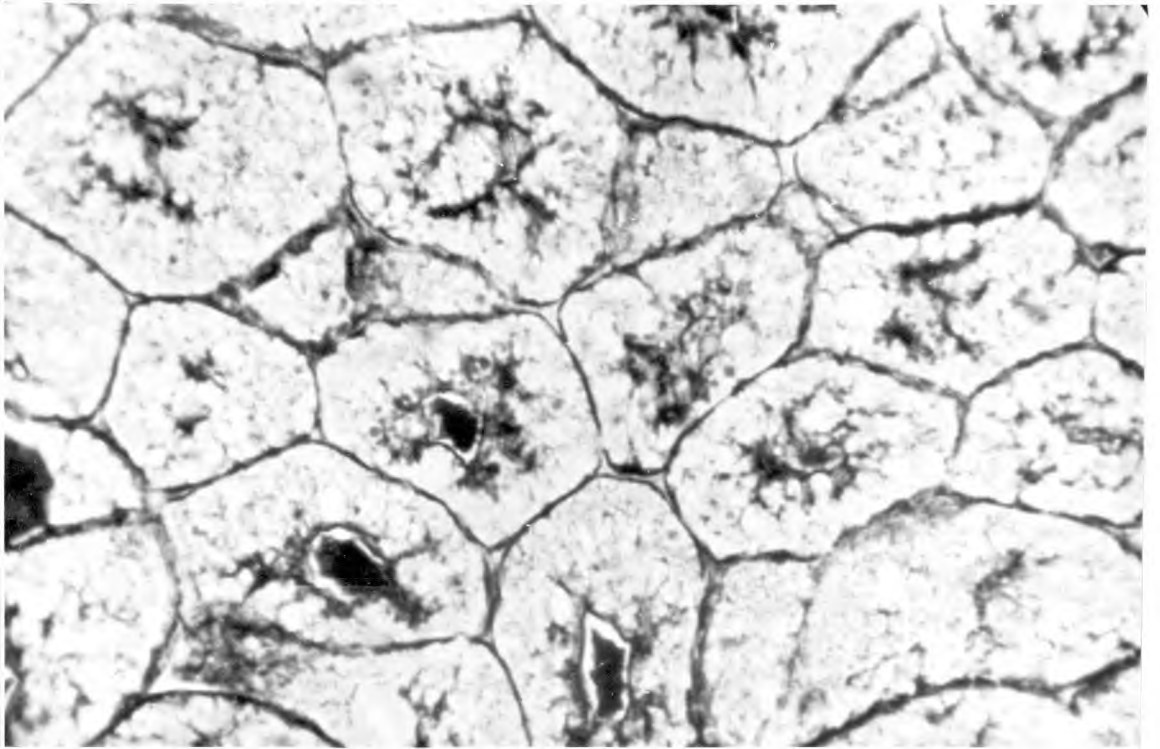
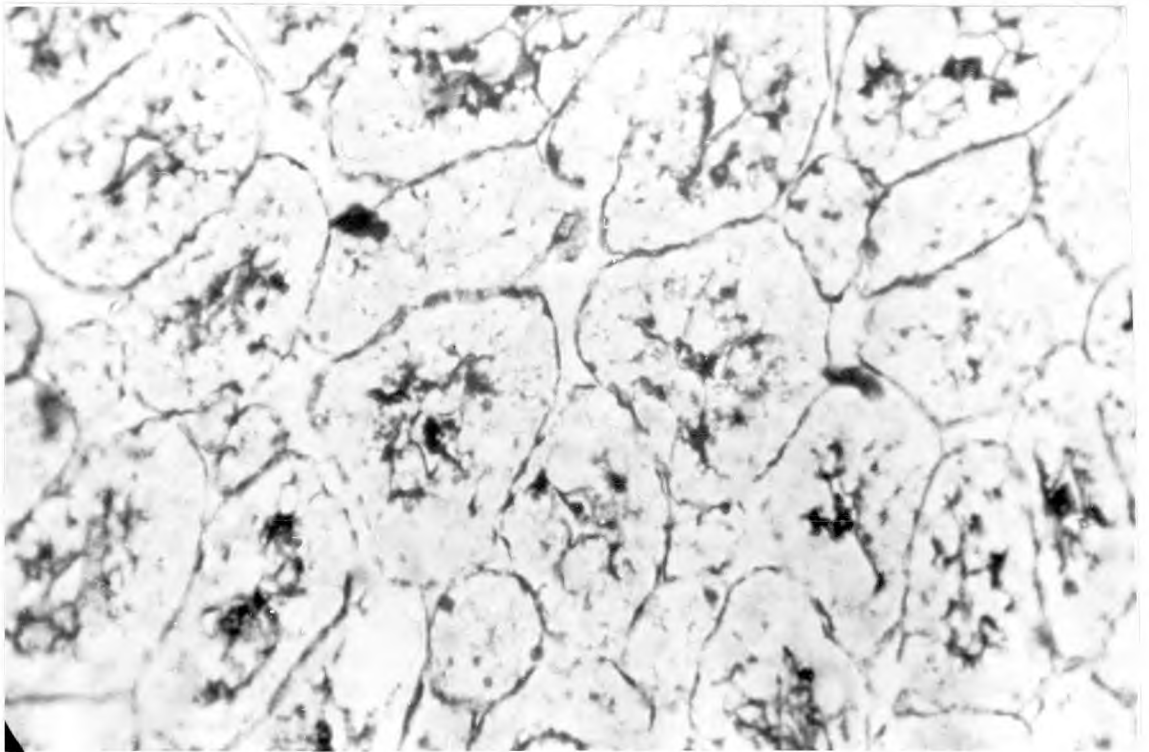
**A****B**

Fig.9. Sections of fat-body cells of (A) a healthy 6-day-old larva and (B) a larva of the same age infected with sacbrood virus when it was one day old.

X400

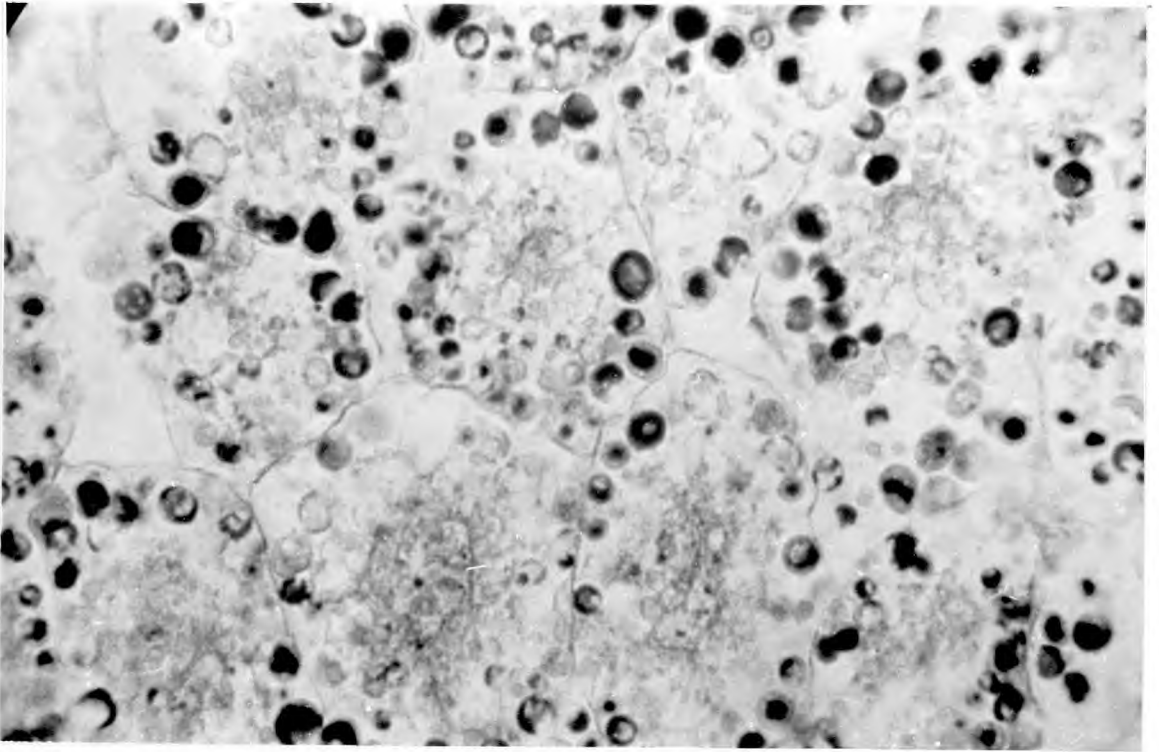
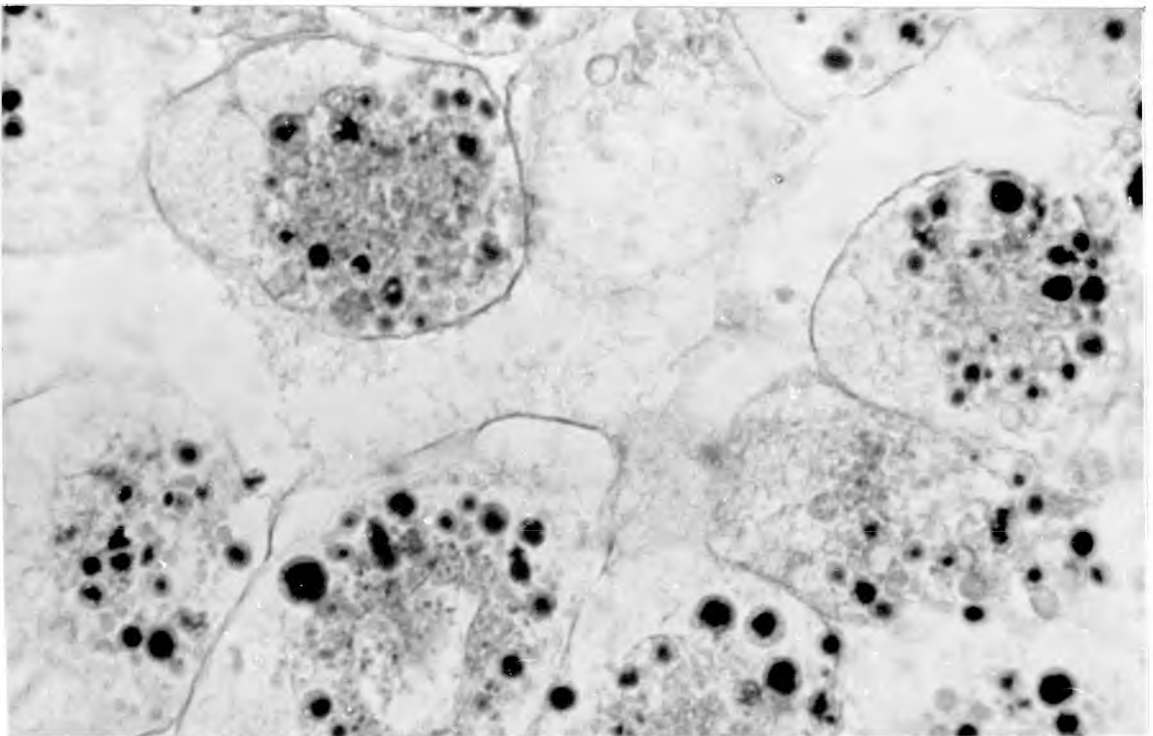
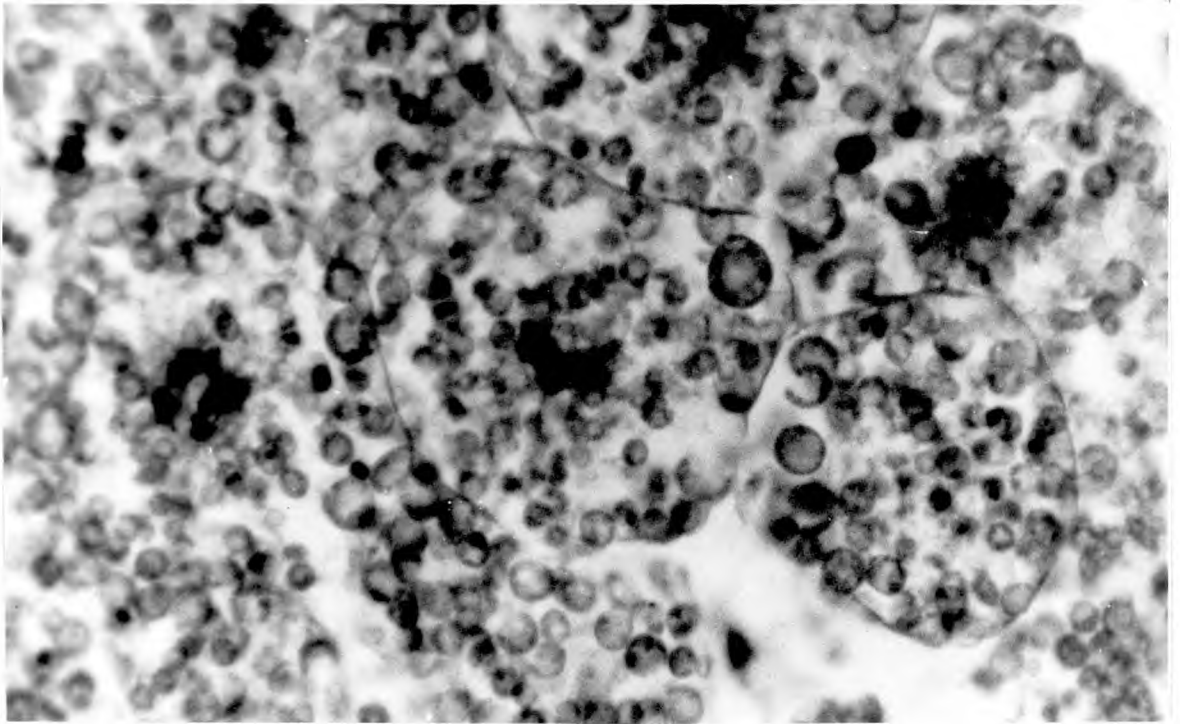
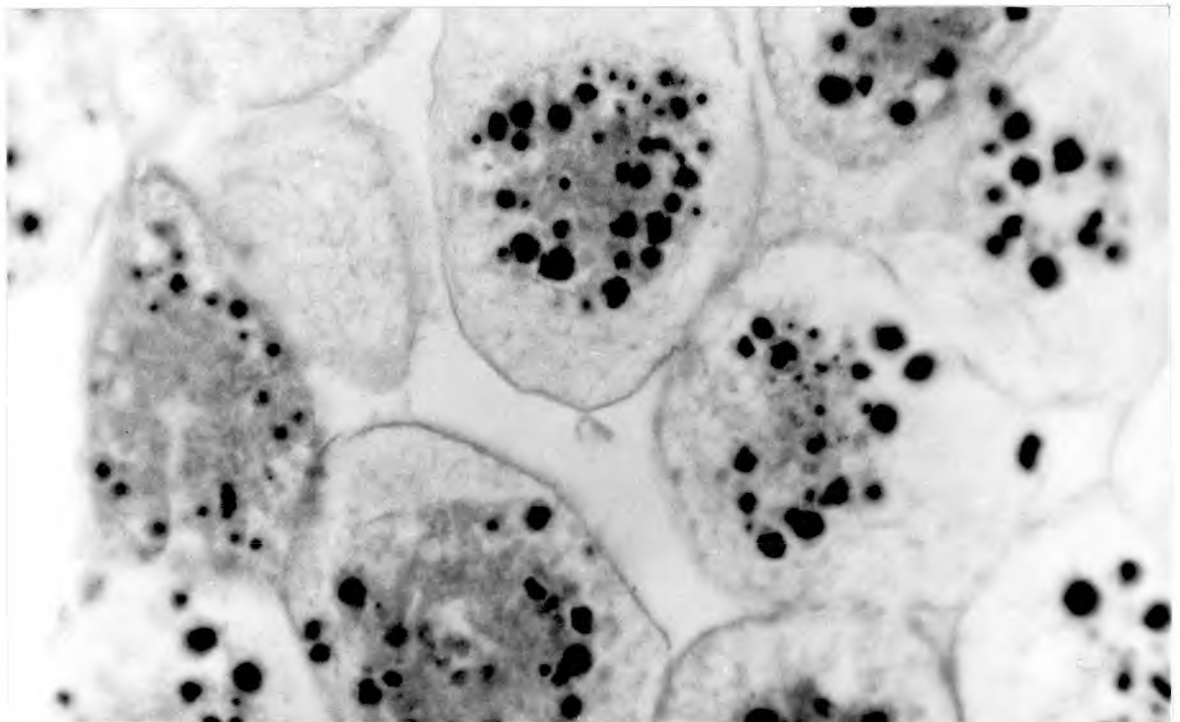
**A****B**

Fig.10. Sections of fat-body cells of (A) a healthy 8-day-old larva and (B) a larva of the same age infected with sacbrood virus when it was one day old.
x400

**A****B**

individuals.

(d) 7-8 day-old larva. The fat-body cells of healthy larvae had further enlarged (Table 3) with less distinct cell membranes, making the tissue appear diffuse. The protein granules also had increased in number but more of them than before had become acidophilic (Table 4). Most appeared as vacuolated spheres with centres staining only slightly and the peripheries staining slightly darker than the surrounding cytoplasm (Fig.10A). The fat-body cells of infected larvae also enlarged further and were still significantly larger than those of healthy individuals (Table 3) and they still maintained distinct cell membranes. The protein granules were more numerous than in the infected 6 day-old larvae but they were still basophilic and they were still fewer than in healthy individuals (Table 4). Moreover, they still remained close to the nucleus (Fig.10B).

All the granules in both infected and healthy larvae stained deeply with bromophenol blue, which indicated they contained much protein.

No other histological abnormalities were observed in infected larvae.

Discussion:

My histological studies produced no evidence of damage to the nervous tissue of infected larvae or to the formation of the pupal cuticle. In fact, the histology of infected larvae seemed normal till they were six days old and were

changing into prepupae. The most important pathological effect of infection is the inhibition of the process of digestion of the endocuticle, possibly by preventing the action of enzymes. This prevents ecdysis and kills the larva.

Infection also causes a marked change in the protein metabolism in fat-body cells of infected larvae. This was shown mainly by the fewer protein granules developing in the fat-body cells of infected larvae than in healthy larvae, and the failure of these granules eventually to dissolve. The latter effect may again indicate failure of the formation or action of enzymes.

These possible effects on enzymes and the comparatively few protein granules in infected larvae may well be caused by loss of protein to the multiplying virus.

Part 4. EFFECT OF SACBROOD VIRUS ON ADULT BEES

4.1. Introduction

According to Bailey (1968; 1969; 1970) bees infected with sacbrood virus, either by feeding or by injection, show no symptoms. However, he showed that young adults fed sacbrood virus before they had eaten pollen subsequently ate no pollen when kept in cages in the laboratory. Moreover, they lived, on average, only three weeks, as uninfected bees deprived of pollen, whereas uninfected bees supplied with pollen lived about eight weeks. Young bees injected with sacbrood virus also ceased to eat pollen, whereas bees injected with sterile water continued to eat pollen and live normally. Bailey also showed that healthy young bees anaesthetized with CO₂ ate no pollen and lived only about as long as those infected with sacbrood virus.

Bailey (1970) also found that the lives of drones, which, unlike worker bees, do not feed on pollen, were shortened by infection only slightly, although significantly, from 25 days to 21 days. Much virus was still in the infected drones at the end of their lives, showing that they had not become free of infection.

