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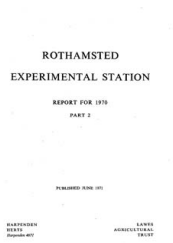

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Virus Diseases of the Honeybee

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Diseases resembling those now known to be caused by viruses were among the first to be noticed in honeybees, and they have been described by beekeepers since more than 100 years ago. Descriptions then made of 'paralysis' of adult bees and of some kinds of 'foulbrood' of their larvae correspond with those of paralysis, as it is still called today, and of sacbrood. In spite of the early diagnoses, however, the viruses causing these diseases were the latest, and may well be the last, of the common pathogens of honeybees to be identified. Most of this work has been done during the past decade.

Paralysis

Early history. The signs of paralysis in a bee colony were described originally as the emergence of many flightless moribund adult bees, usually termed 'crawlers', which often had distended abdomens and sometimes were also hairless, black and shiny. Some of these signs, or very similar ones, soon became associated with one or other of several micro-organisms discovered later, when the hunt for microscopic parasites of bees became popular, about 60 years ago. For example, *Nosema apis*, *Malpighamoeba mellificae* and *Acarapis woodi* were frequently found in colonies showing some of the signs previously ascribed to paralysis and became accepted as the cause of the symptoms. However, the terms 'Nosema disease', 'Amoeba disease' and 'Acarine disease', which are still commonly used, do not specify diseases or hint at symptoms; they were clearly invented to suit the names given to the three newly discovered parasites. In fact recent work has established that the parasites are often very numerous in seemingly healthy bees and colonies and, although *N. apis* and *A. woodi* have been shown to shorten bees' lives somewhat, none has been shown to cause overt signs (Bailey, 1968a). Nevertheless codes of the alleged symptoms of these parasitic infections of bees soon became accepted. Thereupon, the identity of paralysis became obscure and the name became a general term for various apparently uncommon diseases, mostly non-infectious, that seemed to have similar symptoms (Butler, 1943). Morison (1936) noted that only bees with all the symptoms of paralysis had basophilic cytoplasmic bodies in the cells of their hind-gut epithelium, whereas bees infected with the well-known parasites had not. However, the specificity of this sign was disputed soon after it was described because Lotmar (1940) found similar granules in moribund bees in Switzerland that were neither hairless not dark. As the Swiss bees were not infected with any known parasite, they were suspected to have been poisoned and the granules were associated with this.

Etiology. Burnside (1945) made the first successful experiments in the United States with one form, at least, of paralysis. The sick bees in nature were flightless and soon died, although few were hairless, black or shiny. Burnside serially passaged the disease nine times by spraying caged healthy bees with filtrates, free from bacteria and larger organisms, of extracts in water of sick individuals. The incubation period was 8 to 14 days in bees kept at about 23°C and the infected bees soon became trembly and flightless.

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Some were bloated but none became black or hairless. Bailey, Gibbs and Woods (1963) and Bailey (1965a) investigated the same or a very similar disease at Rothamsted where it had appeared spontaneously and very severely in a single colony. The sick bees were flightless and trembly and the trembliness was especially apparent when the bees were kept warm and compared with healthy individuals. More colonies were obtained showing the same severe symptoms from various parts of England. Dark, hairless bees were rare but a few sick individuals with bloated abdomens from the distension of their honey-sacs with liquid, invariably occurred within the colonies. In every way the disease seemed identical with that described by Burnside. Severe attacks were devastating. They were usually seen in summer when thousands of bees crawled and died, leaving the queen with a handful of attendant bees on the neglected combs of colonies that had appeared large and prosperous only a week or two previously.

Chronic bee-paralysis virus. Bailey *et al.* (1963) extracted a virus from paralysed bees by triturating them in water + carbon tetrachloride. They called this virus chronic bee-paralysis virus to differentiate it from another virus, acute bee-paralysis virus (see below), which they discovered at the same time. Later Bailey *et al.* (1968) found that extraction with water + ether followed by carbon tetrachloride was the most efficient method. Clarification of the extract at 8000 g for 10 minutes, followed by sedimentation at 75 000 g for $2\frac{1}{2}$ hours or precipitation with half-saturated ammonium sulphate, gives fairly pure preparation of anisometric particles that resemble prolate spheroids and range in size from 15 to 40 nm wide and 20 to 100 nm long. The average width of the particles, which contain nucleic acid of the ribose type, is about 21 nm and there are three components averaging 42, 52 and 61 nm in length, with sedimentation rates ($S_{20,w}$) of 97, 110 and 125 respectively. The smallest particles are the least infective. Paralysed bees from different sources in nature, and bees injected with fractions of extracts containing mostly large particles, all contain the three virus components in the same relative proportions. Between 10^{10} and 10^{11} particles can be extracted from one paralysed bee and the median lethal dose (LD 50) by injection into the haemolymph for adult bees is about 10^2 particles. The LD 50 of the virus when applied as a spray in water is between 10^9 and 10^{10} particles and when fed in syrup is more than 10^{10} particles. The sprayed virus probably infects via the tracheae and the true LD 50 is probably many fewer particles than all that are sprayed at a bee.

