INTRODUCTION

During the development of a marking technique which necessitated anaesthetizing honey-bees (*Apis mellifica* L.), certain after-effects of anaesthesia were noticed. This work is a comparison of the after-effects of treatments with carbon dioxide, nitrogen and chloroform; it shows how behaviour patterns may be affected by physiological factors.

I am indebted to several of my colleagues at Rothamsted for criticisms and suggestions, and especially to Mr M. J. R. Healy for statistical advice.

METHODS

Bees were held in small specimen tubes with gauze-covered ends, exposed to chloroform vapour until they were anaesthetized, and then marked. Bees exposed to either carbon dioxide or nitrogen were dropped into tall glass jars which had been filled with the gas, kept there for 2 min. after anaesthesia, and then marked immediately. In warm weather they recovered quickly; when necessary, recovery was facilitated by placing them either in sunshine or in an incubator. Young bees were sprinkled with syrup when they recovered, and then dropped through the feed-hole in the crown-board of their hives; foraging bees were left in dishes on the lawn in front of the laboratory, and they flew away as they recovered.

Since the study was intended primarily to be qualitative no attempt was made to expose the bees to graded doses of anaesthetics. It is believed that variations in the activity of a bee at the moment of anaesthesia are likely to play an important part in determining the extent of the effects.

Bees were either group marked (each batch of bees all marked with a small blob of paint of one colour, so that they were recognizable as a group but not as individuals), or individually marked (each bee marked with a different symbol, and recognizable as an individual). For the latter method, with foraging bees, anaesthesia was necessary.

Modification of foraging behaviour by anaesthetics

On 14 and 16 May 1947, 90 bees were caught when they were returning to their hive with various pollen loads, and group marked; 40 untreated bees were marked blue, 20 CO₂-treated bees were marked white, and 30 CHCl₃-treated bees were marked yellow (Exp. 1). The hive was watched periodically on 17, 21 and 23 May, during which times the untreated controls returned from forty-nine trips, carrying
Changes in the behaviour of honey-bees after anaesthesia

pollen loads on 34 occasions (69%), the CHCl₃-treated bees returned from 59 trips, carrying pollen loads on 36 occasions (61%), and the CO₂-treated bees returned from 24 trips, carrying a pollen load only once (4%).

On 29 May (Exp. 2), a further 196 bees, returning to their hive with full pollen loads, were caught and group marked. A N₂-treated group supplemented the treatments previously given. The hive entrance was watched for 2 hr. on each succeeding day until 11 June, on which date only one survivor was seen. The results are detailed in Table 1. These two experiments indicate that treatment with CHCl₃ does not affect the pollen-gathering capabilities of the bees, but that pollen collecting almost ceased after treatment with either CO₂ or N₂. The flying activities of the CO₂-treated bees seemed consistently lower throughout the second experiment, but the treatment with either CHCl₃ or N₂ had no apparent effects upon either activity (as indicated by the number of trips per bee per day) or longevity.

These experiments conveyed no information concerning the extent to which the inhibition of pollen gathering might be associated with changes of crop or impairment of memory. It was therefore decided to treat bees which were working upon a row of cornflowers (Centaurea cyanus L.) from which they were collecting both nectar and pollen (Exp. 3). The bees were collected and divided at random into three groups of 15 bees each, which were treated with CHCl₃, and N₂ and CO₂ respectively, and individually marked, using a differently coloured set of symbols for each group. On the two subsequent days the cornflowers were inspected at half-hourly intervals, and the size of the pollen load of each individual forager was recorded. The presence of pollen was conclusive evidence that it was being collected, but, since any bee might have been seen at the commencement of her trip and before she had gathered a readily visible quantity of pollen, no conclusion could be drawn from its absence until this had been observed repeatedly. For this reason the records of two CHCl₃-treated and two N₂-treated bees, all seen on not more than two occasions, were omitted, and for each bee the maximum amount of pollen recorded was considered to typify its activities.