The results of these laboratory experiments suggested that the lives of adult bees infected with sacbrood virus when young and kept in cages, might be shortened only by their decreased appetite for pollen, which is essential for their longevity (Maurizio, 1950). However, it seems

unlikely that sacbrood virus would have no other pathological effects especially as it multiplies in the brains of adults (Bailey, 1970), so I made some further experiments, first to see whether the virus affected the longevity of mature adult bees that had already eaten pollen. Bees were tested in summer, autumn and winter, for Lotmar (1939) showed that adult worker bees consume much pollen in colonies during autumn and winter, thereby increasing the protein reserves in their fat body. I also investigated the effect of the virus on the pollen consumption of young adult worker bees under natural conditions in colonies.

4.1a. Methods:

1. Length of life of adults

Experiments were done with foraging honeybee workers, captured as they returned with pollen loads, at the hive entrances of normal colonies, and with mature adult worker bees collected from the clusters of colonies during autumn (October) and winter (January).

Each bee was injected with 10^{-6} units of sacbrood virus as described in section 2.5 and incubated in cages in the laboratory at 35°C. as in section 2.6. For control experiments similar bees were injected with an equivalent extract of healthy larvae. These mature bees were not provided with pollen as they do not eat any, at least not in cages, even when they are healthy.

For comparison, similar experiments were done with

newly emerged worker bees fed with sacbrood virus and provided with pollen (section 2.3) and also with young drones either fed (section 2.4) or injected, with a virus suspension as in section 2.5. All cages were incubated at 35°C. as described in section 2.6.

Dead bees in all treatments were removed daily till half the individuals in each cage had gone.

2. Pollen consumption of infected adults in colonies

100 newly emerged worker bees were fed with a suspension of sacbrood virus and another 100 with an extract of healthy larvae as described in section 2.3. After treatment, 50 infected bees together with the same number of uninfected control bees were marked and placed immediately in a normal colony. The remainder were marked differently and placed in the same colony, after incubation in cages without any pollen at 35°C. for 40 hours, as described in section 2.3. This treatment allowed some time for the virus to multiply before the infected bees could find pollen in the colony. Newly emerged bees commence eating pollen three hours after they emerge and, about ten hours later, the pollen grains enter the rectum, from which they are discharged only when the bees begin to fly (Whitcomb & Wilson, 1929; Bailey, 1952; Dietz, 1969), usually not before they are five days old. Therefore, all the marked bees were collected from the colony five days after they had been placed in it and were examined for the shells of ingested pollen grains, the contents of which are

digested by bees, by removing the whole rectum from each bee and macerating each one in 1 ml. of distilled water.

The amount of pollen consumed by each individual worker bee was found by counting the pollen grains in a haemocytometer.

4.1b. Results

1. Length of life

Table 5 shows the effects of sacbroodvirus on the length of life of worker bees and drones. The half-lives of the groups of infected newly emerged worker bees or of the groups of infected mature worker bees from the colonies in autumn and winter were decreased significantly ($P < 0.001$) to about 20 days, about the same as that of groups of uninfected newly emerged worker bees that were not supplied with pollen. The half-lives of groups of infected foraging bees were decreased from about 22 days to 15 days ($P < 0.001$).

The half-lives of groups of newly emerged drones, infected with sacbrood virus either by feeding or by injection, and kept in cages with attendant worker bees, was about the same as that of young healthy worker bees that had not been supplied with pollen, and only very slightly shorter than that of healthy drones.

2. Pollen consumption

Table 6 shows the number of pollen grains in the rectum of worker bees that lived in normal colonies for five days after they had been infected with sacbrood virus when

Table 5. Half-life of groups of adult bees infected with sacbrood virus at different times of the year and incubated in cages at 35°C.

Inoculum	Types of bee and method of infection																
	Newly emerged worker bees				Pollen gatherers (July)	Mature worker bees from colonies (October)				Mature worker bees from colonies (January)				Newly emerged drones			
	supplied pollen		Not supplied pollen														
	Fed				Injected								Fed		Injected		
	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	Repli- cates	Mean	
Sacbrood virus	6	20 ± 1.5	-	-	6	15 ± 0.45	5	20 ± 0.9	8	20 ± 0.6	6	18 ± 0.54	6	18 ± 0.50			
Extract of healthy larvae	6	53 ± 2.5	6	22 ± 0.19	6	22 ± 0.84	5	34 ± 2.6	8	28 ± 1.0	6	21 ± 0.66	6	20 ± 0.32			
Significance of difference between means (P)	< 0.001				-	< 0.001				< 0.001				N.S.		N.S.	

Table 6. Mean number of pollen grains per rectum of worker bees after they were fed with sacbrood virus or a virus-free inoculum when newly emerged, incubated without pollen, and then kept for 5 days in a normal colony.

Inoculum	Period (hrs) incubated without pollen	No. of bees	No. recov- ered	No. exam- ined	Pollen grains per bee $\times 10^5$	Significance of difference (P) between means
Sacbrood virus	0	50	4	4	9 ± 3.6	< 0.001
Extract of healthy larvae	0	50	9	4	20 ± 2.5	
Sacbrood virus	40	50	15	15	0	< 0.001
Extract of healthy larvae	40	50	32	15	11 ± 0.86	

newly emerged. Infected bees that were placed immediately in a colony had eaten less pollen after five days than similar healthy bees, and those placed in the colony after incubation for 40 hours at 35°C. in cages, had eaten none.

4.1c. Discussion

My results are similar to those of Bailey (1969, 1970) for young worker bees and drones infected with sacbrood virus. However, the lives of mature adult worker bees that had already eaten pollen were shortened by the virus. Moreover, comparatively few infected worker bees were recovered from colonies only five days after they were placed in them (Table 6). They disappeared long before they were due to die, according to the results of experiments with infected bees in cages (Table 5). My results show, therefore, that sacbrood virus causes pathological effects in adult bees, and changes their behaviour, in addition to destroying the appetite for pollen.

Further experiments were made, therefore, to see whether there were any observable effects of sacbrood virus on the behaviour of adult bees in colonies.

4.2. Behaviour in the colony

The fact that individual worker honeybees perform a sequence of different duties in the colony has long been established (Danhoff, 1855, in Ribbands, 1953). Since then numerous papers on the division of labour in honeybee colonies has been published or reviewed, and it has been suggested that although several factors influence the particular duty, this depends mainly on the age of bees and the needs of the colony. (Rösch, 1925; Haydak, 1932, 1963; Lindauer, 1953; Ribbands, 1952, 1953; Sakagami, 1953). It has been generally accepted that worker bees usually perform the various duties within the hive, such as feeding larvae and probably the queen and drones with protein-rich secretions of their hypopharyngeal glands (Langer, 1912; Patel et al, 1960) and cleaning and building comb cells, when they are young, whereas when they are about 3 - 4 weeks old they take to foraging activities in the field. One of the tasks in the hive is to attend the queen. A circle of 8 - 18 attendant bees forms round her when she is resting or laying (Rösch, 1925; Lindauer, 1952; Allen, 1955, 1956, 1960; Butler, 1954). All ages of worker bees form the circle of attendants but the queen receives food only from those younger than 12 days, the same age group of worker bees that feed larvae (Rösch, 1925; Lindauer, 1952; Allen, 1955, 1956, 1960).

Perepelova (1928) noted that drones rarely move about in the colony except when soliciting food, and that they do

not take food from comb-cells. They were fed by worker bees of 8 - 14 days of age. Alpatov & Saf'yanova (1950) stated that caged drones were fed by nurse bees but were neglected by foragers, while Oertel et al (1953), Oertel (1956) and Mindt (1962) found that, although old drones fed themselves on honey, young drones were fed by worker bees of similar ages to those feeding the queen and larvae. Ruttner (1964) has stated that drones take their first flight on or after the 8th day following emergence, but they do not become sexually mature till they are 15 - 20 days old.

The effects of sacbrood virus were studied on some of these various aspects of behaviour.

4.2a. Methods

Observations were made using single-comb observation hives (Fig.11) measuring 39 x 5 x 24 cm. with entrances leading out of doors by a narrow tunnel. The sides of the hives and the top of the tunnel were covered with transparent plastic. The hives were each provided with a comb containing larvae, pollen, honey and a healthy, mated queen. A thermostatically controlled heater beneath each hive maintained the temperature of the air on either side of them at $34 \pm 1^{\circ}\text{C}.$, the temperature at the centre of a normal colony.

1. Behaviour of worker bees

Newly emerged worker bees were fed with sacbrood virus and others with an extract of healthy larvae as described in section 2.3. After treatment, groups of

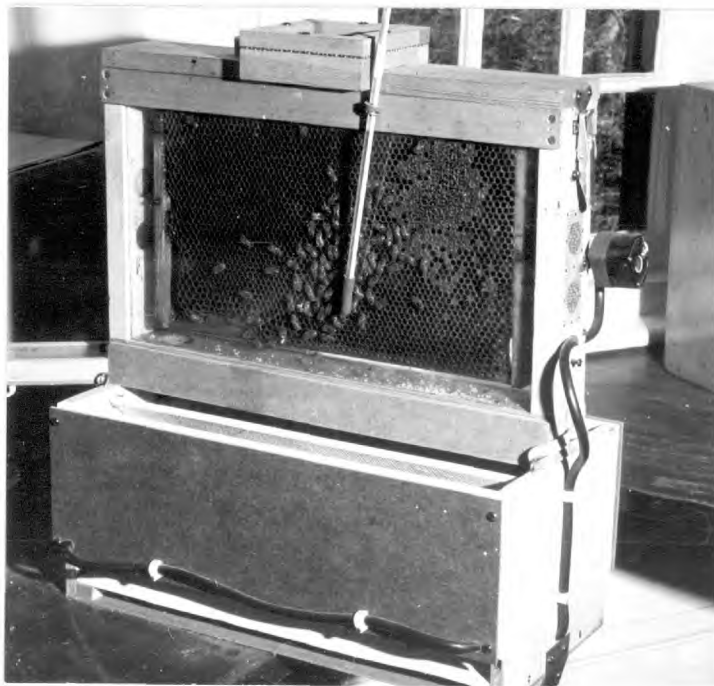


Fig.11. Single-comb observation hive.

bees were marked and placed in observation hives as follows:

Experiment A. 75 infected worker bees + 225 uninfected bees

Experiment B. 200 " " " + 200 "

Experiment C. 225 " " " + 75 "

Experiment D. 350 infected worker bees only in one hive
and 350 uninfected worker bees only in
a similar adjoining hive.

Experiment E. Newly emerged worker bees were kept in cages with pollen and sucrose solution till they were 5 days old when they were anaesthetized by chilling at 4°C. and 30 were each injected with 10^{-6} units of sacbrood virus. A similar number were injected with an extract of healthy larvae (section 2.5). They were then marked and placed in an observation hive among about 500 healthy worker bees of all ages.

The behaviour of all treated bees was observed daily and the number of attendant workers in the court of the queen was observed for one minute every hour for eight hours each day.

Worker bees do not form a court around drones, so their behaviour towards them is difficult to observe. Accordingly the survival of drones attended by infected or healthy worker bees was measured in cages in the laboratory.

Newly emerged worker bees were fed with virus or extracts of healthy larvae (section 2.3), and kept in cages with healthy drones at 35°C. (section 2.6). During the course of the observations, dead worker bees were replaced with similar live individuals. Dead drones were removed daily till half of them in each cage had died.

2. Behaviour of drones: Groups of newly emerged drones were either fed (section 2.4) or injected (section 2.5) with sacbrood virus or with extracts of healthy larvae and placed in an observation hive containing healthy worker bees of all ages. All treated individuals were observed daily for their behaviour in the hive.

3. Length of life and infectivity checks: Groups of additional bees from all experiments were kept in cages at 35°C. (section 2.6) and their half-lives were measured. Six worker bees from each group were removed after 5 days and tested for sacbrood virus by the infectivity and serological method described in section 2.7.

Groups of infected drones were similarly kept with attendant workers in cages at 35°C. as described in section 2.6. and their half-lives measured. The heads of six drones from each experiment were tested serologically (section 2.7).

4.2b. Results

One of the most striking effects of infection with sacbrood virus was its effect on the behaviour of the worker bees towards the queen. Table 7 (experiments A, B and C) shows

that infected worker bees did not join her court (Fig.12). In experiment D one or two worker bees of the colony that consisted entirely of infected bees were occasionally beside the queen, but there were always many fewer than those observed with the queen in the hive containing similar, uninfected bees. On most occasions the queen in the colony of infected bees was not attended at all. In all experiments, about 5 - 9 worker bees from the uninfected groups, whatever proportion they were of the colony, attended the queen (Table 7). Table 8 shows a similar pattern in the behaviour of worker bees that had been injected with sacbrood virus when they were five days old, after they had eaten pollen in cages, and had then been placed in the observation hive.

In all four Experiments, A, B, C and D workers fed with sacbrood virus before they had eaten pollen clustered tightly in the colony (Fig.13) but when sacbrood virus was injected into 5-day-old worker bees after they had eaten pollen they did not cluster (Experiment E.).

Table 9 shows that in all four experiments A, B, C and D, infected bees began to leave their hives from the first day. About half had gone by the sixth day, and all had disappeared by the tenth day, whereas most healthy bees remained after fourteen days. Although normal bees can fly when they are two days old, bees infected with sacbrood virus did not, but dropped to the ground as soon as they left the hive entrance.

Table 7. Mean number of worker bees in the court of the queen on eight hourly occasions on each of eight successive days after they were fed sacbrood virus or extracts of healthy larvae when less than one-day old.

Expt.	Inoculum	No. of bees	Pro- portion %	Days after treatment							
				1	2	3	4	5	6	7	8
A	Sacbrood virus	75	25	0	0	0	0	0	0	0	0
	Extract of healthy larvae	225	75	7	5	9	7	6	5	5	6
B	Sacbrood virus	200	50	0	0	0	0	0	0	0	0
	Extract of healthy larvae	200	50	7	7	9	8	6	7	7	6
C	Sacbrood virus	225	75	0	0	0	0	0	0	0	0
	Extract of healthy larvae	75	25	5	5	5	4	4	3	5	5
D	Sacbrood virus	350	100	1	0	0	0	1	0	0	0
	Extract of healthy larvae	350	100	7	7	7	7	6	7	6	8

Table 8. Mean number of worker bees present in the court of the queen on eight hourly occasions on each of eight successive days after they were injected with sacbrood virus or with extracts of healthy larvae when five-days old.

Experiment E.

Inoculum	No. of bees	Days after injection																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Sacbrood virus	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Extract of healthy larvae	30	3	3	3	4	3	5	4	5	2	2	3	2	3	3	2	3	2	2	2	1	2	1	1	2	0	0	0

Table 9. Number of worker bees present in an observation hive on each of fourteen successive days after they were fed with sacbrood virus or extracts of healthy larvae when less than one day old.

Expt.	Inoculum	No. of bees	Pro- portion %	Days after feeding													
				1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	Sacbrood virus	75	25	73	70	64	44	28	24	12	9	7	5	2	0	0	0
	Extract of healthy larvae	225	75	225	225	225	225	225	225	225	225	225	225	223	223	223	221
B	Sacbrood virus	200	50	193	177	125	100	80	62	42	21	7	0	0	0	0	0
	Extract of healthy larvae	200	50	200	200	200	200	200	200	200	200	200	200	200	200	200	195
C	Sacbrood virus	225	75	220	200	193	178	103	73	62	20	3	0	0	0	0	0
	Extract of healthy larvae	75	25	75	75	75	75	75	75	75	75	75	75	75	75	75	75
D	Sacbrood virus	350	100	341	330	315	183	163	88	35	10	10	0	0	0	0	0
	Extract of healthy larvae	350	100	350	350	350	350	350	350	350	350	345	345	344	344	344	342

Fig.12. The queen in an observation hive with attendant uninfected worker bees (marked yellow). Those infected with sacbrood virus are marked white.

Fig.13. Young worker bees in an observation hive showing those infected with sacbrood virus (marked white) clustering at the centre, and uninfected individuals distributed in the peripheral region.

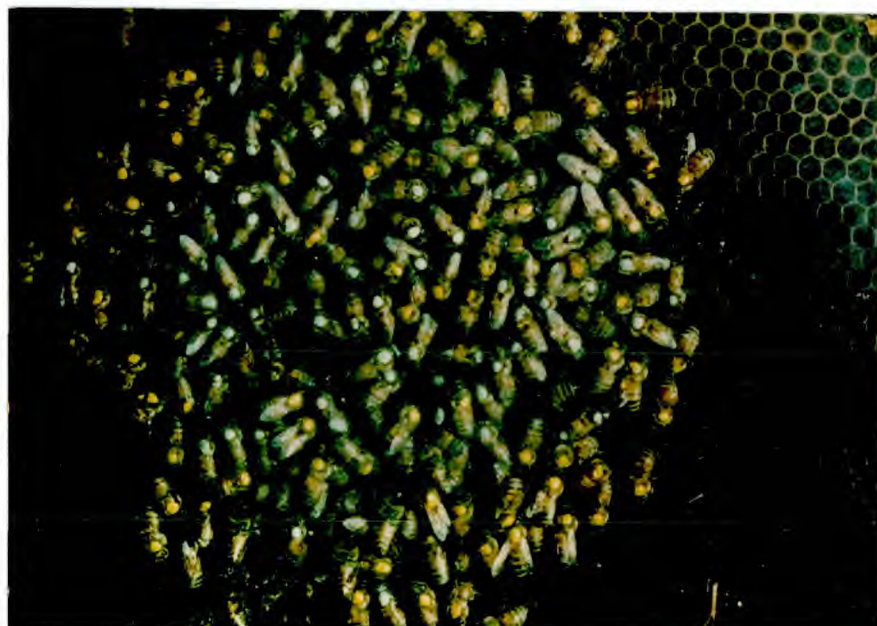


Table 10 shows that half the number of worker bees that had been injected with sacbrood virus when five days old had gone about eleven days after they were placed in the hive, and most disappeared by about seventeen days, whereas more than half the number of healthy worker bees stayed till about twenty days, and some remained even after thirty days.

Table 11 shows that half the number of infected drones disappeared from the hive after about five or six days and all had gone after ten or eleven days. Half the number of healthy drones remained for twenty days in the hive and all had not gone till after twenty-four days.

Table 12 shows the half-life of groups of young healthy drones kept in cages at 35°C. with young attendant worker bees that were infected with sacbrood virus was only about ten days ($P < 0.001$), about half that of drones attended by healthy workers, and about the same as that of young healthy drones kept in cages without any attendant worker bees.

Table 13 shows that the half-lives of groups of caged worker bees and drones that had been injected in the same way as the bees used in the observation hives, were about twenty days, similar to the results of previous experiments with caged bees (section 4.1b).

Serological tests showed that all individuals inoculated with sacbrood virus preparations had become infected.

No other abnormalities in the behaviour of bees

Table 10. Number of worker bees present in an observation hive on each of 30 successive days after they were injected with sacbrood virus or an extract of healthy larvae when they were five days old.

Inoculum	No. of bees	Days after injection																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Sacbrood virus	30	30	30	30	28	23	20	20	17	17	15	10	5	4	4	4	3	2	1	1	1	1	1	0	0	0	0	0	0	0	0
Extract of healthy larvae	30	30	30	30	30	25	25	25	20	20	20	20	20	17	15	15	15	15	15	12	12	12	11	11	11	10	7	5	3	3	3

Table 11. Number of drones present in the observation hive on each of twenty-five successive days after they were inoculated with sacbrood virus or with extracts of healthy larvae when they were newly emerged.