Bees infected by any method and incubated between 30° and 35°C become flightless and trembly about five to seven days after infection and die a day or so later. The lower the temperature the longer they take to die after first showing symptoms. Basophilic cytoplasmic bodies are strikingly evident in the cells of their hind-guts and some of the bees develop bloated abdomens caused by distension of their honey-sacs with liquid. These symptoms correspond exactly with those seen in nature.

Occurrence of paralysis. Much chronic bee-paralysis virus has been detected in bees found with paralysis symptoms and sent to Rothamsted from Australia, China, Mexico, the U.S.A., Scandinavia, Europe, the Mediterranean area and many parts of Britain. Fresh specimens contained as much paralysis virus as there is in bees killed by it in laboratory tests. Similar particles have been extracted by other workers from paralysed bees in the Ukraine, where Aleksenko and Kolomiets (1967) claim to have cultured the virus in 10–11-day-old chicken embryos, France (Giauffret *et al.*, 1966a) and Canada (Lee & Furgala, 1965a). There is no evidence that there are host species other than honey-

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bees: *Bombus*, *Psithyrus* and *Galleria* species were unaffected when injected with doses of virus that were lethal for honeybees (Bailey, 1965a).

Viruses obtained from almost all the countries mentioned above have been compared at Rothamsted and seem serologically indistinguishable from local ones, but Reinganum (1968) observed ring-shaped particles in purified preparations from bees with paralysis in Australia, and he found more long particles in bee samples during June (winter) than during September. Some variation in virus strains may be indicated by this, and their virulence may well differ.

Recent surveys in Britain show that at least 70% of samples of crawling moribund bees or of live bees from colonies producing very many crawlers in nature were of bees suffering from paralysis (Bailey, 1967a). Moreover, some of these samples contained many bees infected with *Acarapis woodi*, a parasitic mite long alleged to cause crawling in bees. However, only the sick bees in one of these samples, which was of many live bees, contained much paralysis virus, whereas *A. woodi* was distributed equally among the sick and apparently healthy individuals. Thus paralysis virus, not *A. woodi*, was causing the crawling.

Notwithstanding the world-wide distribution of paralysis virus, the percentage of colonies that are severely attacked is small, probably not more than 1 or 2%. However, in Britain at least, inapparent infection is very common. Electron microscopy of extracts of bees and serological evidence has suggested that a few particles of paralysis virus occur commonly in seemingly healthy bees (Bailey *et al.*, 1963; Bailey, 1965a). Infectivity tests with extracts of dead bees collected from beneath normal colonies showed that each of about 30% of the individuals contained more than about 10^7 particles of chronic paralysis virus, whereas similar extracts of live bees from the colonies were not infective (Bailey, 1967a). More than 10% of the dead bees contained as much virus as bees killed by chronic paralysis in the laboratory. Very recently (Bailey, unpublished), infectivity and serological tests with extracts of live bees from apparently healthy colonies with no history of paralysis show that during autumn and early winter at least, most bees contain some chronic paralysis virus. This virus is localised, occurring mainly in the post-cerebral and thoracic glands—i.e. the salivary or labial glands—with an average of about 10^5 particles per bee. Hypopharyngeal glands of the same bees contained about 10^4 particles per bee, but the heads of some individuals contained up to 10^8 particles. It may well be, therefore, that a large proportion of the very many bees continually lost from 'normal' colonies die of paralysis, as do many of the comparatively few individuals found dead nearby. 'Good' colonies of today must be only stunted versions of what they would be, were they free from chronic paralysis virus.

Multiplication and spread of chronic paralysis virus. About half the number of particles of chronic paralysis virus in a paralytic bee are in its head (Bailey *et al.*, 1968) which is about 1/10 of the total body weight. Extracts of the brains of paralytic bees are very infective and serological tests indicate that each brain is likely to contain 10^{10} or more particles. However, the only particles yet seen in sections of brain tissue that resemble those of paralysis virus were seen also in sections of brains from healthy bees, and may have been micro-tubules or other components of normal nerve tissue (Bailey & Milne, 1969). Lee and Furgala (1965a) saw similar particles in sections of the nerve ganglia of the thoraces and abdomens of paralysed bees but not in the ganglia of healthy bees. Whether these particles seen in nervous tissue were of chronic paralysis virus remains to be proved, but the infectivity of brain extracts and the symptoms of infection make it probable that the virus multiplies in nerve tissue. Giauffret *et al.* (1967) found many

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basophilic granules in the cytoplasm of the cells of the thoracic ganglia of paralytic bees, whereas the ganglia of uninfected bees contained none. These granules closely resemble those in the cytoplasm of the hind-gut of paralysed bees (Morison, 1936) and contained much nucleic acid of the ribose type.