Of 11 CHCl₃-treated bees observed, 8 were seen with pollen loads, 2 carrying traces of pollen and 1 without pollen. (The number of observations was 97; 37 with pollen, 19 with traces, 41 without pollen). One of the two trace-collecting bees was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. marked</th>
<th>Pollen presence or absence</th>
<th>May</th>
<th>June</th>
<th>Totals</th>
<th>Trips per bee</th>
<th>Percentage of trips with pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>50</td>
<td>+</td>
<td>26</td>
<td>18</td>
<td>10</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>45</td>
<td>+</td>
<td>23</td>
<td>20</td>
<td>11</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>CO₂</td>
<td>58</td>
<td>+</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>N₂</td>
<td>43</td>
<td>+</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
seen on 12 occasions (5 times with a trace and 7 without); this indicates the likelihood that there was a definite group of trace-collecting bees (possibly only collecting pollen accidentally acquired?), and that some of these bees were not merely bees seen in the early stages of collecting a larger load.

The results from the CO₂-treated and N₂-treated bees were quite different. Of 6 CO₂-treated bees observed only 1 was seen with pollen, and 5 without (number of observations 48; 5 with pollen, 43 without pollen). Of 12 N₂-treated bees observed 1 was seen with pollen, 2 with traces of pollen, and 9 without (number of observations 77; 2 with pollen, 6 with traces, 69 without pollen). The differences between the results from the CHCl₃-treated bees and those from the CO₂- and N₂-treated bees were both statistically highly significant ($\chi^2 = 6.4$ and $7.6$ respectively; 1 degree of freedom (d.f.); probability ($P$) = 0.01).

Exp. 4 was a repetition of Exp. 3, using bees which were foraging upon patches of sainfoin (*Onobrychis sativa* L.). They were treated and individually marked on 27 July 1948, and the crop was inspected on the three subsequent days. Three groups of 12 bees were used.

Every one of the 8 CHCl₃-treated bees subsequently observed was seen to collect pollen (number of observations 129; 73 with pollen, 22 with traces, 34 without). None of the 5 CO₂-treated bees collected a pollen load, but one of them was seen with a trace load on the first day of observations (number of observations 62; 1 with a trace, 61 without). Of 8 N₂-treated bees observed, 2 collected pollen, 1 collected traces, 5 were without pollen (number of observations 67; 5 with pollen, 1 with traces, 61 without). The differences between the results from the CHCl₃-treated bees and those from the CO₂- and N₂-treated ones were both statistically significant ($\chi^2 = 5.9$ and 4.6 respectively; 1 d.f.; $P = 0.015$ and 0.03).

A feature of this experiment was that one of the CHCl₃-treated bees, which had collected pollen on two successive days (first day, 2 observations with traces and 4 without pollen; second day, 7 observations with pollen and 2 without) collected nectar only on the third day (5 observations without pollen). The absence of pollen collection on the third day was confirmed by watching this bee continuously for an hour and a half, during which time she obtained a nectar load from 1126 successive flower visits.

As these two experiments had shown that the inhibition of pollen gathering induced by CO₂ and N₂ was not necessarily associated with change of crop, it was decided to study the effects of the treatments upon bees foraging upon nectar-free pollen sources.

Shirley poppies (*Papaver rhoeas* L.) were therefore chosen for Exp. 5. On 31 July 1948, 55 Shirley poppy foragers were captured; 27 were treated with CHCl₃, 14 with CO₂ and 14 with N₂, and all were individually marked. The Shirley poppies were inspected at frequent intervals on the two subsequent days, and on 6 August a search for marked bees was made in the hives of an adjoining apiary, which had supplied a proportion of the foragers. The results, presented in Table 2, show that most of the CHCl₃-treated bees continued to return to the crop, but nearly all the CO₂-treated and N₂-treated bees deserted it, presumably in order to visit nectar crops elsewhere.
Changes in the behaviour of honey-bees after anaesthesia

Table 2. Exp. 5. Return of bees to Shirley Poppies

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bees marked</th>
<th>Bees observed on poppies</th>
<th>Bees seen alive in adjoining apiary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Aug.</td>
<td>2 Aug.</td>
<td>6 Aug.</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>27</td>
<td>17</td>
<td>14 (not seen on 1 or 2 Aug.)</td>
</tr>
<tr>
<td>CO₂</td>
<td>14</td>
<td>1</td>
<td>0 (not seen on 1 Aug.)</td>
</tr>
<tr>
<td>N₂</td>
<td>14</td>
<td>2</td>
<td>1 (not seen on 1 or 2 Aug.)</td>
</tr>
</tbody>
</table>

The effect of carbon dioxide and nitrogen anaesthesia upon the sequence of hive duties

The accepted pattern of honey-bee activity is based upon the researches of Rösch (1925 et seq.), who found that during their lives they undertook a sequence of duties, the last of which was foraging. Wiltze (1882), Nelson (1927) and Rösch (1930) have shown that the typical sequence can be modified to suit the requirements of the colony.