Type and method of treatment	No. of drones	Expt.	Days after treatment																								
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Fed sacbrood virus	20	1	20	19	19	18	18	12	9	4	2	0															
	20	2	20	19	16	14	10	7	6	2	2	1	0														
Fed extract of healthy larvae	20	2	20	20	20	20	20	20	18	15	15	14	14	14	12	12	12	12	9	9	8	5	3	1	0	0	
	20	2	20	20	20	20	20	20	20	19	19	17	15	15	15	15	14	12	11	9	7	5	3	2	2	0	
Injected sacbrood virus	20	1	20	20	19	13	9	6	6	3	1	0															
	26	2	26	20	17	7	6	3	2	2	2	2	2	0													
Injected extract of healthy larvae	20	1	20	20	19	19	19	19	19	17	16	15	15	14	14	13	13	13	13	11	9	8	3	3	1	0	
	26	2	26	26	24	24	24	23	23	22	22	22	20	20	20	16	16	16	15	15	8	8	5	3	0	0	0

Table 12. Half-life of groups of healthy drones kept in cages at 35°C. with healthy attendant worker bees or with bees infected with sacbrood virus.

Type of attendant worker bees	Replicates	Mean (Days)
Fed sacbrood virus	6	10 $\pm$ 0.36
Fed extract of healthy larvae	6	21 $\pm$ 0.45
No attendant worker bees	6	11 $\pm$ 0.36

Table 13. The time in days before half the number of worker bees and drones died in cages at 35°C. after being treated in the same way as bees used in the observation hives (See Tables 9 & 10).

Inoculum	Replicates	Type of bee and method of infection						
		Newly emerged worker bees				5 day old worker bees	Newly emerged Drones	
		Fed				Injected	Fed	Injected
		Experiment A	Experiment B	Experiment C	Experiment D	Experiment E	Experiment F	Experiment G
		Mean	Mean	Mean	Mean	Mean	Mean	Mean
Sacbrood virus	3	20 ± 1.50	19 ± 0.57	21 ± 0.57	20 ± 0.40	21 ± 1.15	20 ± 1.22	18 ± 0.91
Extract of healthy larvae	3	55 ± 3.6	51 ± 1.15	49 ± 1.62	53 ± 1.68	54 ± 1.87	22 ± 0.57	20 ± 0.51
Significance of difference between means (P)		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	N.S.	N.S.

infected with sacbrood virus were observed.

4.2c. Discussion

The results can be discussed under three headings, as follows:

(1) Queen attendance: As young worker bees infected with sacbrood virus do not eat pollen (section 4.1), their hypopharyngeal glands do not develop and so probably do not secrete much protein as in healthy young bees (Langer, 1912; Patel et al, 1960). This could be a reason for the failure of the young virus infected worker bees to attend the queen and it might also explain their inability to prolong the lives of healthy drones, for these probably depend upon protein, supplied from the glands of worker bees (Nixon & Ribbands, 1952), for longevity. A further explanation of the changed behaviour of infected bees towards the queen might be damage to the brain where sacbrood virus may multiply as it does in drones (Bailey, 1970).

Worker bees that were injected with sacbrood virus after they had eaten pollen also failed to attend the queen. Perhaps they lost protein very quickly to the multiplying virus, as suggested when their lives were decreased to about the same as those of worker bees that were not supplied with pollen (Table 5). This might cause their hypopharyngeal glands to atrophy as, for example, they do in bees infected with the microsporidian parasite, Nosema apis Zander (Wang & Moeller, 1970). Worker bees infected with this parasite

also do not attend the queen and leave the hive earlier than usual.

(2) Clustering: Infected worker bees appeared to be chilled because they clustered more than healthy bees. This suggests they are less able to maintain their body temperature than healthy worker bees, perhaps because their metabolic rate is diminished. Infected drones did not cluster but they may well not have the same instinct to do so as worker bees.

(3) Departure from colonies: Infected worker bees left the hive earlier than similar uninfected bees, long before they were due to die according to the results of cage tests (Table 13). This explains the rapid disappearance of infected worker bees from colonies in which they were placed in earlier experiments (section 4.1c), and indicates that sacbrood virus changes the behaviour of bees.

These aspects were investigated further as described in the following sections.

4.3. Protein metabolism

4.3a. Hypopharyngeal glands

The form and function of the hypopharyngeal glands in relation to the life of the healthy honeybee has been the subject of numerous investigations ever since they were discovered by Meckel in 1846. The secretions of the glands were stated to be composed of a protein-rich substance and were well developed only in worker bees that were feeding

larvae (Schiemenz, 1883; Langer, 1912; Patel et al, 1960). Soudek (1927) found that the glands were small and empty in newly emerged bees, but developed very rapidly after they ate pollen, while Kratky (1931) found that the hypopharyngeal glands did not develop in newly emerged worker bees which were fed on sugar solution, but developed when they were fed pollen, provided the bees were less than 10 days old. In addition to secreting the protein component of the food of larvae, these glands were also found to contain enzymes used to convert nectar into honey (Kratky, 1931; Gauhe, 1940; Simpson, ^{et al} 1968).

The structure of the hypopharyngeal glands has been adequately studied by several authors (Soudek, 1927; Kratky, 1931; Beane & King, 1933; Painter, 1945; Wetzig, 1964). The glands are well developed in worker bees but vestigial in queens and absent in drones. They are situated in the forepart of the head closely about the brain in the upper and the anterior parts of the head cavity. The glands are composed of a string of numerous ovate lobules, each lobule consisting of several unicellular acini. The entire lobule is surrounded by a thin, transparent, membranous capsule which extends as a closed sac from the common duct. The acini of each lobule contain numerous intracellular secretory canaliculi which join an intercellular duct that opens into the common duct of the glands.

The existence of a secretory activity is indicated by

the presence of secretory globules in the cytoplasm of the acini (De Robertis et al, 1965). These secretory globules are ^{found} not in the hypopharyngeal glands of newly emerged bees but appear when they are about 3 - 4 days old, after they have eaten sufficient pollen. The glands then became expanded and spherical in appearance (Kratky, 1931; Painter, 1945; Wetzig, 1964; Wang & Moeller, 1969). The duration of the secretory activity of the hypopharyngeal glands in healthy bees under normal conditions is not yet known, but during most of the winter, the glands remain enlarged (Kratky, 1931). Wetzig (1964) stated that the secretory activity of the hypopharyngeal glands in summer bees begins to decrease when the bees are about 11 days old. Wang & Moeller (1969) have stated that a direct correlation exists between the size of the secretory globules and the volume of the lobules. In old bees, especially those engaged in foraging activities, the hypopharyngeal glands become atrophied. Simpson (1954) has stated that anaesthesia with carbon dioxide also hastens the retrogression of the hypopharyngeal glands.

Bailey (1969) who found that much sacbrood virus accumulates in the hypopharyngeal glands of infected worker bees suggested that it may be transmitted in nature in the secretions. The youngest adult bees are the comb-cell cleaners and probably become infected when they ingest parts of dead larvae killed by sacbrood. Some of these worker bees may then subsequently feed healthy larvae with gland

secretions containing the virus.

The effect of sacbrood virus on the hypopharyngeal glands of worker bees was studied as follows:

1. Methods

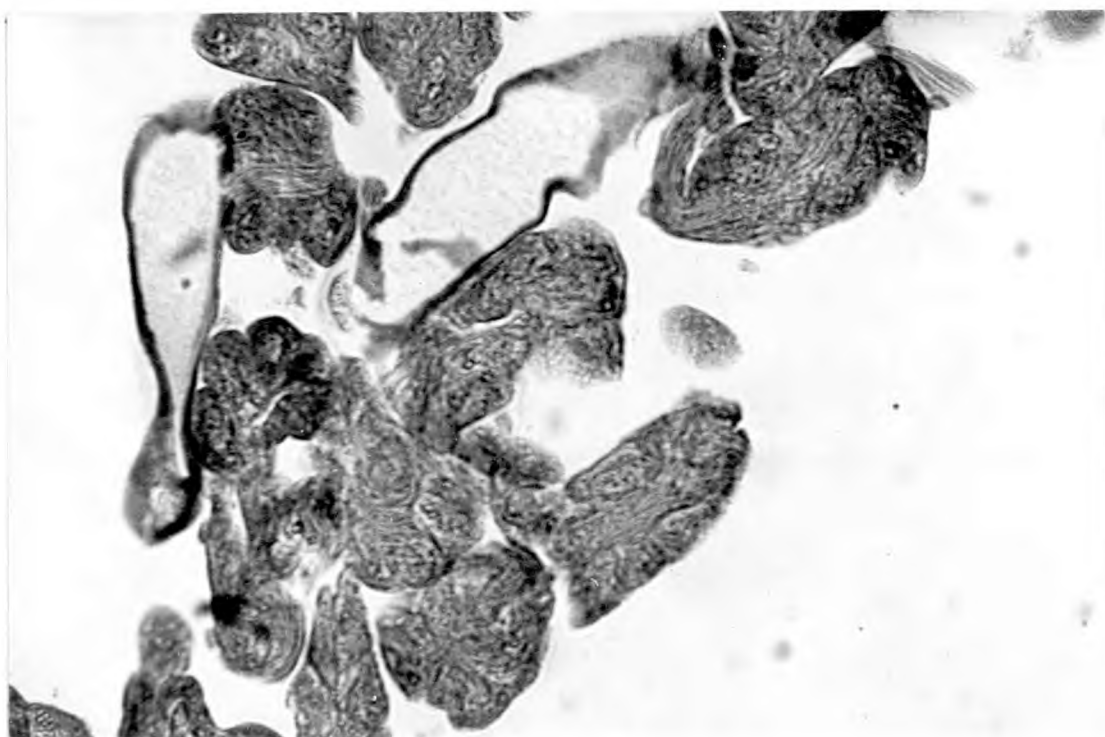
Experiment A: Newly emerged worker bees were fed with a suspension of sacbrood virus or with extracts of healthy larvae as described in section 2.3. Groups of bees were kept in cages at 35°C. (section 2.6) and were provided with pollen twenty-four hours after treatment. Another group of bees that was fed with extracts of healthy larvae were similarly kept in cages without any pollen.

Experiment B: Mature adult bees were collected at random from within colonies during winter (January). Each bee was injected with 10^{-6} units of sacbrood virus while others were injected with an equivalent extract of healthy larvae (section 2.5). After treatment they were kept in cages at 35°C.

Histological studies were made of the hypopharyngeal glands of some bees from all treatments, as described in section 2.8a.

2. Results

Table 14 shows that after 6 days in cages at 35°C. worker bees infected with sacbrood virus when newly emerged had much smaller hypopharyngeal glands, without any secretory globules (Fig.14A), than similar uninfected bees that had consumed pollen (Fig.14B). However, they were slightly



A

Fig.14. Sections of hypopharyngeal glands of worker bees six days after they were fed (A) sacbrood virus, (B) extracts of healthy larvae when they were less than one day old and provided with pollen in cages, and (C) extracts of healthy larvae when less than one day old and not provided with pollen.

X400

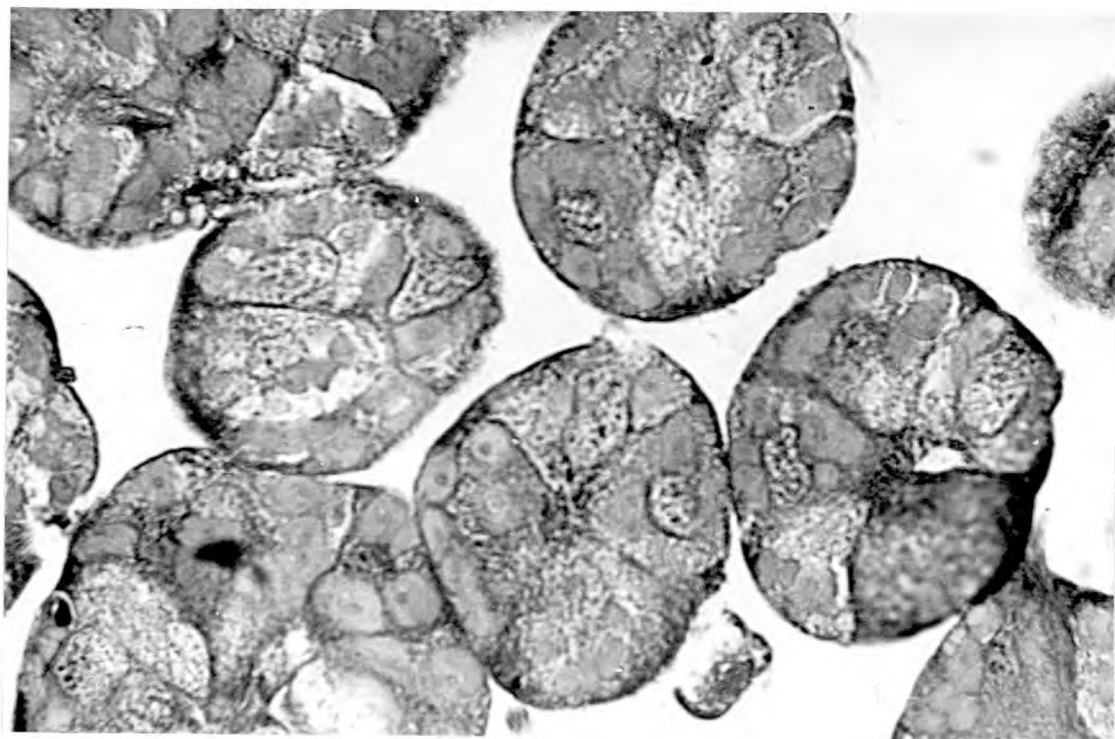
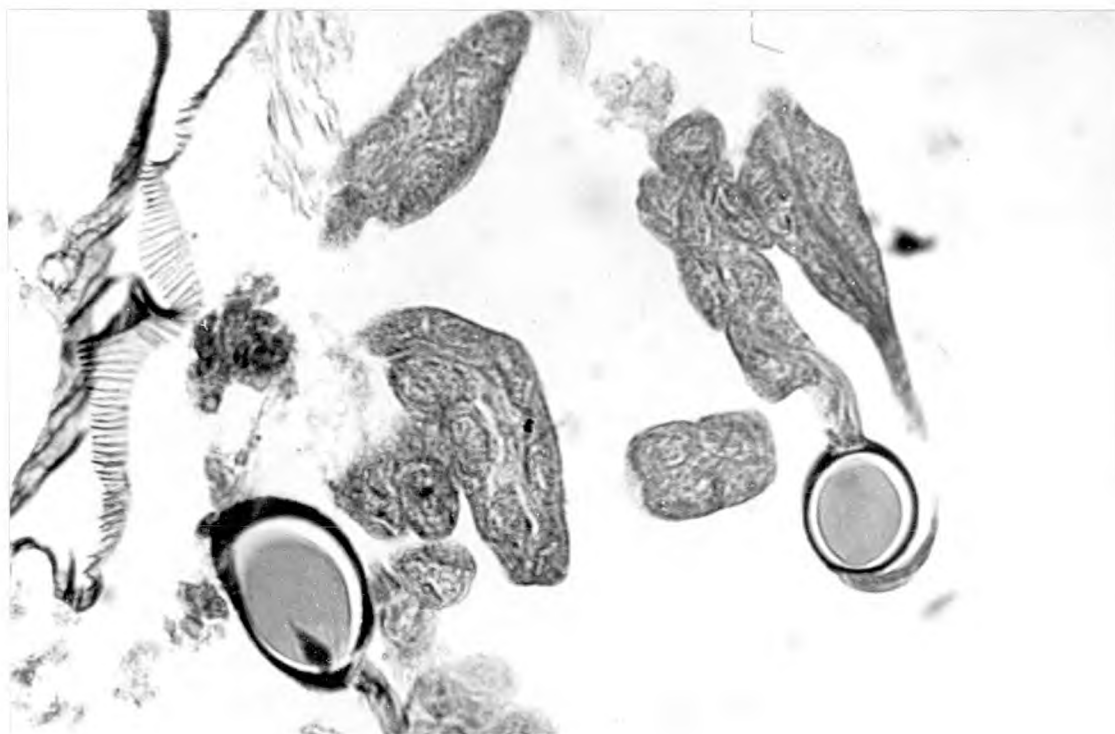
**B****C**

Fig.15. Sections of hypopharyngeal glands of mature worker bees from colonies in autumn six days after they were injected with (A) sacbrood virus and (B) extracts of healthy larvae.
x240

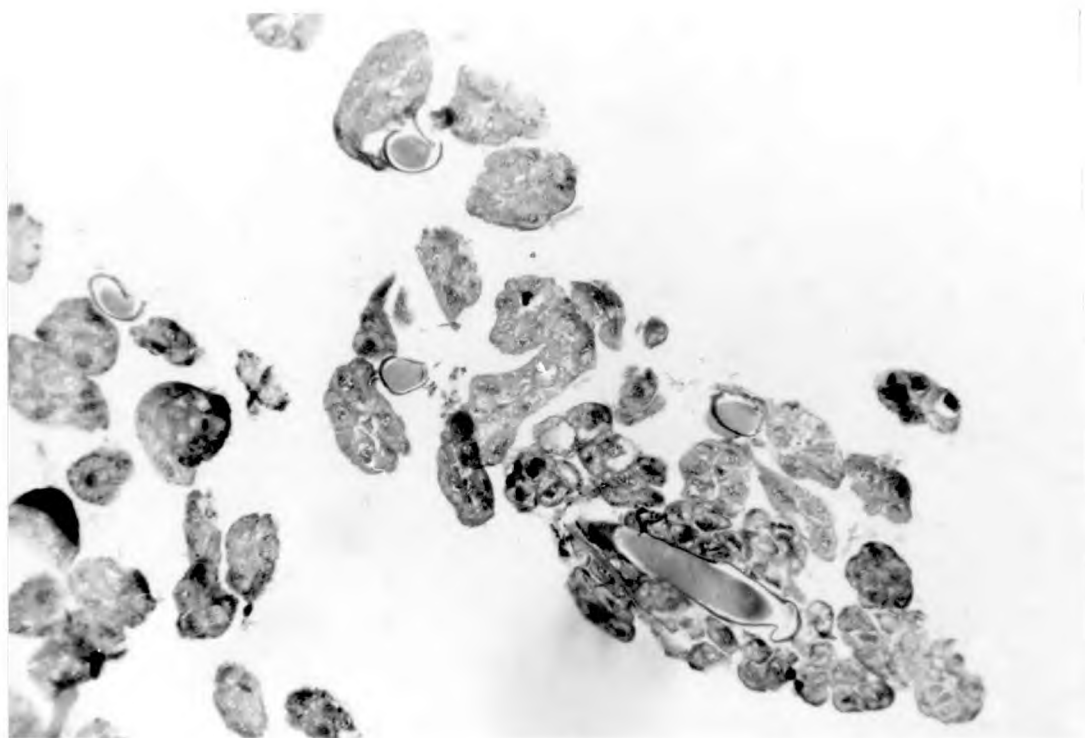
**A****B**

Table 14. Hypopharyngeal gland size, secretory globules and pollen grains in worker honeybees six days after they were fed with sacbrood virus or extracts of healthy larvae when they were less than one day-old.