The spread of paralysis between bees in nature has not been explained. About 10^{11} particles of chronic paralysis virus have been found in the 30 mg or so of liquid distending the honey-sac of the bloated paralytic bees found in nature (Bailey, 1970). Presumably the virus is secreted into the honey-sac from the salivary and possibly the hypopharyngeal glands, where it occurs even in healthy bees on some occasions (Bailey, unpublished), and one of the bloated paralytic bees would seem able to pass several lethal doses of virus in food to a few healthy individuals, according to the LD 50 found by laboratory tests. However, in other tests, bees injected with chronic paralysis virus failed to cause paralysis in healthy bees they fed entirely with food into which they had secreted the virus. Also, it was shown that virus passing into the faeces of paralysed bees soon lost infectivity, that virus accumulating in various tissues of bees fed sub-lethal doses quickly decreased after a few days and that colonies sprayed with virus suspensions suffered trivial and very transient losses of bees (Bailey, 1965a). Burnside (1933) observed that a few healthy bees became paralysed when placed in colonies with severe paralysis, but failed to transmit the disease by putting food from these colonies into healthy ones.

There is much circumstantial evidence indicating that susceptibility to paralysis is closely limited by inherited, possibly matroclinous, factors (Bailey, 1967a). Nevertheless, observations at Rothamsted for several years on colonies headed by queens bred from colonies with severe paralysis show that the onset of disease in them is unpredictable. Significantly more of these colonies have been severely weakened by paralysis than have colonies headed by normal queens but, at any one time, most remained apparently healthy even when kept in the same small area with the few that were severely diseased. Moreover, their bees have proved no more susceptible to artificial infection than bees from other colonies; also, although severely paralytic colonies have recovered when their queens have been replaced with queens from elsewhere, others have recovered spontaneously. One of the recovered colonies relapsed, severely affected and with the same marked queen, after seeming normal for a year (Bailey, 1967b, 1968b, 1969b). Environmental factors were possibly playing a part as they seem to have done when strains of bee susceptible to paralysis showed more disease when kept in uncultivated 'forest' regions in Germany than elsewhere (Drescher, 1964; Bailey, 1967a). Temperature may be important. Chronic paralysis virus multiplies more in bees at 30°C than at 35°C, yet it kills bees quicker at 35°C (Bailey & Milne, 1969). To some extent this reflects events in nature when much virus accumulates in live apparently healthy bees during autumn and winter and severe disease seems to occur mostly during summer, but it does not offer a ready explanation of the sudden and erratic nature of severe attacks. Perhaps extraneous toxins are involved. Maurizio (1945), for example, considered that bees apparently suffering from 'Waldtrachtkrankheit' and 'Maikrankheit', when they contain much chronic paralysis virus (Bailey, 1965a), sometimes had been poisoned with nectar containing glucosides such as saponin. Maurizio found these materials caused paralysis-like symptoms when fed to caged bees, and saponin increases the susceptibility of some cells to virus infection (Way, 1969), possibly by increasing their permeability. However, when bees known to be inapparently infected with chronic paralysis virus were fed toxic concentrations of saponin and other glucosides, the virus did not multiply (Bailey, unpublished). A likely possibility remaining is that susceptibility is not matroclinous and, because the sperm of the several individual drones that usually mate with one queen do

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not become entirely mixed within the spermatheca (Taber, 1955), a queen occasionally produces a flush of susceptible workers when using sperm mostly from a susceptible drone. This could explain the irregular occurrence of paralysis and the spontaneous recovery of a colony with the same queen.

All three castes of the honeybee are susceptible in nature to paralysis, seemingly at any age, and infected queens seem not to transmit the disease to their offspring. For example, some queens reared from severely paralysed colonies at Rothamsted have been found with paralysis only a day or so after they emerged from their cells, whereas others have mated successfully, laid many eggs, from which more apparently healthy queens and workers were reared, and have then died of paralysis. Extracts of the heads of the paralysed queens contained as much chronic paralysis virus as extracts of heads of paralysed worker bees and of paralysed mature drones. Young, moribund workers and drones, most probably less than 24 hours old, and even immature worker and drone pupae, found beneath paralytic colonies have contained as much virus as paralysed mature adults (Bailey, 1969b).