Rösch was unable to distinguish any definite sequence in the foraging activities, but in view of his observations there was a possibility that such a sequence existed, and that the changes in foraging habits after CO₂ and N₂ treatments might be a consequence of artificial ageing, and accompanied by changes in longevity. Exps. 6–9 were designed to test this hypothesis.

In each experiment either sealed brood or eggs of bees of one colour were introduced into a colony of bees of another colour. The approximate emergence dates of the introduced bees were calculated, and then confirmed by inspection. The young bees were then treated and individually marked, and their subsequent foraging activities determined by observations made at the hive entrance.

During these observations foraging trips, which were repeated at intervals, could be distinguished from occasional cleansing or exercise flights, which were single trips and on which the time between exit and entrance was usually less than two minutes. Foraging trips only will be considered in the presented results.

In view of Rösch’s researches, the first foraging dates were divided into four periods:

(a) Bees not more than 12 days old = foraging within usual brood-rearing period.
(b) Bees 13–18 days old = foraging within usual wax-secreting period.
(c) Bees 19 and 20 days old = foraging during usual entrance guarding period.
(d) Bees at least 21 days old = foraging after completion of full sequence of hive duties.

The first of these experiments, no. 6, was a comparison of CO₂-treated and CHCl₃-treated bees. A comb of sealed brood of leather-coloured bees was introduced into a colony of black bees. 6 April 1948 is considered to be their date of emergence, but a few had emerged on the previous day, and some emerged subsequently. Between their fourth and seventh day 80 bees were treated with CHCl₃, and on the ninth day 30 bees were treated with CO₂; all these bees were individually marked.

Hive observations were made daily from the 10th to the 39th day, except on the
12th, 13th, 20th, 25th, 26th, and 27th days. The usual observation times were a 2 hr. period each morning and afternoon.

19 CO$_2$-treated and 55 CHCl$_3$-treated bees were observed. 5 out of 19 CO$_2$-treated bees but only 1 out of 55 CHCl$_3$-treated ones commenced foraging when less than 13 days old, during their normal brood-rearing period. All the CO$_2$-treated bees had commenced foraging within 19 days, but 51% of the CHCl$_3$-treated bees commenced on or after 21 days. Both of these differences are highly significant ($\chi^2 = 8.3$ and $13.5$; 1 D.F.; $P = 0.005$ and $< 0.001$).

The pollen-gathering propensities of the bees were also analysed. No significant differences were apparent between the proportion of pollen-gatherers among the early- and late-foraging groups of the CHCl$_3$-treated bees, but the two groups were separately recorded because the propensities of the CO$_2$-treated bees could only legitimately be compared with those of the CHCl$_3$-treated ones which commenced flighting at the same time, because forage available at different dates may have varied. Three out of 19 CO$_2$-treated bees and 15 out of 27 CHCl$_3$-treated early foragers gathered pollen loads on at least 10% of their observed trips. This difference is significant ($\chi^2 = 5.8$; 1 D.F.; $P = 0.016$).

It was decided to repeat this experiment with the addition of a group of N$_2$-treated bees (Exp. 7). A comb of eggs of black-coloured bees was introduced into a colony of golden bees. The date of emergence of the black bees was 1 June 1948 (1 day.). On 2, 3 and 5 June three groups, each of 20 bees, were individually marked after treatment with either N$_2$ or CO$_2$ or CHCl$_3$. Observations were impeded by inclement weather, and confined to 7-9, 14-17, 22, 23, 25 and 26 June and July 1-3, 6, 8, and 14 July. Twelve bees from each group were observed.