Inoculum	Pollen supply	No. of bees examined	No. of lobules per bee examined	Dimensions of lobules (μ)		Dimensions of secretory globules (μ)		No. of pollen grains in rectum	
				Mean length	Mean breadth	Mean diameter		Mean x 10^5	
Sacbrood virus	+	12	5	52.1 ± 2.2	31.8 ± 1.2	0	-	0	-
Extract of healthy larvae	+	12	5	122.4 ± 2.5	94.9 ± 3.2	8 ± 0.15		8 ± 1.10	
Extract of healthy larvae	-	12	5	40.1 ± 1.2	23.1 ± 1.0	0	-	0	-

Table 15. The size of hypopharyngeal glands and their secretory globules in mature adult worker bees, from colonies in winter, six days after they were injected with sacbrood virus or an extract of healthy larvae.

Inoculum	No. of bees examined	No. of lobules per bee examined	Dimensions of lobules (μ)		Secretory globules (μ)
			Mean length	Mean breadth	Mean diameter
Sacbrood virus	12	5	71.4 ± 2.1	52.8 ± 1.5	3.0 ± 0.12
Extract of healthy larvae	12	5	117.9 ± 1.8	91.2 ± 2.5	6.3 ± 0.10
Significance of difference between means (P)			<0.001	<0.001	<0.01

though significantly ($P < 0.002$) larger, than the hypopharyngeal glands of healthy worker bees that had not been supplied with pollen (Fig. 14C).

Table 15 shows that the hypopharyngeal glands of mature adult worker bees six days after they were injected with virus were significantly ($P < 0.001$) smaller (Fig. 15A and 15B) and their secretory globules had also become much smaller ($P < 0.01$) than in uninfected bees.

No other cytological abnormalities were observed in the hypopharyngeal glands of infected bees.

The half-lives of surplus groups of bees prepared for these investigations were similar to those in previous tests (Table 5 and Table 13) indicating that sacbrood virus had multiplied in the inoculated individuals.

Tests on the hypopharyngeal glands with bromophenol blue indicated that the secretory globules contained much protein.

3. Discussion

It was now clear that the hypopharyngeal glands remained undeveloped in worker bees that were infected when newly emerged with sacbrood virus. This was expected as they failed to eat pollen. However, their hypopharyngeal glands became larger than those in healthy worker bees deprived of pollen, probably because of the accumulation or active secretion of the virus. The atrophy of the hypopharyngeal glands of bees infected with sacbrood virus after they had

eaten pollen and the disappearance of the protein-rich secretory globules are probably caused by loss of protein to the multiplying virus. Therefore this could well explain one reason for the failure of worker bees infected with sacbrood virus to attend the queen and drones (section 4.2c) and their shortened lives (section 4.1b and 4.2c).

4.3b. Fat-bodies

Lotmar (1939) found a positive correlation between the size of the hypopharyngeal glands and that of the fat-bodies in overwintering bees although this relationship no longer exists when they begin to rear larvae. Maurizio (1954) found a positive correlation between the length of life of worker honeybees and the size of their fat-bodies.

The physiology and changes undergone by the fat-body tissue have been less studied in the worker honeybee during the course of its life than in larvae. Koehler (1921) stated that the fat cells of overwintering worker bees contain many protein granules which are probably protein reserves. They disappear in spring, probably being converted into secretions of their hypopharyngeal glands, especially in bees that feed larvae. These protein reserves are not found in the fat cells of worker bees during summer. Similar observations have also been made by Snodgrass (1925) and Lotmar (1939).

The fat-body tissue of the honeybee consists of whitish layers of cells against the body wall of the abdomen. The

cells are much smaller, with less defined cell outlines, than those on full grown larvae. They contain very little fat, especially in overwintering bees (Snodgrass, 1956).

Observations on the effect of sacbrood virus on the fat bodies of bees were made as follows:

1. Methods and Results

The fat body tissue was removed from the same group of winter bees, both infected and uninfected, from which the hypopharyngeal glands were removed for histological studies (section 4.3a.1, Experiment B), and was treated as described in section 2.8a.

The fat-bodies of drones which had been kept with worker bees in cages at 35°C. when newly emerged were removed six days after they each had been injected when 5 days old with 10^{-6} units of sacbrood virus, or with an equivalent extract of healthy larvae (section 2.5).

Table 16 shows there were no protein granules in the fat cells of infected worker bees (Fig. 16A) whereas there were about 20 granules per fat cell in similar uninfected bees (Fig. 16B). The fat-body cells degenerated in both infected and healthy bees after they had been in cages for twelve or more days.

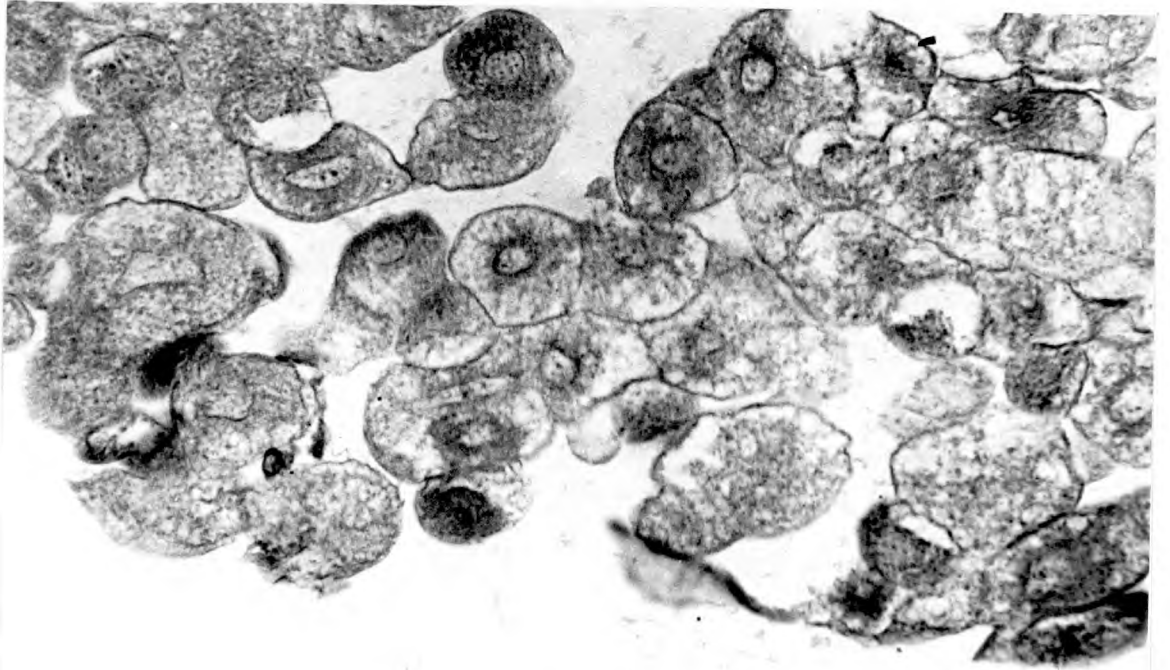
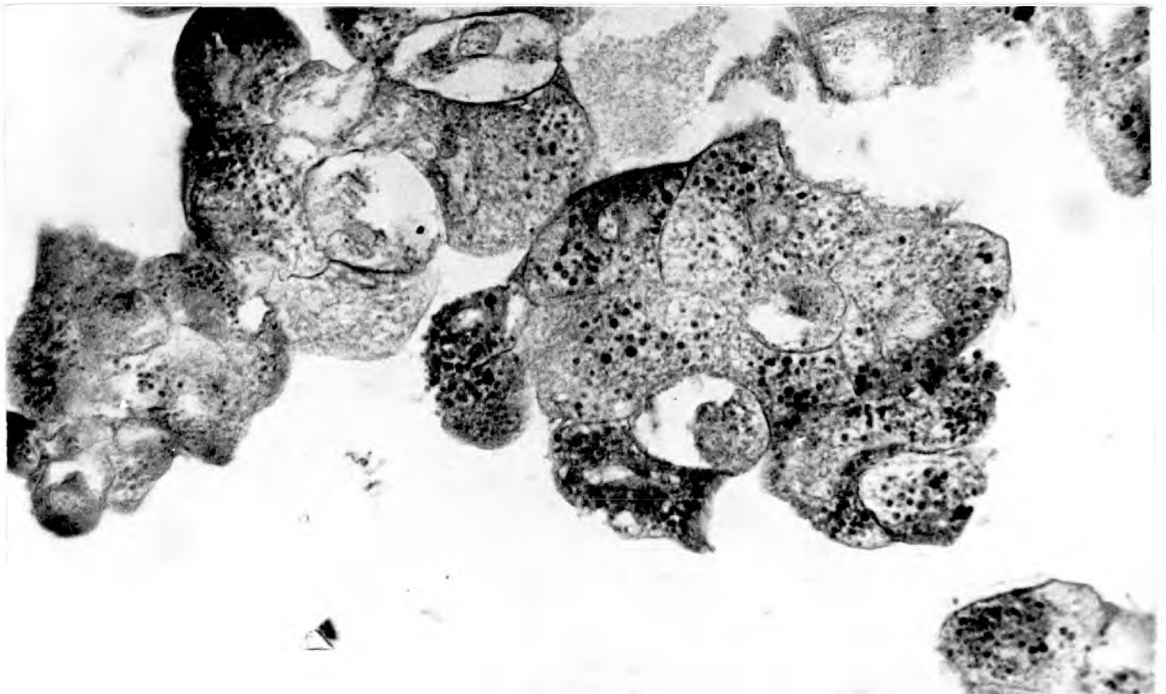
All granules stained deeply with bromophenol blue, indicating they contained protein.

There were no protein granules in the fat-body cells of infected or healthy drones, either before or after treatment.

Table 16. Numbers of cytoplasmic protein granules per fat cell of mature worker bees six days after they were injected with sacbrood virus or with an extract of healthy larvae.

Inoculum	No. of bees	No. of fat cells per bee	Mean no. of protein granules
Sacbrood virus	6	10	0
Extract of healthy larvae	6	10	20 ± 0.70
Significance of difference between means (P)			<0.001

Fig.16. Sections of fat-body cells of mature worker bees from colonies in autumn six days after they were injected with (A) sacbrood virus and (B) extracts of healthy larvae. x400

**A****B**

No other histological abnormalities were observed in the fat-body tissue of either infected worker bees or drones

2. Discussion

These observations indicate that protein is lost from the fat-body of infected bees probably to the multiplying virus. The loss of protein from the fat-body would prevent secretory globules from forming in the hypopharyngeal glands (Koehler, 1921) and it would explain the shortening of the lives of worker bees injected with sacbrood virus (section 4.1b). The absence of protein granules from the fat cells of healthy drones may well explain the comparatively slight effect that sacbrood virus has on their length of life.

4.4. Clustering

Honeybees in a normal colony form a cluster which contracts or expands with a decrease or increase respectively in the environmental temperature (Gates, 1914). A minimum of about fifty worker bees cluster at 20°C; smaller numbers cluster at lower temperatures (Sladen, 1901; Lecomte, 1950). In summer, the temperature at the centre of a cluster, which is the warmest part of a colony, is usually about 34-35°C. (Gates, 1914; Hess, 1926; Himmer, 1932), and in the outer regions of the cluster it is about 25-30°C. (Ribbands, 1953). In winter, the temperature at the centre of the cluster fluctuates between 20-30°C., being more often nearer to 30°C. than to 20 C. (Gates, 1914; Wilson & Milum, 1927; Corkins, 1930). Various observers have reported that bees form a

compact cluster within the hive when the environmental temperature decreases from 18°C . to 10°C . (Phillips & Demuth, 1914; Himmer, 1926; Wilson & Milum, 1927; Free & Spencer-Booth, 1958). The ability of a honeybee cluster to regulate its temperature enables it to survive in conditions when individual bees would succumb. Heran (1952) stated that the thermal preference of young bees varies from 35°C . to 37°C . and that of older bees between 31.5°C . and 36.5°C . Himmer (1926) reported that individual bees stop moving at 11°C . to 12°C . and become rigid at 6°C . to 7°C .

The fact that bees infected with sacbrood virus clustered tightly in the centre of the colony (section 4.2) suggested that they were more susceptible than healthy worker bees to chilling and so the effect of low temperature on them was tested as follows:

a. Methods

Experiment A: Newly emerged worker bees were fed with a suspension of sacbrood virus or with equivalent extracts of healthy larvae as described in section 2.3. After treatment, they were kept in cages (Fig.3) at 35°C . Each cage contained one hundred worker bees and was provided with pollen. Similar numbers of newly emerged worker bees were fed with an extract of healthy larvae and kept in cages at 35°C ., but were not supplied with pollen. At six and twelve days after infection the feeders were replaced with a short glass rod in all cages which were then kept at a constant temperature of 5°C . The

time taken for all worker bees in each cage to cluster round the end of the glass rod, without any remaining on the sides of the cage, was noted.

A few worker bees that had been treated similarly were confined six and twelve days after infection in glass vials, two bees per vial, and placed in a mixture of ice and water. The time taken for each bee to become immobilized was noted.

Experiment B.

Experiment A was repeated with mature adult worker bees, from a colony in autumn, each infected with 10^{-6} units of virus or equivalent extracts of healthy larvae as described in section 2.5.

b. Results and Discussion

Table 17 shows that newly emerged worker bees six and twelve days after they were infected with sacbrood virus or similar healthy bees deprived of pollen clustered earlier ($P < 0.001$) than healthy bees that had eaten pollen. Mature adult worker bees from colonies in autumn also clustered earlier than similar uninfected bees six days after they were infected, but they did not cluster earlier than healthy bees after twelve days.

Table 18 shows that individual newly emerged worker bees infected with sacbrood virus and similar healthy worker bees that had not been supplied with pollen were more susceptible to chilling than healthy worker bees supplied with pollen. Mature individual worker bees from colonies in

Table 17. Time taken (minutes) for one hundred worker bees (4 replicates) to cluster at 5°C. after they were inoculated with sacbrood virus or virus-free extracts, and kept in cages at 35°C.

Inoculum	Type of worker bee and treatment					
	Newly emerged - Fed				Mature - Injected	
	Days after treatment					
	Pollen		6	12	6	12
	supplied	consumed	mean	mean	mean	mean
Sacbrood virus	+	-	5.4 ± 0.18	4.3 ± 0.15	6.0 ± 0.36	4.3 ± 0.18
Extract of healthy larvae	+	+	10.8 ± 0.86	11.0 ± 1.4	11.7 ± 0.33	4.7 ± 0.18
Extract of healthy larvae	-	-	5.6 ± 0.13	4.6 ± 0.12	-	-

Table 18. Time taken in minutes for individual worker bees to become immobilized at 0°C. after they were inoculated with sacbrood virus or virus-free extracts, and kept in cages at 35°C.

Inoculum	Pollen		Type of worker bee and treatment					
			Newly emerged - Fed			Mature - Injected		
			Days after treatment					
			6		12		6	
	supplied	consumed	No. of bees tested	Mean	Mean	No. of bees tested	Mean	Mean
Sacbrood virus	+	-	12	1.8 ± 0.08	1.8 ± 0.11	10	4.7 ± 0.25	3.7 ± 0.17
Extract of healthy larvae	+	+	12	3.8 ± 0.15	4.6 ± 0.20	10	7.8 ± 0.51	4.0 ± 0.20
Extract of healthy larvae	-	-	12	1.9 ± 0.08	2.0 ± 0.20	-	-	-

autumn also became immobile at 0°C. sooner ($P < 0.001$) when infected with sacbrood virus than healthy bees. After twelve days, however, there was no difference between the times that infected and comparable healthy mature worker bees took to become immobilized.

As bees respond to decreased environmental temperature by increased metabolism and heat production, and consequently by increased food (sugar) consumption (Phillips & Demuth, 1914; Himmer, 1926; Free & Spencer-Booth, 1958; Allen, 1959), the rate at which worker bees assimilated sugar when infected with sacbrood virus was studied as follows:

4.5 Sugar consumption

a. Methods

Experiment A: Groups of newly emerged worker bees were fed with a suspension of sacbrood virus or with an equivalent extract of healthy larvae as described in section 2.3. They were then kept in cages (Fig.4) at 30°C., with twenty bees per cage and provided with 60 per cent sucrose solution and water. Pollen was supplied forty hours after treatment. A further group of newly emerged bees were also fed with an extract of healthy larvae as before, but were kept in cages at 30°C. without any pollen. The quantities of sucrose solution and water consumed were measured daily and the daily rate per worker bee was calculated from the number of bees remaining alive. Correction for evaporation from the feeders was made by measurements with similar cages

containing no bees.

Experiment B: Similar experiments were made with mature adult worker bees collected in autumn each being injected with 10^{-6} units of sacbrood virus or an equivalent extract of healthy larvae as described in section 2.3. After treatment, the bees were incubated at 30°C. or 35°C. The volume of sucrose solution and water consumed by the bees were measured as before.

Experiments A and B were terminated after fourteen days when many bees infected with sacbrood virus or deprived of pollen began to die.

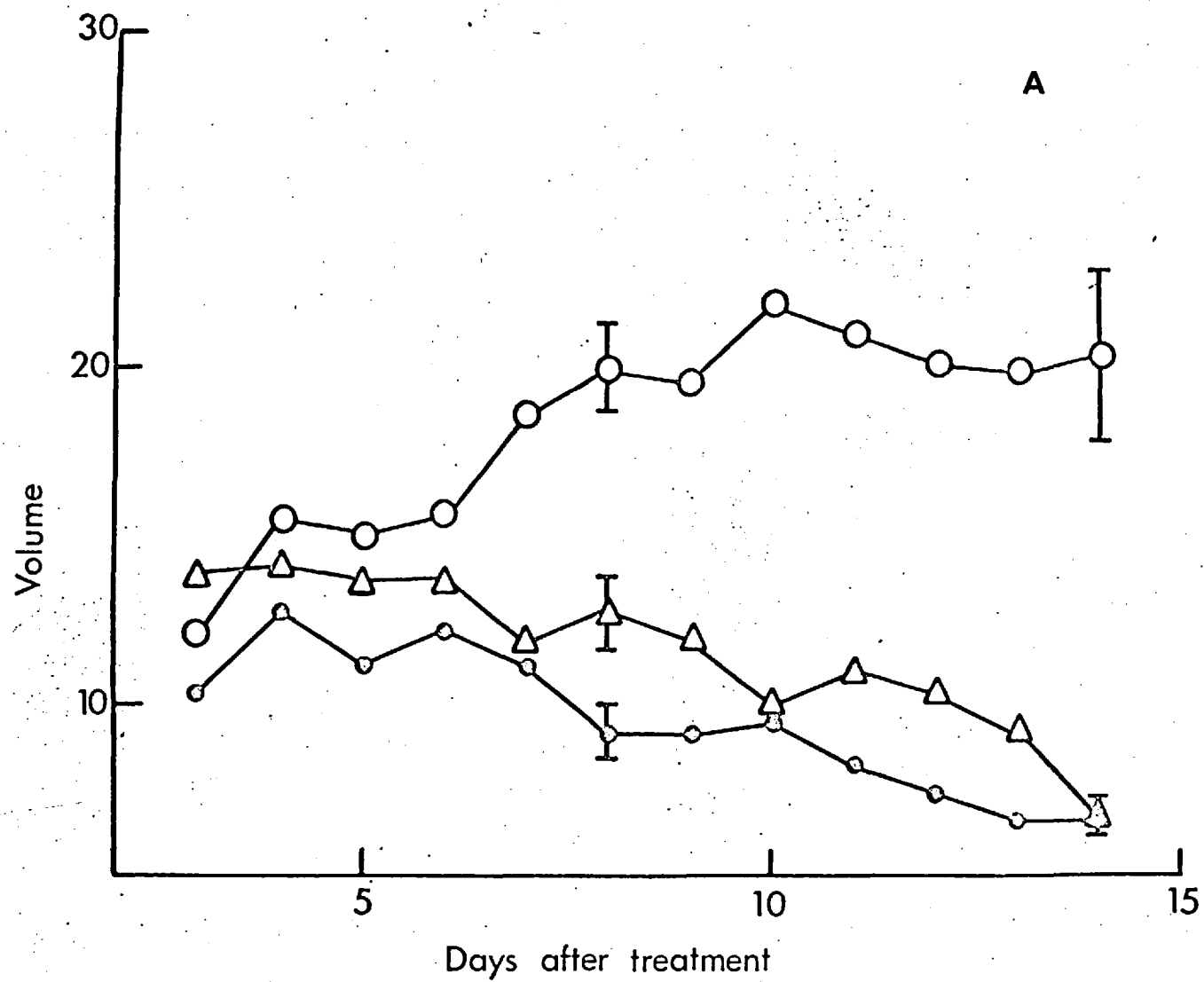
The half-lives of the groups of bees in all cages were noted, and the rectal contents of a few worker bees from Experiment A were examined for ingested pollen grains at the end of the experiment.