The hereditable factors causing susceptibility are probably recessive, otherwise queens that transmit them would soon be killed by paralysis. Drones, however, are haploid, so those carrying the factors would presumably be very vulnerable to infection. This seems an important mechanism selecting against the transmission of harmful genes and may well account for the rarity of colonies that are very susceptible to paralysis and to other disorders that are closely limited by genetic factors. Several years of observation on paralytic colonies at Rothamsted have produced no evidence that proportionately very many more drones die of paralysis than workers, but many may become infected, die and disappear while they are larvae. Larvae must become infected, otherwise the virus could not multiply, as it does, in pupae, although many attempts to infect worker larvae artificially in paralytic colonies, and so cause their deaths as pupae or adults, have failed. In fact, almost all of very many adults obtained by incubating pupae from paralysis colonies have been healthy. On one occasion, however, about half of about 100 newly emerged bees that had been left for a few hours on their comb became paralysed after a few days in cages, whereas further bees, caged as soon as they emerged from the same comb, remained healthy (Bailey, 1969b). This suggests that bees can become infected from the comb, and recently (Bailey, unpublished) infective chronic paralysis virus was found in pollen collected in the field by bees and removed from them before they could deposit it in their combs. The bees probably secrete the virus into the liquid they add to the pollen they collect. They probably infect larvae similarly, perhaps more efficiently than can be done artificially. Nevertheless, the spread and rapid multiplication of paralysis virus in nature seems very fickle. As other pathogens of bees, it seems to need several coincident factors before it causes much harm.

Retrospect. With our present knowledge of paralysis in mind it is interesting to consider some past accounts of adult bee diseases. One hundred and more years ago, but especially about 1905 when the so-called 'Isle of Wight disease' was alleged to have spread quickly throughout Britain and destroyed most of the bees, many amateur accounts in various journals, together with a few professional reports (e.g. Imms, 1907), described symptoms that match those of paralysis today. Although the allegations, often made then and subsequently, of a single infectious disease spreading rapidly throughout Britain, were never substantiated (Bailey, 1963), there is no doubt that disease quickly spread through some colonies of bees, as paralysis sometimes does today. The sight is impressive and soon trains the eye to notice far less severe examples. The virulence of paralysis virus

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when it multiplies, and the speed with which it can ruin a colony, contrasts especially with the nature of *Acarapis woodi*, which is popularly believed to have caused very severe and swift epidemics, but which can produce only five or six offspring per female and has a life cycle in its symptomless host of about 14 days. During this period, when *A. woodi* can multiply very little, even when circumstances for it are most advantageous, paralysis virus can multiply by many millions and almost destroy a large colony.

Strains of bees very susceptible to paralysis but free from virus may sometimes survive and multiply. Alternatively, and perhaps more probable, strains of bee may arise that are very susceptible to some strains of paralysis virus from elsewhere. In either event, spectacular losses can be expected when exotic virus strains are introduced. Local incidents of this kind seem to occur today when beekeepers propagate certain bee strains for various reasons and sometimes inadvertently select strains specially susceptible to paralysis (Bailey, 1967a). To replace them is a simple remedy when most other bee strains are resistant, and attempts made by beekeepers in these circumstances to find strains insusceptible to paralysis would soon succeed without any knowledge of the pathogen. However, the losses of bees in the past and the search for resistant strains by beekeepers are of unknown extent: the few records of losses suggest they were much exaggerated in most accounts (Bailey, 1963). Not very long afterwards, bees with the same symptoms were found to contain Morison's cell inclusions (Morison, 1936; Lotmar, 1940), which indicates that they were severely infected with chronic paralysis virus as we know it today, because the inclusions are specifically associated with chronic paralysis (Bailey, 1965a). There seem to be very good reasons, therefore, for suspecting that many of the colonies with crawling bees diagnosed in the past as suffering from the 'Isle of Wight', 'Nosema', Acarine' or 'Amoeba diseases' were really suffering from paralysis.

No other parasite of bees has been shown to cause such a severe and quickly fatal disease of the individual in which it multiplies, to be so constantly lethal to bees in nature, and to be so widespread as chronic bee-paralysis virus. Ironically it has attracted the least attention but it would presumably have had greater priority in research on bees long ago had it been as easy to detect as the well-known, large parasites of bees.

Sacbrood

White (1913, 1917), in the U.S.A., first described sacbrood clearly. He observed that larvae with the disease fail to shed their final skin about two days after they are sealed in their cells. The skin becomes a transparent sac, accumulating unusually much ecdysial fluid around the pupal integument. The larva then dies within a day or so and soon dries to form a flat brown scale in its cell, unless adult bees detect and eject it. White caused severe outbreaks of sacbrood by feeding bee colonies with sucrose syrup containing bacteria-free filtrates of diseased larvae. These larvae lost their infectivity about three weeks after they died.