The dates on which foraging was first observed were:

(i) CO$_2$-treated bees: 9 bees < 12 days, 3 bees between 13 and 18 days.
(ii) CHCl$_3$-treated bees; 2 bees < 12 days, 4 bees between 13 and 18 days, 6 bees > 21 days.
(iii) N$_2$-treated bees: 9 bees < 12 days, 1 bee between 13 and 18 days, 2 bees > 21 days.

The differences between CHCl$_3$-treated bees and the CO$_2$- and N$_2$-treated bees are both significant ($\chi^2 = 6.04$; 1 D.F.; $P = 0.015$).

Pollen-gathering at this period was reduced and only nine loads were gathered by all the 36 bees with which we are now concerned, so no statement can be made concerning pollen-gathering propensities.

The relation between changes in behaviour and longevity

In Exp. 6 the expectation of life of the CO$_2$-treated bees was significantly reduced. Analysis indicated that this was not necessarily a direct effect of this treatment, but could be attributed to the change of behaviour which was induced. Comparison of the early foragers and late foragers among the CHCl$_3$-treated bees showed that although the mean foraging life of the former was greater than that of the latter ($15.0 \pm 1.2$ days compared with $10.8 \pm 0.8$ days) the mean total life was less ($30.1 \pm 1.2$ days compared with $37.1 \pm 0.6$ days). This implies that foraging life was
Changes in the behaviour of honey-bees after anaesthesia

more hazardous and/or more exhausting than life within the hive. The mean foraging life of the CO$_2$-treated bees ($15.1 \pm 1.9$ days) was not significantly different from that of the early-foraging group of CHCl$_3$-treated bees.

The problem of longevity was investigated in greater detail in Exps. 8 and 9, in which eggs of black bees were introduced into a leather-coloured colony, and the black bees were collected from the hive entrance soon after they had commenced foraging. Half of these bees were treated with CO$_2$, and half with CHCl$_3$, and all were individually marked. Regular observations were made at the hive entrance, and the longevity was calculated from the date on which each bee was last seen.

In Exp. 8 the mean foraging life, from the date of marking, was $10.2 \pm 0.8$ days for 57 observed CHCl$_3$-treated bees and $10.8 \pm 0.8$ days for 64 observed CO$_2$-treated bees. The adequacy of the treatments is indicated by the fact that the former collected pollen on $21\%$ of their trips, the latter only on $4\%$.

In Exp. 9 the periods were $10.2 \pm 0.4$ days for 50 observed CHCl$_3$-treated bees and $9.8 \pm 0.4$ days for 48 observed CO$_2$-treated bees. The former collected pollen on $12.3\%$ of their trips, the latter only on $1.4\%$.

These results indicate that the CO$_2$ treatments, in the doses administered, had no direct effect on longevity.

Conclusions

Exps. 1 and 2 were considered to provide adequate evidence that the treatments with CHCl$_3$ had no after-effects upon either the pollen-gathering capacity or the longevity of the treated bees, and Exps. 3 and 4 showed that the memory of bees so treated was not impaired. CHCl$_3$-treated bees were therefore used in subsequent experiments as controls; such bees have since been used successfully in investigations of bee behaviour (e.g. Ribbands, 1949).

The conclusion that CHCl$_3$ anaesthesia did not impair memory, and that bees so treated returned normally to their own hives and to the crops which they had worked before treatment, conflicts with the view of von Buttel-Reepen (1900). He claimed that anaesthesia with either ether, chloroform or fumes of saltpetre destroyed all then-existing memories, because he was able to induce treated bees to enter hives not their own. Bees not completely recovered from anaesthesia will fan and crawl into any accumulation of bees; this fact would explain von Buttel-Reepen’s results, and the conclusion which he drew from them was erroneous.

The experiments showed that CO$_2$ anaesthesia induced a permanent change in foraging behaviour, with the elimination or very marked reduction of the pollen-collecting tendency of bees so treated. Exps. 3 and 4 showed that this change was not associated with an impairment of memory for the crops which the bees had worked before treatment. The CO$_2$-treated bees returned to the crops which they had worked before treatment, but collected only nectar.