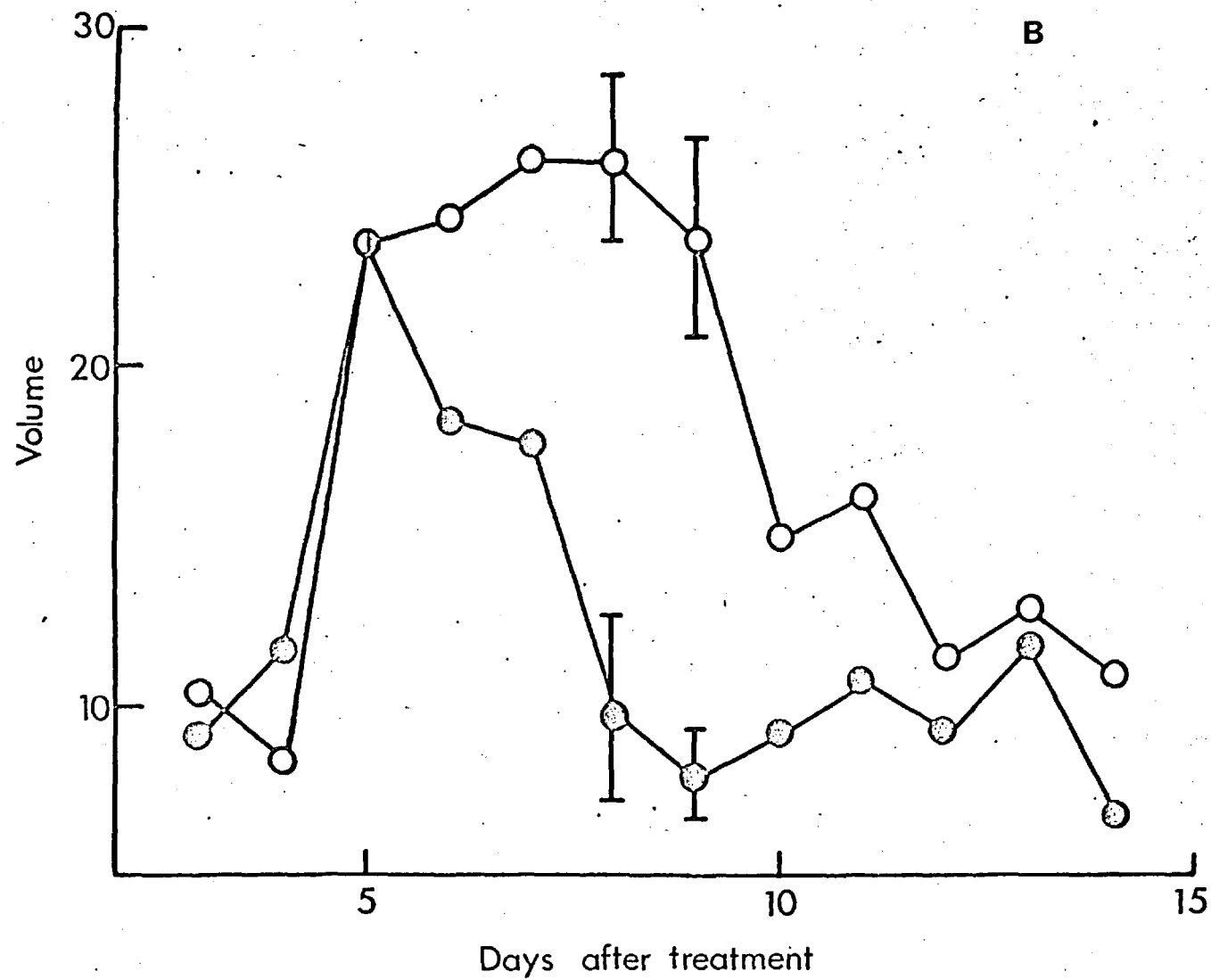
Tests were made with additional groups of bees as described in Experiment B. The size of their honey stomachs were measured after three and eight days of treatment. During the removal of the honey stomachs the bees were kept immobilized at 4°C. after they had been anaesthetized with carbon dioxide.

b. Results

Fig. 17A shows that newly emerged bees that had been infected with sacbrood virus for six days consumed the same amount of sucrose as similar healthy bees deprived of pollen, and much less than similar healthy bees that had eaten pollen.

Fig.17. The volume (mm^3) of sucrose solution (60% w/w) consumed per caged bee per day by (A) newly emerged worker bees at 30°C., (B, page 94) and (C, page 95) mature worker bees from colonies in autumn at 30°C. and 35°C. respectively. Each point is the mean of 6 to 8 groups each of twenty bees that were healthy (O), infected with sacbrood virus (●) or healthy and deprived of pollen (Δ). Vertical bars indicate standard deviations of the means and delimit the points when differences between volumes consumed by healthy and test worker bees were significant ($P < 0.001$ to < 0.002).





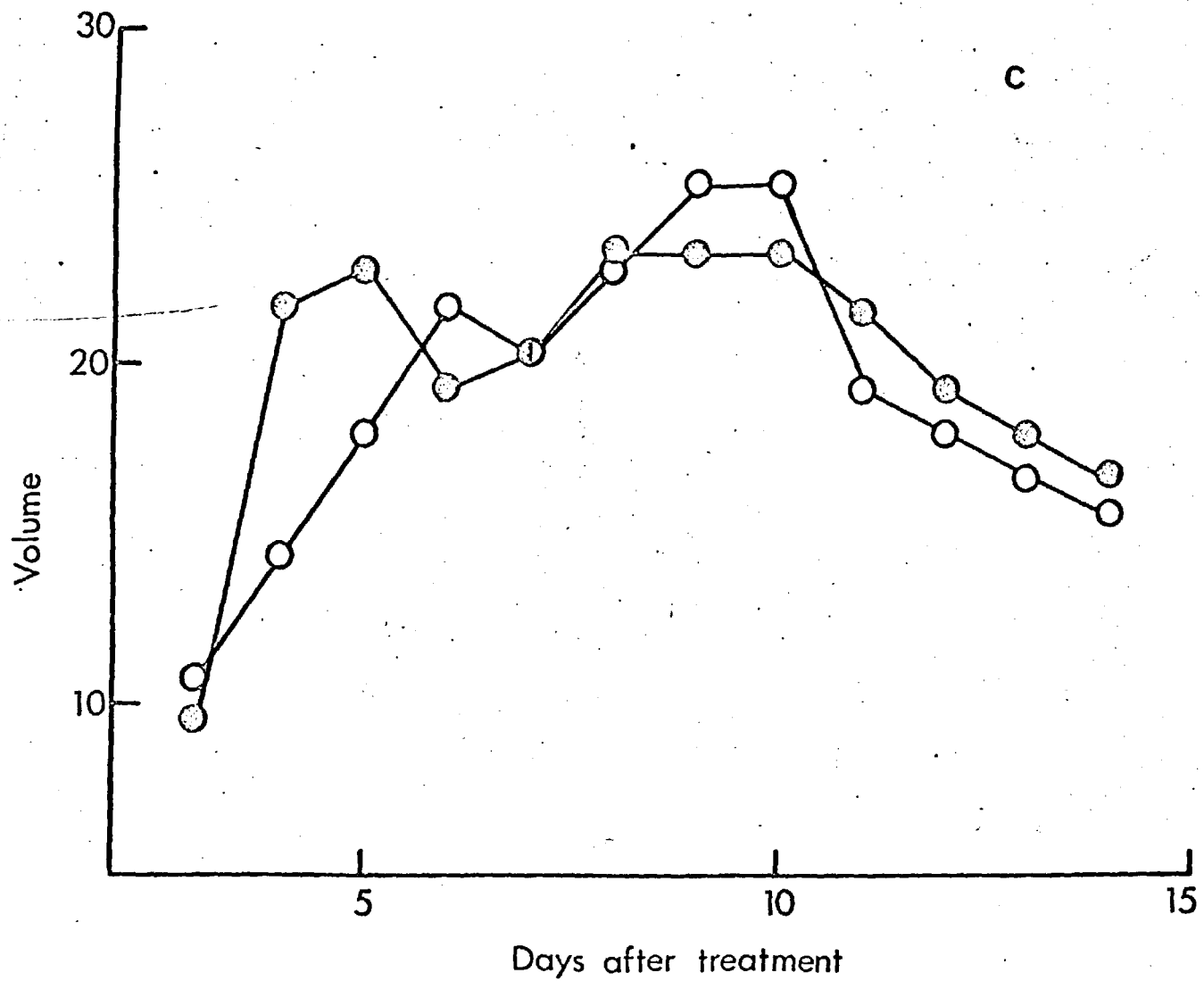


Fig.17B shows that at 30°C. mature worker bees from colonies in autumn also consumed less sucrose when infected with sacbrood virus than similar uninfected bees. This difference became greatest ($P < 0.001$) by the 8th and 9th day of infection, after which the sucrose consumption of the healthy worker bees gradually decreased to that of bees infected with the virus. However, at the normal brood-nest temperature of 35°C. bees infected with sacbrood virus consumed a similar volume of sucrose as healthy individuals, about the same as that consumed by infected worker bees at 30°C. (Fig.17C).

Healthy and infected worker bees consumed the same volume of water at 30°C. and equally increased volumes at 35°C.

During the experiments I noticed that whereas the half-lives of the groups of mature worker bees three days after they were infected with sacbrood virus was about twenty-four hours when their feeders containing sucrose and water were removed, similar to uninfected worker bees, they died within seven hours of starvation eight days after infection with the virus (Table 19). Table 20 shows that the volumes of the honey-stomachs of mature worker bees infected with sacbrood virus became smaller than those of similar uninfected worker bees eight days after infection.

The rectum of uninfected worker bees fed with extracts of healthy larvae when newly emerged contained much pollen,

Table 19. Time taken (in hours) for half a group of healthy worker bees or one infected with sacbrood virus to die of starvation after they had been deprived of food.

Inoculum	Days after injection			
	3		8	
	No. of replicates	Mean	No. of replicates	Mean
Sacbrood virus	5	24.0 $\pm$ 0.09	5	7.0 $\pm$ 0.68
Extract of healthy larvae	5	24.6 $\pm$ 0.63	5	25.0 $\pm$ 0.68
Significance of difference between means (P)		N.S.		<0.001

Table 20. Volume of the honey stomachs of mature worker bees after they had been injected with sacbrood virus or extracts of healthy larvae.

Inoculum	Days after injection			
	3		8	
	No. of bees examined	Mean (mm ³)	No. of bees examined	Mean (mm ³)
Sacbrood virus	12	52.6 ± 4.7	12	16.4 ± 5.5
Extract of healthy larvae	12	59.5 ± 7.3	12	69.3 ± 6.7
Significance of difference between means (P)		N.S.		<0.001

whereas the rectal contents of similar worker bees infected with sacbrood virus were devoid of pollen. The half-lives of groups of worker bees infected with sacbrood virus were also much decreased (similar to results shown in Table 5). These results indicated that sacbrood virus had multiplied in the inoculated bees.

c. Discussion

My results show that infection with sacbrood virus prevents worker bees from maintaining an increased metabolic rate when they are kept at 30°C. It is interesting that young bees deprived of pollen are similarly affected as are mature worker bees kept in cages for long. These worker bees do not form protein reserves in, or lose them from, their fat-body cells (section 4.3) in common with worker bees that are infected with sacbrood virus. The susceptibility to chilling appears, therefore, to be closely associated with the lack of protein reserves.

The smaller honey stomachs of infected worker bees and their inability to live for long when deprived of sucrose at 30°C. shows that they keep less sucrose in reserve in their bodies. This may be related to their low metabolic rate.

The consumption of water by bees was unchanged by infection with sacbrood virus. Water consumption, therefore, is probably more dependent on water loss by evaporation from bees than on their metabolic rate.

4.6. Behaviour in the field

The early departure of bees infected with sacbrood virus from their colony, as seen in my previous experiments (section 4.2C.), suggests that they may commence field duties earlier than usual.

The age at which worker bees first take to field duties differs greatly according to the conditions in the colony, but it has generally been observed that worker bees older than fourteen days usually take to field duties (Rosch, 1925; Ribbands, 1952; Lindauer, 1952; Sakagami, 1953). There is no clear sequence of foraging duties with age although they usually start by collecting pollen and most bees collect both pollen and nectar sometime during their lives. However, during a single trip and often for several trips in succession, they are constant to pollen only, nectar only, or both, and some even collect water (Rosch, 1925; Ribbands, 1949, 1952, 1953; Singh, 1950). Foraging activity has also been reported to shorten the life of worker bees (Ribbonands, 1952).

The hypopharyngeal glands of worker bees atrophy in individuals that begin to forage even when such bees are relatively young (Rösch, 1925, 1930). Moskovljevic-Filipovic (1956) found that the hypopharyngeal glands in about half the worker bees between 18 and 27 days old had degenerated. Factors that change the state of the development of its hypopharyngeal glands also influence a bee's occupation. For example, Fyg (1950) and Simpson (1954)

found that anaesthetizing worker bees with CO_2 causes their hypopharyngeal glands to retrogress sooner than usual, and, such bees curtail or cease their hive duties and begin to forage earlier, with markedly fewer of them gathering pollen, than usual (Ribbands, 1950).

Bailey (1971a) has shown that worker bees fed sacbrood virus when young did gather pollen, and as much as 10^6 particles of sacbrood virus were present in each pollen load 5-10 days after infection. This, he stated, was good evidence that infected worker bees secreted the virus from the glands in the fluid they add to the pollen they gather.

In view of the similarity of the effects of sacbrood virus to CO_2 on the hypopharyngeal glands (section 4.3a) and of it causing the worker bees to leave the colony early (section 4.2c), experiments were made to find the effect of infection by the virus on the field activities of worker bees.

a. Methods

Experiment A. 30 pollen gatherers from a normal colony were each injected with 10^{-6} units of sacbrood virus and another thirty bees were injected with an equivalent extract of healthy larvae after they were anaesthetized by chilling at 4°C . (section 2.5). They were then marked, returned to the same colony, and their behaviour was observed for the next five days, when very few were left. Then the pollen loads of six of the treated bees were collected on their return from foraging and were tested for sacbrood virus by

macerating them in 0.1 ml. distilled water and 0.2 ml. carbon tetrachloride, clarifying the extract by centrifuging at 8000 g. for 15 mins. and injecting 1 μ l. of the supernatant fluid into each of several 3-day-old drones. The heads of these were tested serologically for sacbrood virus (sections 2.7b. and ~~2.8~~) after keeping them in cages with attendant workers for seven days at 35°C.

Experiment B. Newly emerged worker bees were kept in cages at 35°C. with sucrose solution, water and pollen till they were five days old, when 30 were each anaesthetized by chilling at 4°C. and injected with 10⁻⁶ units of sacbrood virus. A similar number of worker bees were injected with an equivalent extract of healthy larvae (section 2.5). They were marked and placed in an observation hive (section 4.~~2~~) containing larvae, a laying queen and about 300 healthy worker bees of all ages.

Experiment C. One hundred and fifty 5-day-old worker bees were injected with sacbrood virus and a similar number were injected with an extract of healthy larvae as in Experiment B. They were then marked and placed in a normal colony in the field.

Experiment D. A similar number of worker bees were treated as for Experiment B, and a further one hundred and fifty 5-day-old worker bees were anaesthetized for five minutes with carbon dioxide. All these groups of bees were then marked and placed in a normal colony.

The foraging behaviour of the treated worker bees in the Experiments B, C and D was observed daily for one hour at a time of day when the rest of the worker bees in the observation hive or in the colony were most actively foraging under favourable field conditions.

Groups of worker bees receiving the same treatment as those in Experiments B and C were also kept in cages without pollen at 35°C. (section 2.6) and their half-lives recorded.

b. Results

Fig.18 shows that the proportion of pollen gatherers that changed to nectar gathering during the five days after they had been injected with sacbrood virus, did not differ significantly from that of the control group, although the tests for sacbrood virus in their pollen loads were strongly positive.

However, Fig.19 shows that worker bees infected with sacbrood virus when 5 days old and then placed in an observation hive among other worker bees of different ages, began to forage sooner than similar uninfected worker bees. Moreover, very few of them collected pollen, whereas many of the similar uninfected individuals foraged mainly for pollen, as healthy worker bees normally do, especially when they began their foraging duties. Some of the worker bees infected with sacbrood virus that were returning to the hive without pollen loads were heavily dusted with pollen, and several performed vigorous recruiting dances (von Frisch, 1968) on

Fig.18. Numbers of foraging worker bees that continued to gather pollen or changed to nectar gathering. Observations were made during a period of one hour each day for six successive days after pollen-gatherers captured at the hive entrance were injected with (a) sacbrood virus and (b) extracts of healthy larvae.