Sacbrood virus. Steinhaus (1949) saw spherical particles about 60 nm across in extracts of larvae with sacbrood and Brčák *et al.* (1963) found isometric particles about 30 nm across, whereas they found no particles in extracts of healthy larvae. They made no tests to show that these particles would cause sacbrood. Bailey, Gibbs and Woods (1964), independently of Brčák *et al.*, also found isometric particles 30 nm in diameter and showed that purified preparations of them caused sacbrood when fed to honeybee larvae. Two-day-old larvae are the most susceptible and the median lethal dose for them by mouth is between 10^7 and 10^8 particles. About 10^{13} particles can be extracted from one larva

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with sacbrood (Bailey, 1969a). The nucleic acid of sacbrood virus is of the ribose type (Lee and Furgala, 1965).

Occurrence of sacbrood. Sacbrood was once thought to be rare, at least in Britain. Tarr (1937) thought most disease resembling sacbrood was a non-infectious hereditary fault—'addled brood'—because he failed to spread it by placing combs containing many diseased larvae in healthy colonies. However, as sacbrood does not spread readily this way (Hitchcock, 1966), and as the photographs shown by Tarr clearly resemble those of larvae known to have sacbrood (Bailey, 1967a), he was probably dealing with this disease.

Recent surveys show that over 80% of diseased honeybee larvae containing no visible organisms from England and Wales have sacbrood and that during summer 10 to 30% of apparently normal colonies, within about 20 miles of Harpenden, contain a few larvae with sacbrood (Bailey, 1967a). A signicantly greater proportion ($P = 0{\cdot}001$) of colonies of an imported strain of bees had sacbrood than local strains. Severe outbreaks occur during spring (White, 1917), but recent tests show that up to 6% of larvae from apparently healthy colonies in August have sacbrood (Bailey, unpublished). These tests were done by removing brood combs from their colony, without adhering bees, when individually identified larvae in them had just been sealed in their cells, and incubating them at 35°C for four days. Extracts of larvae that failed to pupate were then tested serologically for sacbrood virus. The incidence of sacbrood in a form severe enough to be easily diagnosed has now surpassed that of European foulbrood (Ministry of Agriculture, Fisheries and Food, 1969) and as both diseases are most severe during spring and early summer, it is not surprising that they sometimes occur together in the same colony (Bailey & Locher, 1967). The virus found in larvae with European foulbrood in France and thought by Giauffret *et al.* (1966b) to be a likely cause of this disease was, in fact, sacbrood virus (Bailey, 1969b).

Multiplication and spread of sacbrood virus. The ecdysial fluid in the sac that surrounds the dying larvae is rich in sacbrood virus but little is known of where the virus multiplies within the body of infected larvae. Lee and Furgala (1967a) saw particles, sometimes in crystalline array, in the cytoplasm of fat, muscle and tracheal-end cells 48 and 72 hours after 12–36 hour old larvae had been kept in the laboratory on food rich in virus. However, most of these larvae were classified as 'sick' or dead after 72 hours, at an age when they would still have been unsealed in the colony, so they were atypical of sacbrood larvae in nature, perhaps because they had ingested overwhelming amounts of virus. Fyg (1962) considered that infection upsets the production of moulting and juvenile hormones because analagous symptoms to those of sacbrood can be caused by ligatures that prevent the secretions of the brain and corpora allata from diffusing into most parts of the body of healthy larvae. Therefore, sacbrood virus may typically multiply in and derange these parts of the nervous system. The neurotropism of the virus was recently demonstrated by results of infectivity and serological tests showing that sacbrood virus multiplies in the brains of drones when it is injected into their haemolymph (Bailey, 1970).

White (1917) showed that one larva killed by sacbrood could infect about 3000 others when crushed in sugar solution and fed to the adult bees of a colony. Nevertheless, in natural circumstances, sacbrood usually disappears spontaneously during summer, and does not spread much even when combs containing many diseased larvae are placed in colonies (Hitchcock, 1966). In spite of this slight ability to spread during summer, of the rapid loss of infectivity of sacbrood virus in dead larvae, and of the absence of

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larvae during winter, sacbrood persists from year to year. A probable way it does so is by multiplying in adult bees. Bees of any age become infected when injected with about 10^3 particles and individuals younger than about eight days become infected when they ingest 10^8 particles of sacbrood virus (Bailey, 1969a). They show no symptoms, but much virus accumulates in their hypopharyngeal glands and, as they feed larvae with secretions —'royal jelly'—of these glands, they seem a probable source of infection. The youngest bees are not only the most susceptible to infection by ingesting sacbrood virus, but are also the ones most likely to ingest much in nature because they clean honeycomb cells vigorously on their first day of life (Lindauer, 1952; Sakagami, 1953). They detect and remove most sacbrood larvae within a day or two after the larvae die, when the virus is still infective, and they probably ingest ecdysial fluid when this is released from sacs that are ruptured in the removal process. The virus from less than 0·1 mg of a larva freshly killed by sacbrood is sufficient when ingested to infect one of these bees, and much sacbrood virus collects in their hypopharyngeal glands within two days after they ingest an infective dose (Bailey, unpublished). However, although infected adults show no symptoms, they eat little or no pollen after infection, so bees that are infected when they are the youngest and most susceptible are the least likely to eat pollen (Bailey, 1969a). They will, therefore, secrete the least royal jelly and so presumably will not feed many larvae. Moreover, their lack of protein reserves shortens their life and makes them least likely to survive the winter. Further, adults seem unable to infect each other with sacbrood virus when they exchange food. Probably only a comparatively few adults that have already eaten pollen and that then ingest sacbrood virus before they become immune are able to transmit virus to larvae. There seem to be many factors, therefore, that combine to prevent the rapid spread of sacbrood.

Although adult bees are probably the reservoir of sacbrood virus, no direct evidence has been obtained that they transmit virus they contain to larvae. The successful spread of sacbrood by adults fed on suspensions of sacbrood virus in sucrose solution is equivocal evidence because larvae may then be infected with virus carried mechanically by nurse bees. Small colonies composed entirely of worker bees injected with sacbrood virus reared only healthy larvae (Bailey, 1970) but the behaviour of the injected bees seemed abnormal and the very few larvae successfully reared in their colonies may have been fed by comparatively few bees in which virus had failed to multiply. Infection of adult bees in nature may well have more subtle effects than by injection.

Except for alternative host species, which seem improbable, the only other obvious way that sacbrood virus might be transmitted is through the queen, but many attempts to show this at Rothamsted have failed (Bailey, 1968b, 1970). The virus was injected into laying queens or fed to young individuals, which successfully mated and produced larvae. None of the queens transmitted sacbrood, although infectivity and serological tests with extracts of their heads showed that sacbrood virus had multiplied in them.

Drones, as workers, are most susceptible to infection by mouth when young and become immune to infection this way when they are about seven days old. However, the fact that sacbrood virus can multiply in drones is probably of no epidemiological significance, because it is improbable they will secrete virus from their vestigial salivary glands, and their chances of ingesting an infective dose in nature are small because they do not clean combs. None of 30 drones from a colony with severe sacbrood was infected (Bailey, unpublished). None of this present information about the multiplication and spread of sacbrood virus helps to explain why severe outbreaks usually occur only during spring and early summer, but the proportion of susceptible young adults and larvae may then be greatest because colonies are growing fastest.

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Acute paralysis

Acute paralysis was discovered as a laboratory phenomenon during work on chronic paralysis virus (Bailey, Gibbs & Woods, 1963). When 1 μl of bacteria-free, concentrated extracts of whole, apparently healthy, bees—e.g. ten bees extracted in 1 ml water—were injected into similar bees, most of these became flightless after about five or six days at 30°C, and then died within about a day. By contrast, bees injected with chronic paralysis virus remain flightless and trembly for a few days at 30°C before they die. The two kinds of paralysis are most easily differentiated when infected bees are kept also at 35°C. Those with chronic paralysis then die a little sooner than at 30°C, whereas those with acute paralysis, especially when injected with terminally infective dilutions, continue apparently normal for many days, some living as long as uninjected bees. When concentrated extracts of chronically paralysed bees are injected into apparently healthy bees, chronic paralysis virus multiplies, but after several serial passages of similarly concentrated extracts of the resulting paralysed bees, acute paralysis usually prevails.

Acute paralysis virus. Bailey, Gibbs and Woods (1963) extracted acute paralysis virus by grinding acutely paralysed bees in water + carbon tetrachloride, clarifying the extract at 8000 g for 10 minutes and then sedimenting the virus at 75 000 g for 2 hours. Infective virus can also be precipitated from clarified extracts by adding 1½ volumes of ethanol. It maintains infectivity for years in acutely paralysed bees preserved at −20°C. Acute paralysis virus has isometric particles about 30 nm in diameter which separate into two fractions with sedimentation rates ($S_{20,w}$) of 160 and 80, corresponding to particles that appear 'full' and 'empty' respectively when negatively stained with neutralised phosphotungstic acid and examined with the electron microscope. The particles closely resemble those of sacbrood virus but they are serologically distinct, and sacbrood virus kills only larvae whereas acute paralysis virus kills only adult bees (Bailey *et al.*, 1964). About 10^{12} particles, mostly full, can be extracted from one acutely paralysed bee; about 10^2 cause paralysis when injected into the haemolymph but about 10^{11} are needed to cause paralysis when ingested by a bee.