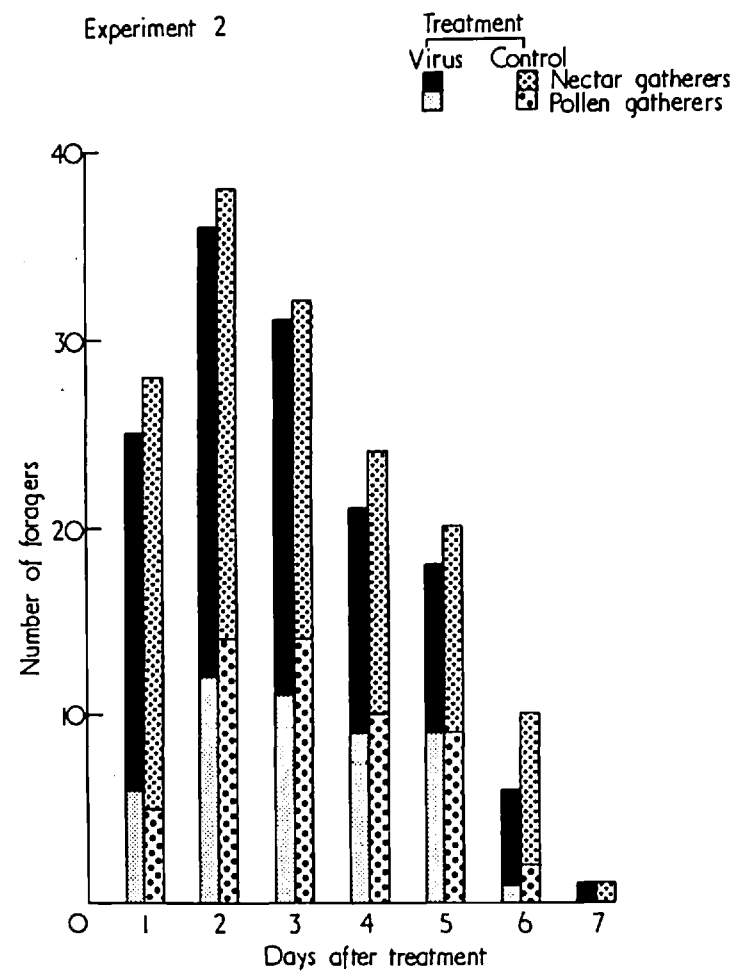
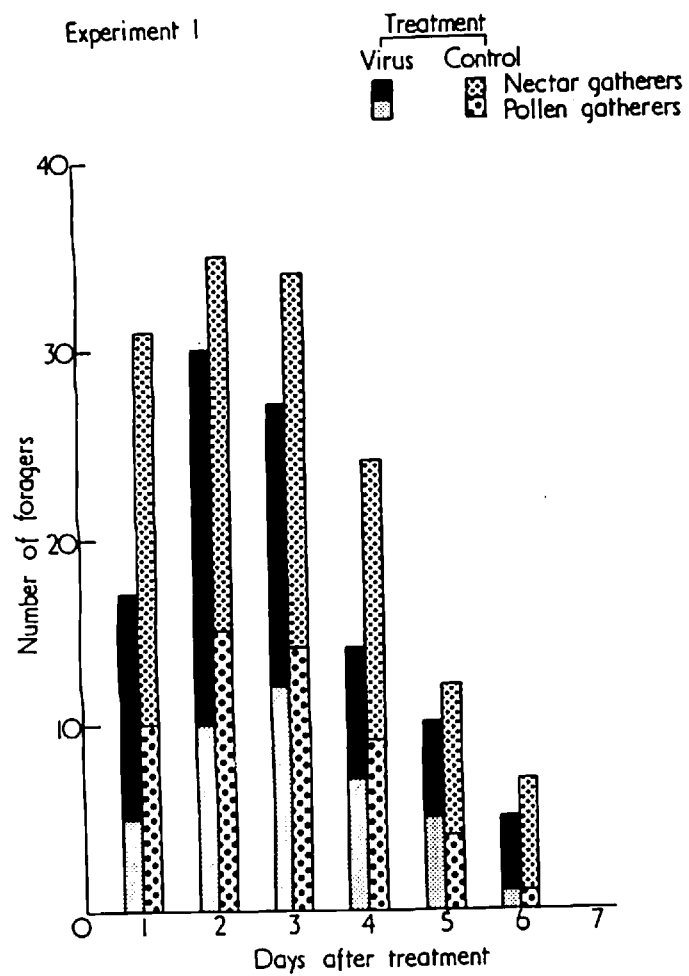
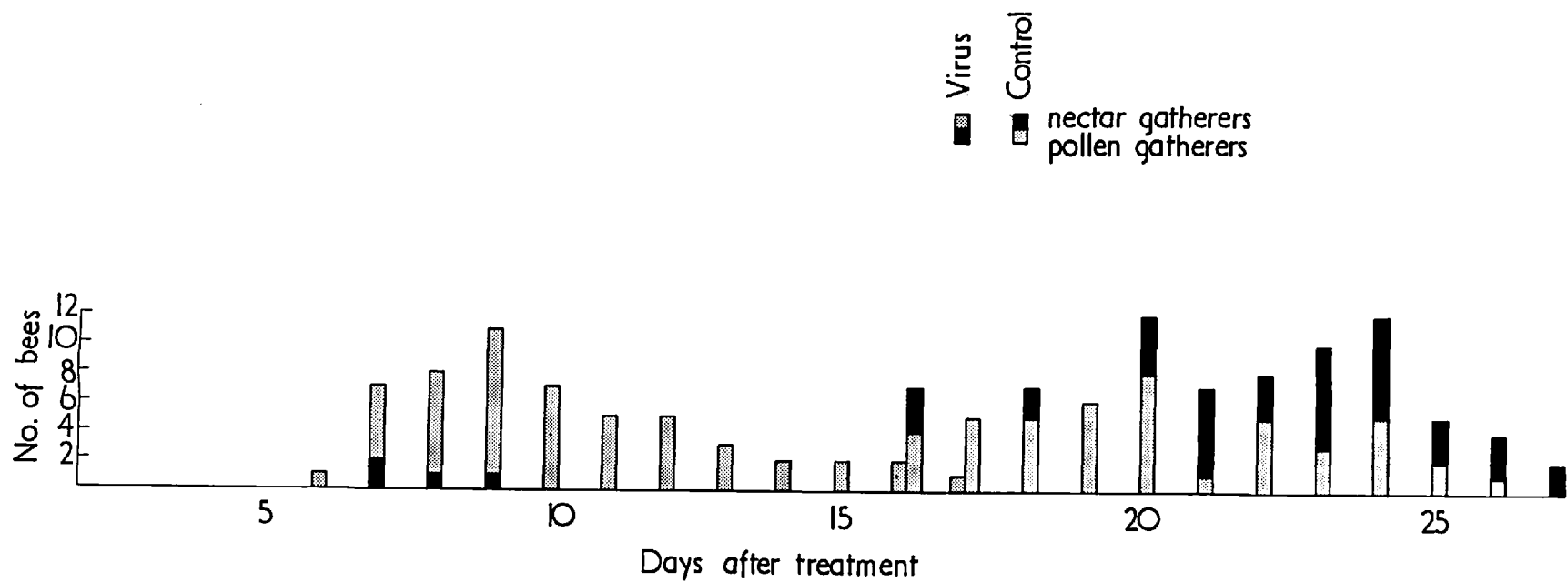


Fig.19. The numbers of marked worker bees seen returning to the observation hive from foraging flights during one hour each day after equal numbers had been injected with sacbrood virus or extracts of healthy larvae, when they were 5 days old.



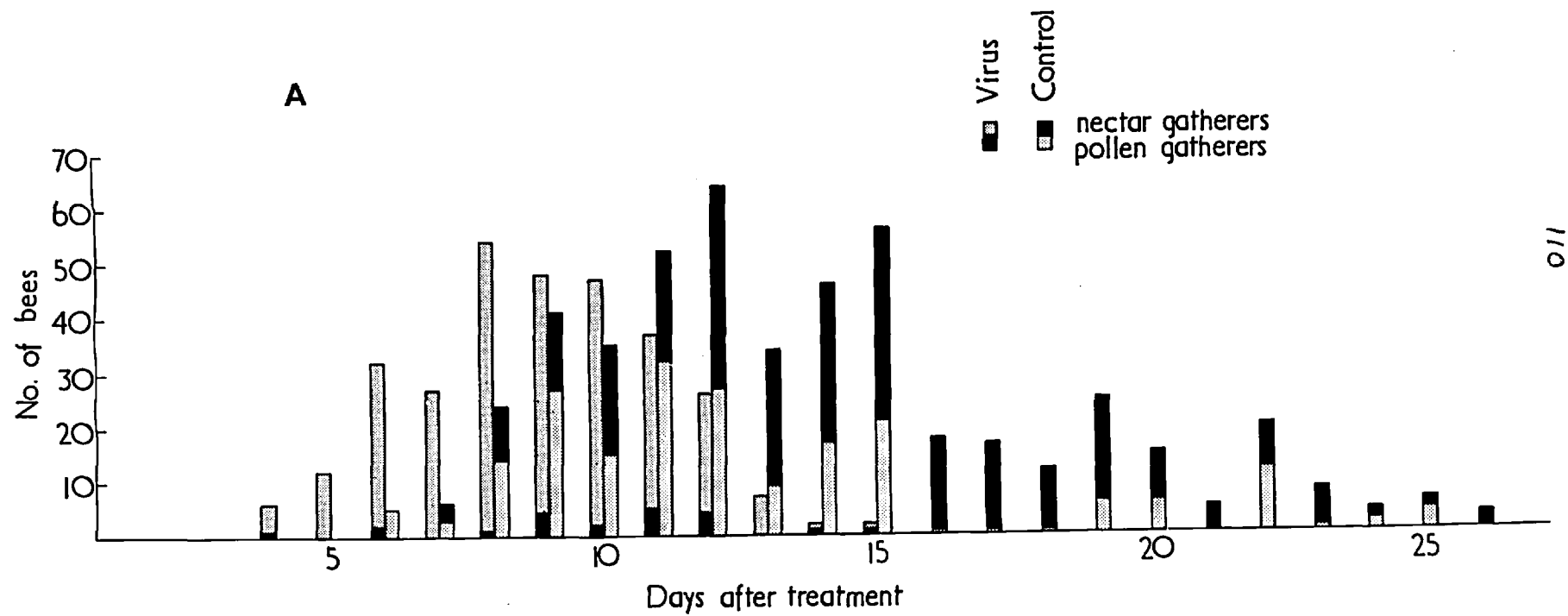
the combs when they returned. This showed that the bees had foraged for nectar and were not behaving abnormally.

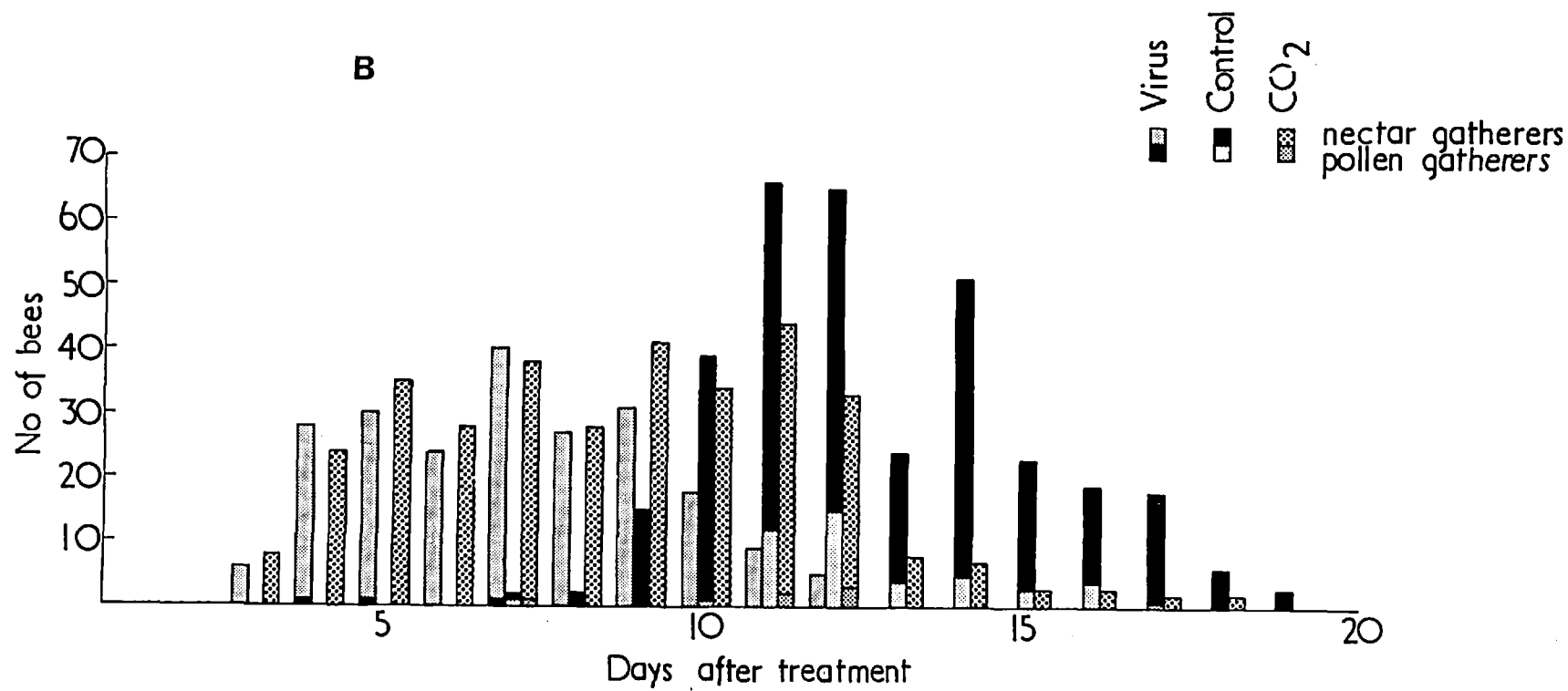
Fig.20A shows that worker bees infected with sacbrood virus when 5 days old and placed with similar uninfected worker bees in a normal colony in the field also foraged earlier than the uninfected worker bees, and, again very few collected pollen.

Fig.20B shows that worker bees infected with sacbrood virus when 5 days old and similar uninfected bees anaesthetized with carbon dioxide began foraging simultaneously and earlier than untreated bees and hardly any collected pollen. In this experiment only about 11 per cent of marked untreated bees collected pollen when they became most active, between eleven and fourteen days; but by this time, only 11 per cent of the unmarked worker bees forming the rest of the colony, were also collecting pollen in deteriorating weather conditions (Fig.21). This showed that marked untreated worker bees accurately reflected the behaviour of the rest of the colony and that the failure of the virus-infected and anaesthetized worker bees to collect pollen when they were most active between days four and eleven can be confidently contrasted with the average of 35 per cent of the unmarked bees that were gathering pollen at the same time.

Table 21 shows that half the number of worker bees that had been infected with sacbrood virus and placed in the observation hive had disappeared after about eleven days, and all had disappeared by eighteen days, whereas more than half

Fig.20. (A and B, page *III*). The numbers of marked worker bees seen returning to their colonies from foraging flights during one hour each day after equal numbers had been injected with sacbrood virus or extracts of healthy larvae, or had been anaesthetized for five minutes with carbon dioxide when they were 5 days old.





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Fig.21. The percentages of marked bees seen returning to their colony with pollen loads during each day after 150 had each been injected with sacbrood virus ($\square$), or with extracts of healthy larvae ($\odot$) or had been anaesthetized for five minutes with carbon dioxide (X) (data from Fig.20B.). The percentages of unmarked, untreated worker bees gathering pollen (O) seen during the same periods was at least 500 bees per day.

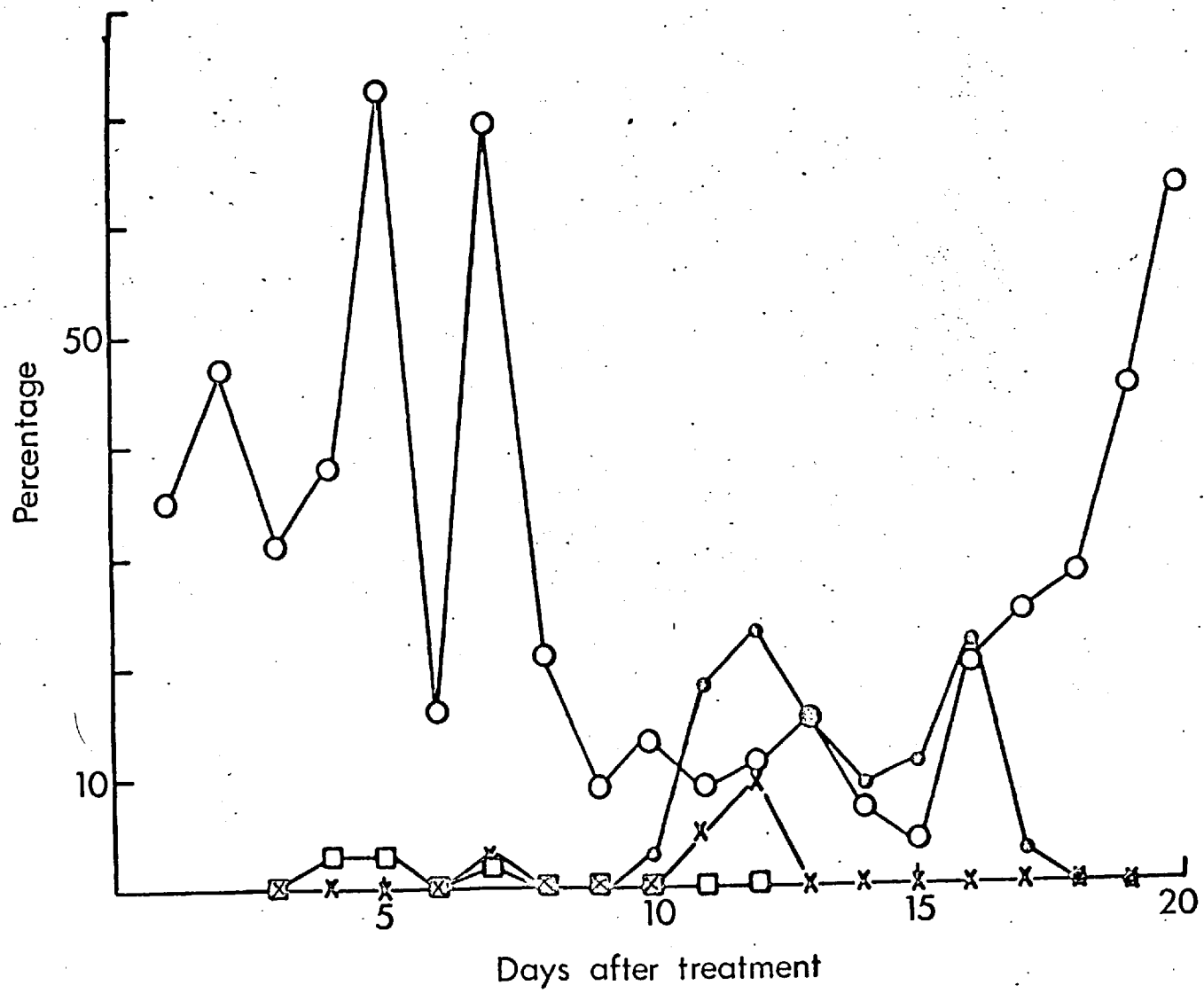


Table 21. Number of worker bees present in the observation hive on each of 30 successive days after they were injected when 5 days old with sacbrood virus or an extract of healthy larvae (Experiment B).

Inoculum	No. of bees	Days after injection																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Sacbrood virus	30	30	30	30	30	30	27	25	20	20	18	15	10	8	5	5	3	3	2	0	0	0	0								
Extract of healthy larvae	30	30	30	30	30	30	30	30	30	30	28	28	27	22	22	22	22	22	21	20	20	20	18	17	12	12	8	6	6	6	5

Table 22. Half-lives (in days) of groups of worker bees kept in cages at 35 C. without pollen after receiving the same treatment as bees placed in colonies for the study of their behaviour in the field (see Figs. 26 & 27).

Treatment	Replicates	Experiment	
		C	D
		Mean	Mean
Injected sacbrood virus	3	20 ± 0.40	21 ± 0.57
Injected extracts of healthy larvae	3	52 ± 1.5	51 ± 1.3
Anaesthetized with CO ₂ for 5 min. ²	3	-	49 ± 1.7

the number of uninfected bees stayed till about twenty-four days.

In the colony experiments, about half the number of worker bees infected with sacbrood virus or anaesthetized with carbon dioxide or that were injected with virus-free extracts had disappeared by about eleven, twelve and sixteen days respectively (Fig.20), whereas Table 22 shows that the half-lives of similar groups of worker bees that had been infected with sacbrood virus and kept in cages at 35°C. were about twenty days and the healthy worker bees, including those anaesthetized with carbon dioxide, lived about fifty days.

c. Discussion

The bees that were already gathering pollen when they were infected with sacbrood virus did not change to gathering nectar as happens almost immediately when such bees are anaesthetized with carbon dioxide (Ribbands, 1950). Even though the bees were secreting sacbrood virus into their pollen loads, there had been insufficient time for the virus to have seriously affected their behaviour. This parallels the effect of sacbrood virus on metabolism, which is not significantly changed, for example, in caged bees till after about seven days (section 4.5). For this reason, the subsequent experiments were made with young bees before they were due to start foraging.