Occurrence. Acute paralysis virus has been detected in all of many samples of bees in Britain, by injecting apparently healthy individuals with concentrated extracts of similar bees from the same source. Similarly bees from N. America and Italy also contained acute paralysis virus (Bailey, 1965b) but bees from Asia and Australia did not (Bailey, 1964, 1967b). Several *Bombus* species in Britain are susceptible to acute paralysis virus and resemble British honeybees in being inapparently infected by it in nature, but *Vespula*, *Galleria*, *Achroia*, *Tenebrio* and *Blatta* species are insusceptible (Bailey and Gibbs, 1964).

Different colonies of bees differ greatly in the percentages of infected individuals (Bailey & Gibbs, 1964). Although the virus is widespread, bees suffering from acute paralysis have not been diagnosed in nature, but the very transitory symptoms of the disease would not make diagnosis easy in the field.

Results of several recent tests (Bailey, unpublished) show that most bees injected with concentrated extracts of live bees from winter clusters at Rothamsted do not become acutely paralysed, even after three serial passages of extracts in winter bees, whereas most test bees from the same source and at the same time were killed by acute paralysis virus when injected with similar extracts of apparently healthy bees that had been collected during summer.

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Multiplication and spread of acute paralysis virus. There are probably not more than about 10^5 particles of acute paralysis virus per apparently healthy bee, and most seem to be in the abdomen, perhaps limited to the intestine (Bailey & Gibbs, 1964). However, bees showing symptoms have much virus in several different tissues, such as the cytoplasm of the fat-body cells (Furgala & Lee, 1966), the brain and especially the hypopharyngeal glands (Bailey & Milne, 1969). Virus accumulates in various tissues of bees, shortly after these are fed sub-lethal doses, but then slowly decreases without causing apparent harm. It becomes systemic and lethal probably only when some particles enter the haemolymph, because antiserum, prepared in rabbits against the virus and injected into the haemolymph protects bees equally against infection by mouth or by injection (Bailey & Gibbs, 1964).

Acute paralysis virus has recently been detected by infectivity and serological tests in pollen freshly gathered by bees (Bailey, unpublished). There were at least 10^5 particles per gram. Although other sources of the virus have not been excluded, bees probably secrete it from their glands, as they do chronic paralysis virus, into the fluid they add to pollen as they collect it. Thus acute paralysis virus seems to multiply and spread most during summer, probably from gland secretions because bees injected with lethal doses of virus transmit it in the food they pass to uninfected individuals in cages (Bailey & Gibbs, 1964). There may be a relationship between the summer multiplication of acute paralysis virus and temperature, because more than three times as much virus can accumulate in living bees at 35°C, which is the usual temperature within the summer cluster, than in bees killed by acute paralysis at 30°C (Bailey & Milne, 1969). These effects of temperature are the converse of those on chronic paralysis virus, which multiplies more at 30°C than at 35°C but kills bees sooner at 35°C. However, an important point of similarity between both viruses is that the severity of their effect is not simply related to the amount that they multiply.

In view of the physical similarities between the particles of acute paralysis and sacbrood viruses and the fact that both multiply in adult bees, their effects, especially on drones, are unexpectedly different. Acute paralysis kills drones at 30°C within a week of injecting the virus and the brain contains about 10^{11} particles, whereas sacbrood virus, which also multiplies in the brain and other tissues at least as much as does acute paralysis virus, causes no symptoms at any temperature and shortens the life of a drone by only a few days (Bailey, 1970).

Some evidence (Bailey & Gibbs, 1964) seemed to indicate that acute bee-paralysis virus could be activated by injecting adult bees with either foreign proteins or concentrated preparations of sacbrood virus (Bailey, 1967c). Later, however, some preparations of sacbrood virus failed repeatedly to cause acute paralysis whereas other preparations always did (Bailey, 1969b), so it seems that sacbrood preparations are frequently contaminated with acute paralysis virus. Similar evidence of contamination was obtained recently when one preparation of sacbrood virus caused chronic paralysis when injected into adult bees (Bailey, unpublished). Possibly the particles seen by Lee and Furgala (1967b) in sections of the fat-body of apparently healthy bees they had injected with sacbrood virus were also particles of acute paralysis virus, especially as they had incubated their bees at 35°C, at which temperature bees infected with acute paralysis virus do not soon die.

As acute paralysis virus occurs commonly in apparently healthy bees, at least during summer, it is likely to contaminate their products and be transmitted when extracts of these are injected into adult individuals. However, transmission can usually be avoided by diluting the extracts, e.g. so that no more than about 10^5 of the extract of an adult

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or a larva is injected. Alternatively, the virus can be neutralised by mixing extracts, before they are injected, with antiserum to it prepared in rabbits.

Conclusions

As there are at least three viruses that can cause severe diseases of the honeybee but that commonly persist without causing symptoms, many similar viruses might be expected to occur in other insects, but very few have yet been found. The only good examples seem to be a virus of termites (Gibbs, Gay & Wetherly, 1970) and 'Sigma' virus of *Drosophila melanogaster* (Seecoff, 1968). The termite virus, moreover, has particles resembling those of acute bee-paralysis virus and kills its host when injected into the haemolymph. Sigma virus kills its inapparently infected host only when the insect is anaesthetised with CO_2, which is at least reminiscent of sacbrood virus causing effects similar to those of CO_2 in adult bees. The best known viruses that attack insects are their peculiar polyhedrosis and granulosis types, which are enclosed in large protein crystals, and the large iridescent types (Smith, 1967). There is some evidence of inapparent infections by some of the polyhedrosis viruses but none has consistently and unequivocably been shown to persist this way. Social insects, especially those with perennial colonies, would be very severely handicapped by host-specific parasites that invariably caused severe disease, so inapparent virus infections are perhaps most likely to evolve among them.

Honeybee viruses seem to have less in common with pathogenic viruses of other insects than with the very many viruses of other animals and plants. They resemble especially the arthropod-borne viruses of plants and animals, not only in being of similar size and in being unenclosed in protein crystals, but in multiplying as inapparent infections and accumulating in the salivary glands of adults. Sacbrood virus is especially striking in this respect, as it does not cause symptoms in adults when it multiplies greatly, even in the brain tissue. The declining concentration of chronic and acute paralysis viruses with time in various tissues of bees that have been fed sub-lethal doses of virus, resembles the behaviour of Semliki forest virus in *Aedes aegypti* (Mims *et al.*, 1966). This behaviour may also be analagous to that of some plant viruses, which, although persistent in vectors that feed for long on infected plants, become established only temporarily in vectors that feed briefly. This phenomenon has been advanced as evidence against the multiplication of such plant viruses in their vectors (Black, 1959). Whether sub-lethal doses of the bee-paralysis viruses multiply or simply accumulate temporarily in their host is uncertain, but the persistence of the viruses through generations of seemingly healthy individuals, with no known alternative hosts, makes it probable they usually multiply and spread as inapparent infections. Only rarely, when the unknown mechanism that limits their spread within the body of a bee is overwhelmed or bypassed, do they attack vital centres. It might be supposed that the gut of adult bees absorbs all of a sub-lethal dose of paralysis virus whereas some of a lethal dose passes through to the haemolymph and becomes systemic, as arboviruses do in mosquitoes (Chamberlain, 1968). This supposition is supported by the ability of antiserum injected into the haemolymph of bees to protect them against lethal oral doses of virus. However, sub-lethal doses of chronic and acute paralysis viruses become temporarily established in several tissues additional to the gut (Bailey & Gibbs, 1964; Bailey, 1965a) so the infective process may not be very clearly demarcated between tissues.

The bee viruses probably cause their severe pathological effects by multiplying in nervous tissues, but they must affect these in different ways. This is suggested by the converse effects of changes of temperature on the multiplication of chronic and acute

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paralysis viruses and on the speed at which they kill when infected bees are incubated at 35° and 30°C, and by sacbrood virus multiplying but causing no overt symptoms in adults at any temperature. These contrasting responses of infections by different viruses in the same host to the same environmental conditions suggest that precise specific factors control the multiplication of each virus. They do not support the concept of 'stress', in its usual non-specific sense, as an activator of virus diseases of insects (Smith, 1967). Nor does the appearance and disappearance of paralysis on different occasions in different colonies within the same small area during summer, when most colonies are flourishing. Additional unknown factors are obviously required for chronic paralysis virus, and probably sacbrood virus, to spread and cause severe disease, but they are probably specific, as are the known factors required for most other pathogens of bees to multiply and spread (Bailey, 1968a). To label them all as stress is retrograde, especially as there are very many circumstances that warrant the same description but do not influence, or sometimes even inhibit, the multiplication of parasites.

As most other parasites of bees, sacbrood virus multiplies freely within individuals it infects and its spread within the population is controlled by various activities of bees, including the frequent replacement of susceptible individuals during summer and their lack during winter. The proportion of susceptible individuals increases or decreases seasonally, which probably causes disease outbreaks or spontaneous recovery, and hereditary factors seem of secondary influence. By contrast with this mainly dynamic process of disease control, the multiplication of the paralysis viruses is usually checked within each of the very many infected individuals by innate resistance factors.

It might be easy to select strains of bees containing substantially less chronic paralysis virus than average by rearing queens from colonies that lose fewest workers from paralysis or that contain least virus. In this way it may be possible quickly to produce much more vigorous colonies than those of today. Virus-free colonies may be attainable by some non-genetical method but to protect them against reinfection from surrounding inapparently infected bees seems a formidable task.

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