The lives of worker bees infected with sacbrood virus

were shorter in the observation hive and colonies than they were in cages, but this could well have been due to the hazards of foraging, as suggested by the lives of healthy worker bees also being shortened, by a similar proportion, in colonies (Figs.19 & 20 and Tables 21 & 22). The fact that infected worker bees began foraging sooner than healthy worker bees, was expected in view of earlier results (sections 4.2 and 4.3) and might be due to a process of physiological ageing expressed perhaps by the loss of protein reserves and the atrophy of hypopharyngeal glands, possibly similar to the results of Hassenhein (1950) and Wang & Moeller (1970) on bees infected with Nosema apis Zander. However, my results show that most worker bees infected with sacbrood virus fail to collect pollen and this change in behaviour is exactly the same as that caused by anaesthesia with carbon dioxide. It seems reasonable to suggest that a common mechanism is involved. A temporary accumulation of acid metabolites may be caused by anoxia due to carbon dioxide and may trigger the change in behaviour (Ribbands, 1950), and this sequence of events could also be the result of the impaired metabolism in worker bees infected with sacbrood virus (sections 4.4 and 4.5) possibly because of damage to oxidative pathways. This damage may be centred in the brain, especially as much sacbrood virus accumulates there (Bailey, 1970).

Accordingly, studies were made to find out if there was any visible damage to the brain of bees infected with sacbrood virus.

4.7. Effect of sacbrood virus on the brains of adult honeybees.

As discussed in the previous section (4.6c), sacbrood virus may affect the behaviour of bees by deranging oxidative metabolism.

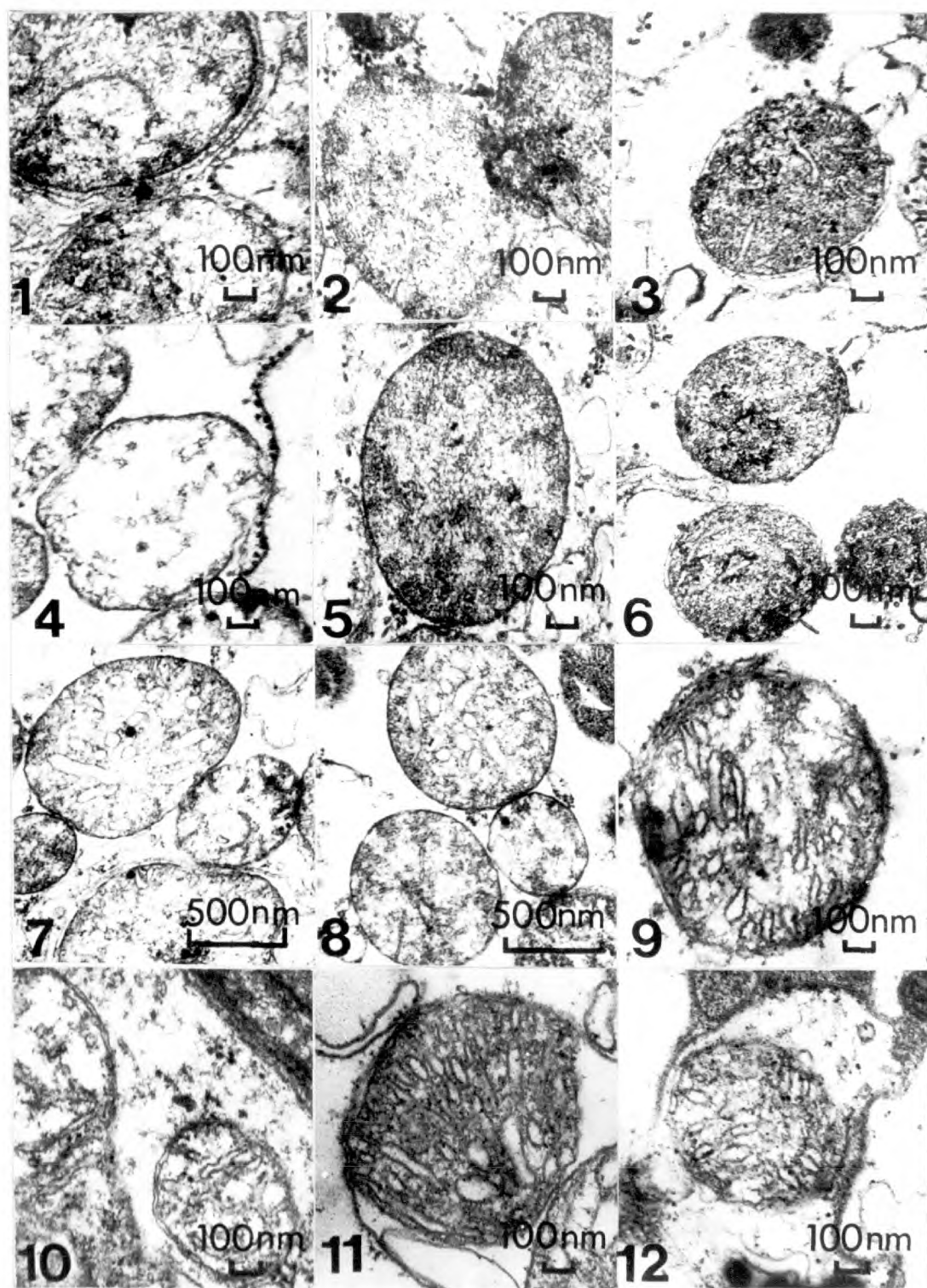
It is a well known fact that the mitochondria are the chief, if not the exclusive, sites of oxidative phosphorylation. Cytochrome c oxidase, which is vital for the respiratory chain, and several other enzymes of the Krebs cycle appear to be localized in these structures exclusively and are, therefore, considered to be the main sites of enzymes linking phosphorylation to oxidation (Siekevitz, 1952; du Bry & Hasselbach, 1956; Schneider, 1956, 1959; Schneider & Hogeboom, 1956). Damage to mitochondria first impairs oxidative phosphorylation and eventually abolishes it completely. The decrease of phosphorylation parallels the degree of destruction of the cristae mitochondriales, whose structural integrity seems to be a requirement for this process (Karlson, 1963; Bursell, 1970).

Observations on the brains of adult bees, particularly on the mitochondria, infected with sacbrood virus were, therefore, made as follows:

4.7a. Methods

Newly emerged drones and 5-day-old worker bees, which had been fed with sucrose and pollen, were each anaesthetized with carbon dioxide and injected with 10^{-6} units of sacbrood

Fig.22. Electron-microphotographs of mitochondria
from brain cells of drones seven days after
they were injected with (1-6) sacbrood virus
and (7-12) with extracts of healthy larvae.



virus or an equivalent extract of healthy larvae (section 2.5), and then incubated in cages at 35°C. (section 2.6). Their brains were removed seven days later. One half of each brain was treated for histological studies with the light microscope or with the electron microscope (section 2.8), and the other half of drone brains was tested serologically (section 2.7) to make sure that much virus had multiplied. The infectivity of the brains of worker bees was tested serologically (section 2.7) with entire brains of similarly treated individuals of the same group.

No histological abnormalities in the brains of worker bees or drones were observed with the light microscope. Studies with the electron microscope showed that many mitochondria of the brain cells of infected drones had no cristae (Fig.22.1-6) but they were very distinct or numerous in the mitochondria of similar uninfected drones (Fig.22.7-12). However, this difference was not evident in the brains of worker bees infected with sacbrood virus.

Serological tests for sacbrood virus on the brains of drones and worker bees were all positive.

b. Discussion

The results indicate that sacbrood virus may cause some damage to the mitochondria of brain cells. This seemed most evident in drones perhaps because more virus multiplies in them than in the brains of worker bees (Bailey, 1970). Damage to mitochondria would damage oxidative pathways and

could result in an accumulation of acid metabolites.

However, these results are inconclusive, and quantitative studies on the metabolism of isolated mitochondria from bees infected with sacbrood virus would, perhaps, give more definite information.

4.8. Effect on the ovaries of the queen honeybee

As some of the effects of sacbrood virus and carbon dioxide anaesthesia on worker bees seem very similar, studies were made on the effect of the virus on queen honeybees, in which it multiplies (Bailey, 1970), especially on their ovaries, as it has been stated that anaesthesia with carbon dioxide causes unmated queens to lay unfertilized eggs earlier than similar untreated individuals.

The reproduction of individuals in a normal honeybee colony depends on a single fertile female or "queen", which is usually the only female with well developed, active ovaries. The ovaries of worker bees usually remain vestigial and inactive, although in a queenless colony they often develop and lay unfertilized eggs which produce drones.

Queen honeybees usually mate in flight within about seven days after they emerge from their cells, but this flight may be delayed up to about three weeks. Mated queens start to oviposit 3 to 4 days after mating, whereas unmated queens prevented from leaving the colony start to lay unfertilized eggs only after several weeks (Ribbands, 1953; Butler, 1954).

The ovary of the queen honeybee has been adequately described by Snodgrass (1956). The ovary is polytrophic and the mature elongated ova are towards the proximal end of the ovarioles.

The effect of sacbrood virus on the ovaries of the

queen were studied as follows:

a. Methods:

Four 3-day-old unmated queen honeybees were each injected with 10^{-6} units of sacbrood virus and another four were similarly injected with an equivalent extract of healthy larvae after they had been anaesthetized by chilling at 4°C . After treatment each queen was placed in a queenless colony, one queen per colony, and the entrance of each colony was covered with a metal grid with slots wide enough to allow worker bees to pass through but too narrow for the queen. The combs in all colonies were examined seven days and fourteen days after treatment for eggs. The ovaries of all treated queens were removed fourteen days after treatment and histological studies made on them with a light microscope as described in section 2.8a.

Four mated, ovipositing queens were also injected with sacbrood virus and a similar number with an extract of healthy larvae as before. After treatment each queen was kept in a separate cage at 35°C . with attendant workers. The ovaries of all queen bees were removed fourteen days after treatment and examined histologically.

b. Results and Discussion

Only three unmated queens infected with sacbrood virus and one uninfected unmated queen were alive fourteen days after treatments. This was not because of their treatment but because the colonies they were in were small and many had absconded or had been robbed to death by large neighbouring

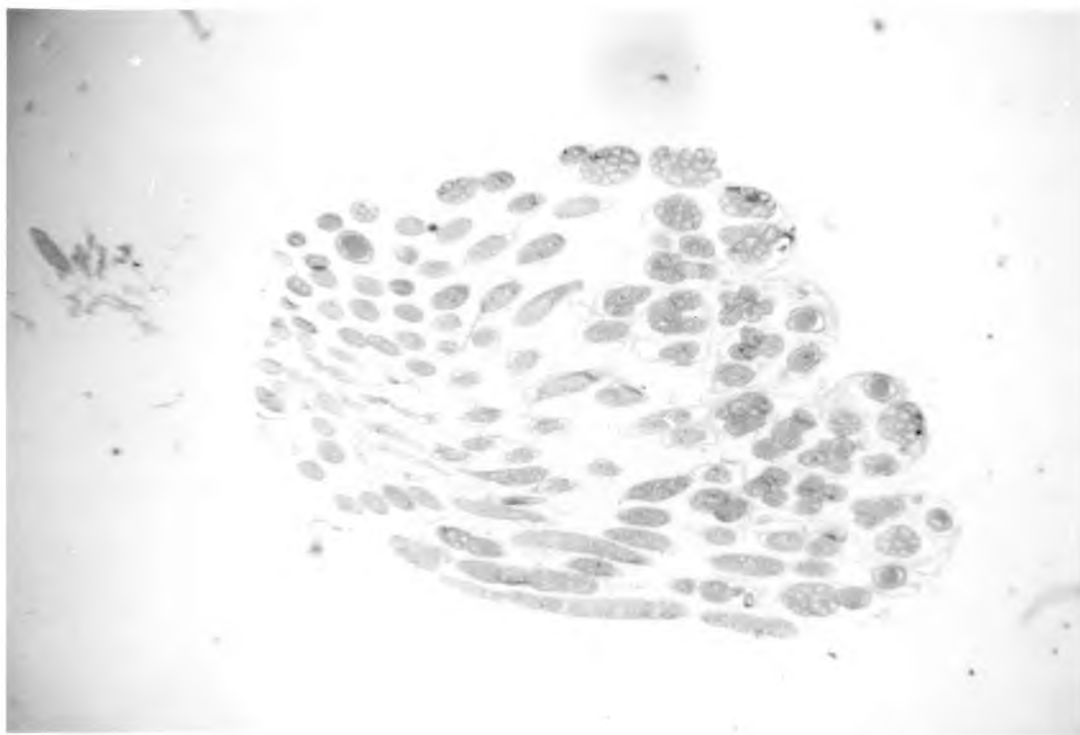
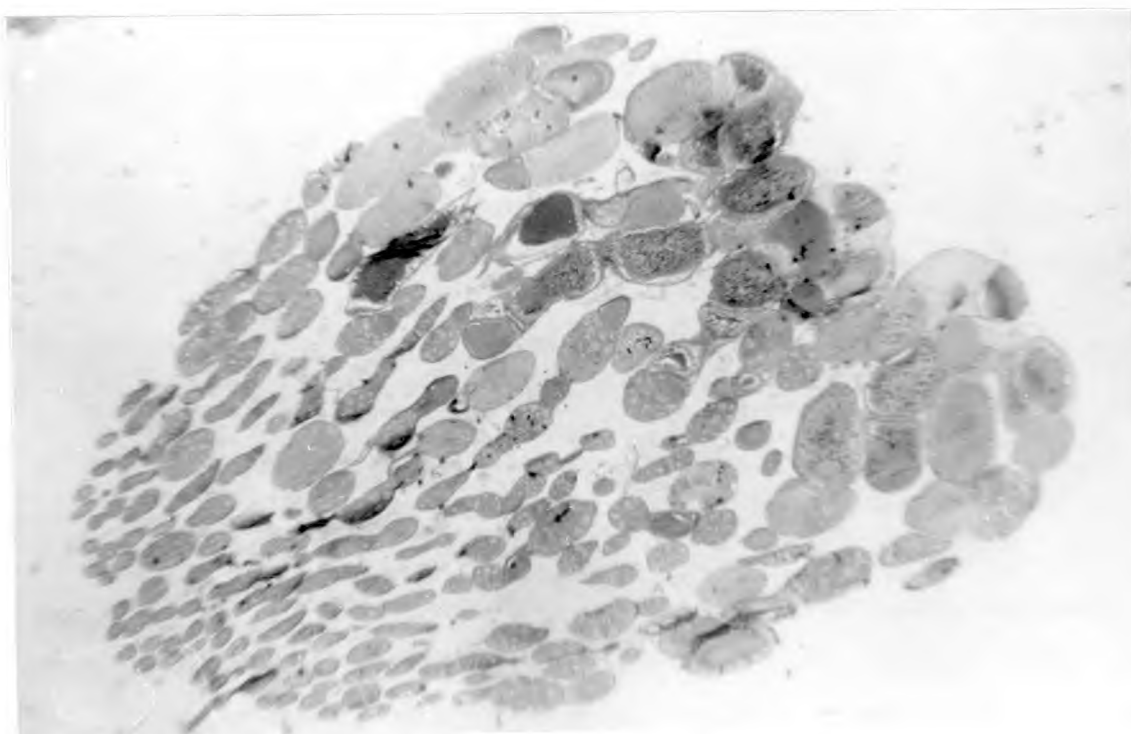
colonies. Nevertheless, in spite of this, and although none of the surviving queens had oviposited, Table 23 and Fig.23 show that the ovaries of the unmated queens infected with sacbrood virus were larger and the ova more mature (Fig.24, Ai) than those of the unmated healthy individual (Fig.24 Aii). Table 23 also shows that the ova of unmated queens infected with sacbrood virus became similar in size to those of mated ovipositing queens (Fig.24B) and that the ovaries of mated, ovipositing queens were unaffected by the virus.

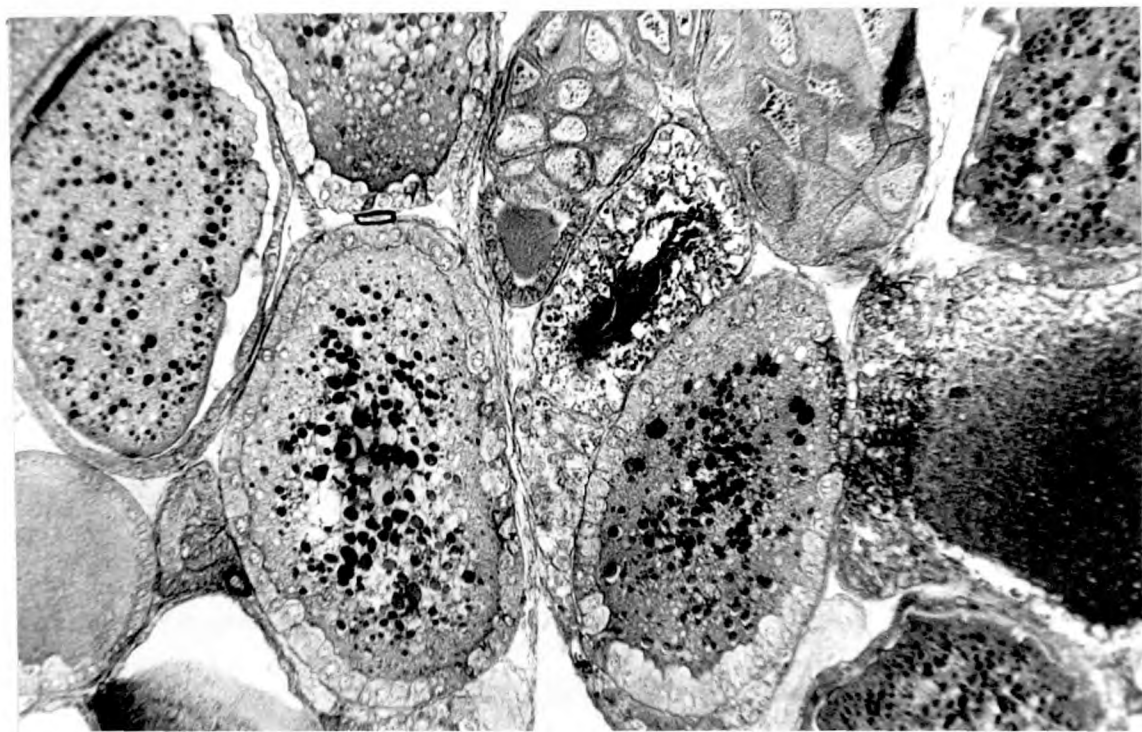
The fact that sacbrood virus causes ova to mature early in unmated queen honeybees resembles the effect on their ovaries of anaesthesia with carbon dioxide, demonstrated by Mackenson (1947). This result, and the fact that sacbrood virus did not affect the ovaries of mated queens also shows that the virus does not multiply at the expense of protein destined for the ovaries.

Table 23. Size of ova (in μ) at the proximal end of the ovarioles of queen honeybees 14 days after they were injected with sacbrood virus or extracts of healthy larvae.

Inoculum Inoculum	Type of Queen Bee					
	Unmated			Mated		
	No. of Queens	Mean length	Mean breadth	No. of Queens	Mean length	Mean breadth
Sacbrood virus	3	478.8 $\pm$ 34.0	228.6 $\pm$ 3.0	3	463.5 $\pm$ 25.8	212.5 $\pm$ 3.0
Extract of healthy larvae	1	95.4	63.0	3	473.4 $\pm$ 32.3	224.4 $\pm$ 5.7
Significance of difference between means (P)		<0.01	<0.01		N.S.	N.S.

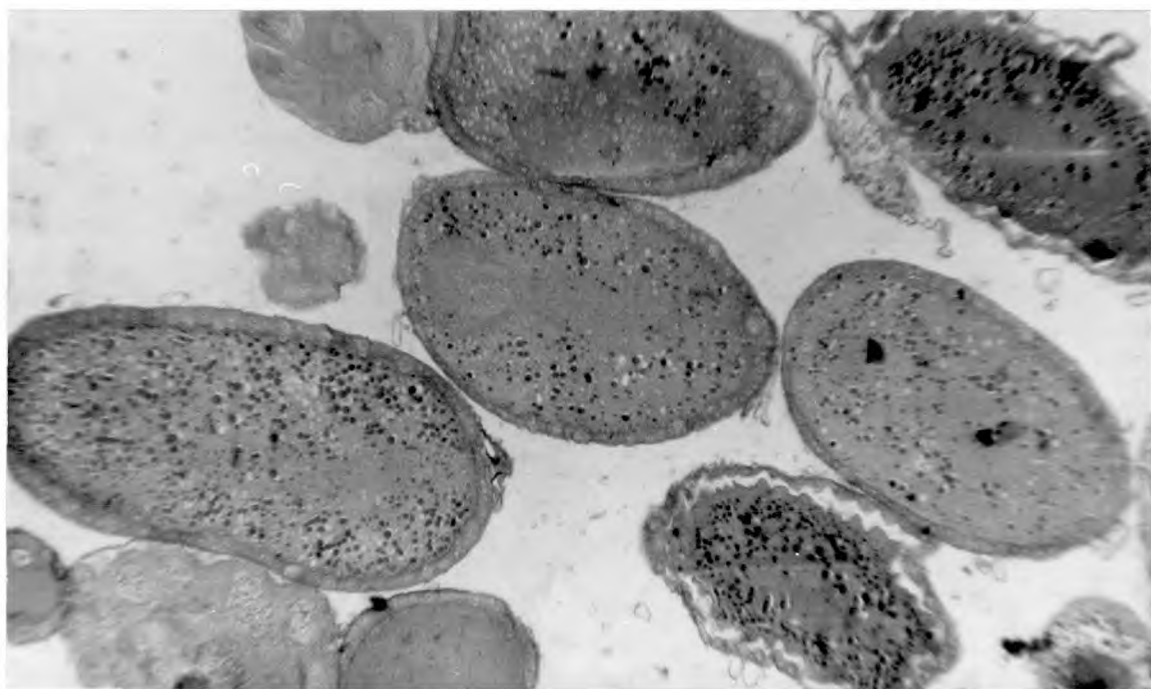
Fig.23. Sections of ovaries of unmated queen honeybees
fourteen days after they were injected with
(A) sacbrood virus and (B) extracts of healthy
larvae. x80

**B****A**



A

Fig.24. Sections of the ova at the proximal end of the ovaries (A) of unmated queen honeybees fourteen days after they were injected with (i) sacbrood virus, (ii) extracts of healthy larvae, and (B) of mated, ovipositing, healthy queen honeybees.
x400

**Aii****B**

4.9. Some comparisons of the effects of sacbrood virus with those of acute bee paralysis virus.

The particles of sacbrood virus and of acute bee paralysis virus are identical in size and shape and have similar sedimentation rates (Bailey, Gibbs & Woods, 1964) and both multiply in adult bees. Sacbrood virus multiplies in the hypopharyngeal glands of worker bees and in the brains of drones (Bailey, 1969, 1970) and much acute bee paralysis virus has also been shown to be present in the hypopharyngeal glands of worker bees and in the brains of worker bees and drones (Bailey & Milne, 1969). Particles of acute bee paralysis virus has also been found in the fat-body cells of worker bees (Furgala & Lee, 1966). Notwithstanding these similarities, however, some of their effects on bees are unexpectedly different. For example, whereas groups of bees infected with acute bee paralysis virus and kept at 35°C. remain apparently healthy, with a half-life of about 21 days (Bailey, 1965) bees similarly injected and kept at 30°C. all die of acute paralysis within about seven days of infection (Bailey & Milne, 1969). Sacbrood virus, which also multiplies in the hypopharyngeal glands of worker bees and in the brains of drones at least as much as acute bee paralysis virus, causes no visible symptoms at either temperature.

In view of my results with sacbrood virus, therefore, I made some experiments to see what the effects of acute bee paralysis virus were, on the pollen consumption of young

worker bees and on the hypopharyngeal glands and fat bodies of workers.

a. Methods

Preparation of virus extract

A suspension containing acute bee paralysis virus was prepared by grinding 10 heads of acutely paralysed worker bees (Bailey & Gibbs, 1964) in 10 ml. distilled water and a quarter volume of carbon tetrachloride, clarifying the extract at 8000 g. for 10 min. and diluting 0.1 ml. in 100 ml. of sterile distilled water, producing a suspension containing the equivalent of 10^{-3} of a head per ml.

1 μ l. of this suspension, equivalent to 10^{-6} of a head, was injected into the haemocoel of each worker bee after it had been anaesthetized with CO_2 . Some of the injected bees were tested, to see that the virus had multiplied, by serological tests with extracts of their heads (section 2.7) 6 days after injection, using antiserum prepared in rabbits against purified acute bee paralysis virus (Bailey & Milne, 1969) and by measuring their mortality in cages at 30°C .

Experiments

A. Young worker bees: As newly emerged worker bees are too delicate to be injected easily, 4-day-old worker bees, which had been deprived of pollen, were each injected with 1 μ l. of the extract of acute bee paralysis virus. A similar number of worker bees were also injected with 10^{-6} units each of sacbrood virus and another group with extracts

of healthy larvae (section 2.5). All treated cages were kept at 35°C. and provided with pollen 48 hours later.

Their rectal contents were examined for pollen (section 4.2⁶) and the histology of their hypopharyngeal glands studied (section 2.8) six days after treatment.

b. Results

Table 24 shows that after six days in cages at 35°C. young worker bees infected with acute bee paralysis virus and provided with pollen 48 hours later, had consumed the same quantity of pollen as healthy individuals, whereas those infected with sacbrood virus had not consumed any pollen, as usual. However, the hypopharyngeal glands of the bees infected with acute bee paralysis virus were significantly ($P < 0.001$) smaller than the healthy bees and contained no secretory globules (Fig. 25 B and C); they resembled the hypopharyngeal glands of worker bees infected with sacbrood virus (Fig. 25A) or those of worker bees that had not eaten any pollen (Fig. 14C.).

Table 25 shows that the hypopharyngeal glands and their secretory globules of mature worker bees six days after infection with acute bee paralysis virus (Fig. 26) were similar to those of healthy bees (Fig. 15B), whereas bees infected with sacbrood virus had significantly smaller ($P < 0.001$) hypopharyngeal glands with smaller ($P < 0.02$) secretory globules (Table 15, Fig. 15A).

Fig. 27 shows that no protein granules remained in the

Table 24. Size of hypopharyngeal glands, secretory globules (in μ) and the amount of pollen in the rectum of 4-day-old worker bees, caged at 35°C. and supplied with pollen, six days after they were injected with sacbrood virus, acute bee paralysis virus or extracts of healthy larvae.

Inoculum	No. of bees examined	Mean length	Mean breadth	Mean size of secretory globules	Mean number of pollen grains $\times 10^5$
Sacbrood virus	12	48.0 ± 1.90	28.1 ± 0.94	-	-
Acute bee paralysis virus	12	47.8 ± 0.85	27.3 ± 0.79	-	9 ± 1.80
Extract of healthy larvae	12	125.8 ± 2.69	89.6 ± 1.85	5.0 ± 0.15	9 ± 0.81



A

Fig.25. Sections of hypopharyngeal glands of worker bees six days after 4-day old worker bees that had not eaten pollen were injected with (A) sacbrood virus, (B) acute bee paralysis virus, and (C) extracts of healthy larvae, and provided with pollen two days after injection.

x240

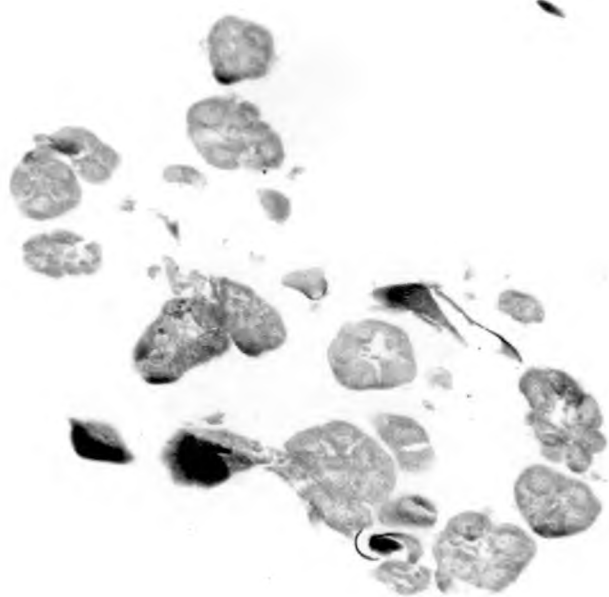
**B****C**

Table 25. Size of hypopharyngeal glands and secretory globules (in μ) of mature adult worker bees six days after they were injected with sacbrood virus, acute bee paralysis virus or extracts of healthy larvae (cf. Table 15).

Inoculum	No. of bees examined	Mean length	Mean breadth	Secretory globules
Sacbrood virus	12	71.4 ± 2.1	52.8 ± 1.5	3.0 ± 0.12
Acute bee paralysis virus	12	117.0 ± 3.2	82.8 ± 1.3	6.3 ± 0.10
Extract of healthy larvae	12	117.9 ± 1.8	91.2 ± 2.5	6.3 ± 0.19

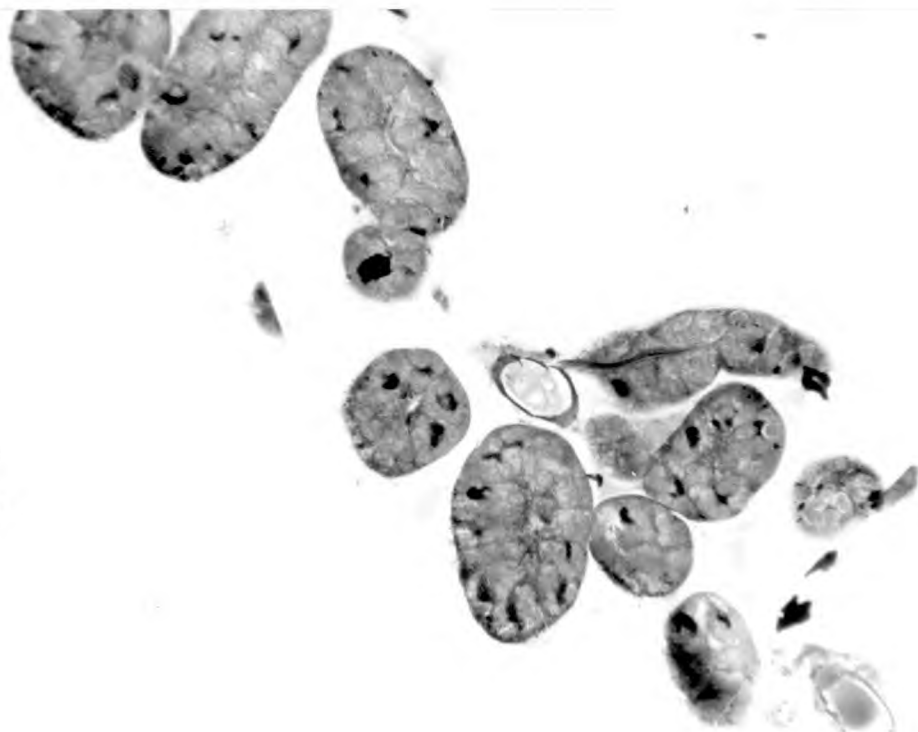


Fig.26. Sections of hypopharyngeal glands of mature worker bees from colonies in autumn six days after they were injected with acute bee paralysis virus. (See Fig.15). X240



Fig.27. Sections of fat-body cells of mature worker bees from colonies in autumn six days after they were injected with acute bee paralysis virus. (See Fig.16). X400

Table 26. Half lives (in days) of groups of worker bees in cages at 35°C. and 30°C. receiving the same treatments as worker bees in experiments A and B.

Inoculum	No. of tests	Temperature and experiment			
		35°C.		30°C.	
		A	B	A	B
		Mean	Mean	Mean	Mean
Sacbrood virus	3	20 ± 0.41	21 ± 0.41	19 ± 0.58	20 ± 0.58
Acute bee paralysis virus	3	19 ± 0.41	19 ± 0.41	6 ± 0.58	6 ± 0.58
Extract of healthy larvae	3	50 ± 1.23	35 ± 2.11	53 ± 0.88	32 ± 0.88

fat-body cells of mature worker bees six days after they had been infected with acute bee paralysis virus, as in those infected with sacbrood virus (Fig.16A), whereas similar healthy worker bees had about 20 granules per fat-body cell (Table 16, Fig.16B.).

Serological tests of worker bees infected with acute bee paralysis virus were positive.

The half-life of groups of bees infected with acute bee paralysis virus were about six days at 30°C., whereas at 35°C. their half-lives were nineteen days (Table 26).

c. Discussion

Some effects of acute bee-paralysis virus resemble those of sacbrood virus. For example, at 35°C. they shorten the lives of young bees or mature bees to about three weeks, equivalent to that of bees deprived of protein. This probably reflects the fact that the viruses cause the loss of stored protein from the fat-body cells. However, acute bee paralysis virus does not cause young bees to lose their appetite for pollen. Moreover, unlike sacbrood virus it does not cause the loss of secretory globules from the hypopharyngeal glands of mature worker bees. This, and the failure of young worker bees infected with acute bee paralysis virus to accumulate secretory globules in their hypopharyngeal glands even though they eat much pollen, shows that the virus probably disrupts protein transport within and between tissues more fundamentally than sacbrood virus.

This seems compatible with the fact that acute bee paralysis virus effects or causes damage to bees far more seriously than sacbrood virus, at least at 30°C.

Part 5. GENERAL DISCUSSION AND CONCLUSIONS

Sacbrood virus multiplies in larvae of the honeybee without severely affecting their vital physiological mechanisms. Infected larvae grow and develop almost normally, dying ultimately (a) with failure to ecdyse and (b) to undergo special changes necessary for pupation, such as the digestion of protein granules and the lysis of fat cells containing these granules.

Similarly, the virus does not severely affect adults. Much sacbrood virus multiplies in their brains, especially of drones, and it changes their behaviour in colonies, but it does not shorten the lives, in cages, of drones or of workers that have been deprived entirely of pollen. By contrast, infection decreases the prolonged lives of workers that have consumed pollen to little more than the length of life of individuals deprived of pollen, or of drones, which do not eat pollen. Therefore the main effect of infection with sacbrood virus is to neutralise the life-prolonging system that depends upon the assimilated constituents of pollen, probably protein. Infected bees then die apparently of senility, as healthy bees. Lack of protein has other secondary effects such as causing hypopharyngeal glands to retrogress or atrophy which in turn probably accounts for the failure of infected bees to attend the queen and drones. These effects of infection with sacbrood virus also correspond with those in senile bees, such as their failure to

maintain the usual metabolic rate of bees at temperatures below 35°C. or to resist chilling.

The most striking effect of sacbrood virus on adults is on their behaviour. This, although changed remains apparently normal; it does not seem likely to be caused by lesions to the brain in which the virus multiplies. The failure of young bees to eat pollen, the early foraging activities of worker bees without gathering much pollen, and even the early maturation of the eggs in unmated queens also resemble events in healthy but senile individuals, and most significantly perhaps, in those anaesthetized with carbon dioxide.

Presumably sacbrood virus and anaesthesia with carbon dioxide cause these effects through a common mechanism. As anaesthesia with carbon dioxide does not shorten the lives of healthy bees that have eaten pollen before they are anaesthetized, its effect more probably corresponds to a result of senility that, in turn, affects behaviour.

Accumulated acid metabolites have been suggested as a cause of bees changing from pollen-gathering to nectar-gathering and of bees abandoning hive duties after anaesthesia with carbon dioxide or other forms of anoxia (Ribbands, 1953).

Were the decreased metabolic rates of senile or virus-infected bees caused by damage to oxidative processes then the same accumulated acid metabolites caused by anaesthesia with carbon dioxide could well be the result. Damage to mitochondria, evident in the brains of drones infected with

sacbrood virus, may even be due to changes associated with senility and such damage would derange oxidative mechanisms.

The change in behaviour observed in bees infected with Nosema apis Zander has also been suggested to be due to a process of "physiological ageing" (Wang & Moeller, 1970). This process of ageing may well be due to the loss of protein reported by these and earlier authors (Lotmar, 1939).

A mechanism activated in this way as a result of senility may be important in healthy colonies by making pollen unattractive to the oldest individuals, which otherwise might be attracted to returning pollen-gatherers (von Frisch, 1968), thereby hindering recruitment of younger bees. Perhaps the younger bees are more able to perform the involved mechanical processes of pollen-gathering. Senile bees may similarly be diverted from hive duties as are bees that have been anaesthetized with carbon dioxide, and so they probably continue most usefully as nectar-gatherers. The same unknown mechanism is probably activated by infection with sacbrood virus.

Whatever causes the change in behaviour of infected bees, its effect is probably important in the epizootology of sacbrood. Young bees clear away the larvae that died of sacbrood probably by ingesting much of the virus during the process, so becoming infected. The comparatively very few infected bees that gather pollen contaminate their loads with infective sacbrood virus. Therefore, were very many of them

to collect pollen, which is readily consumed by young susceptible individuals in the colony (Butler, 1954) the virus would soon find its way to the larvae and kill more of them. Sacbrood virus that may be introduced into the nectar gathered by infected bees would be much diluted among the rest of the incoming nectar, which is quickly and widely distributed (Ribbands, 1953), whereas pollen loads that are stored remain entirely within the cell where they are placed. Any virus in them would remain concentrated and more likely than virus in nectar to infect a bee. However, sacbrood, although endemic and common, rarely becomes very severe in nature (Bailey, 1967, 1969) partly because of the effect of the virus on the behaviour of adult bees and partly due to the shortened lives of infected bees and their susceptibility to cold, all of which probably decreases their chances of spreading infection. These effects also show that infection of adult bees with sacbrood virus could cause unusual losses of bees, especially during winter when their lives become longest after they consume much pollen in autumn (Lotmar, 1939).

The contrasting effects of infection by sacbrood virus and acute bee paralysis virus in the same host and environmental conditions suggest that precise specific factors control the multiplication of each virus. Acute bee paralysis virus appears to affect more vital metabolic processes of the bee than sacbrood virus, resulting in

severe pathological effects, at least at low temperatures.

Sacbrood virus resembles the arthropod-borne viruses of animals and plants, not only in being of similar size and in being unenclosed in protein crystals, unlike most of the known pathogenic viruses of other insects, but also in multiplying as an inapparent infection and accumulating in the salivary glands of adults. Adult honeybees may be considered as vectors of sacbrood virus, and insect vectors of other animal and plant viruses may well suffer behaviour changes analogous to those in bees, and that are similarly important to the spread of disease.

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(* Original not seen.)