Another effect of CO$_2$ upon the honey-bee has already been recorded, by Mackensen (1947). He showed that queen honey-bees which have been instrumentally inseminated commence laying a week or more earlier after CO$_2$ anaesthesia than they do after some other anaesthetics, and that uninseminated virgins subjected to
similar treatment are soon caused to lay unfertilized eggs. Mackensen’s results are complementary to those of Janisch (1924). Janisch exposed to CO₂ for short periods (less than 1 min) the females of three species of grain pests; he concluded that these insects were ‘artificially aged’, so that they went through their potential mating period, and that the treatments thus made them sterile.

Untreated worker honey-bees have a marked tendency to transfer their foraging from pollen sources to nectar sources (Ribbands, 1949), and it has frequently been supposed that the well-known sequence of duties within the hive is followed by a sequence of foraging duties. This result was associated with the results of Mackensen and Janisch, and led to the hypothesis that the CO₂ and N₂ treatments were artificially ageing the bees. Exps. 6 and 7, designed to test this hypothesis, proved that treatment of recently emerged bees with CO₂ induced them to forage at an earlier age than the CHCl₃-treated controls, so that all or a portion of their brood-rearing and wax-secreting activities were eliminated. Foraging life is more hazardous, and perhaps more exhausting, than life within the hive, and therefore the expectation of life of these newly emerged CO₂-treated bees was reduced. However, there was no observable difference between the longevity of these bees and that of those of the CHCl₃-treated bees used as controls which also went foraging at an early age.

Exps. 8 and 9 demonstrated that when the CO₂ treatments were given to bees of known age which had already commenced foraging they had no effect on their expectation of life. In addition, other experiments now in progress are indicating that there is no usual sequence of foraging duties in the field—some bees collect pollen throughout their foraging life, others never do, others sometimes do. For these reasons I conclude that the ageing hypothesis, initially so attractive, is incorrect.

Exps. 2–5 proved that N₂ anaesthesia also produced permanent partial or complete inhibition of the pollen-collecting tendency of foraging bees, and again the memory of the treated bees was not impaired. Exp. 7 suggested that N₂ treatments of newly emerged bees also induced them to forage at an earlier age than they otherwise would have done. There was no evidence of any direct effect of the N₂ treatments upon longevity.

DISCUSSION

The close similarity of the results of the CO₂ and N₂ treatments indicates that the effects are produced by the same mechanism in both cases. The factor common to both treatments is that they deprive the bee of oxygen; the effects may therefore be ascribed to oxygen lack. They are not produced by CHCl₃, which acts upon the nervous system. Dr Wigglesworth, in conversation, has suggested the possibility that oxygen lack prevents the oxidation of acid metabolites produced by the activity of the bee, and that temporary high accumulation of these metabolites gives rise in some way to permanent effects.

An important characteristic of the CO₂ and N₂ treatments is that they do not give rise to abnormal behaviour; they change the behaviour from one normal pattern to the other. Thus one control bee in Exp. 4 collected pollen and nectar on one day,
Changes in the behaviour of honey-bees after anaesthesia

and nectar only on the next day, although her companions continued to collect supplies of both; again, when young bees were treated in Exps. 6 and 7 a few of the controls went foraging at an early age, just as all the CO₂-treated bees did. The results gain in importance from this conclusion, because in consequence the treatments may shed light on the normal mechanism through which the duties of the individual workers are regulated and integrated into a harmonious whole in accord with the requirements of each colony. They suggest a possible physiological basis of the observed behaviour patterns.

The outstanding attribute of the social insects, the differentiation and regulation of the activities of the individuals which compose the colony, has been the subject of much discussion. Roubaud (1916, 1924) and Wheeler (1918, 1928) have emphasized the predominant role of nutritional factors in the development of the community life of the social insects; their opinions were mainly derived from studies of wasps, ants and termites. In the honey-bee the few facets of the problem which have yielded to analysis have been shown to have a nutritional basis. Examples are the differentiation between queen and worker bees, and the subsequent 'nutritional castration' of the latter (Marchal, 1897), and the phenomenon of swarming which can be largely explained on the brood food theory which Gerstung propounded in 1891 (Morland, 1930). In another field, the time sense of bees is controlled by their rate of metabolism (Grabensberger, 1934; Kalmus, 1934). Now that the present experiments have shown that the regulation of some of the workers activities can be altered, apparently as a consequence of extreme oxygen lack, possibly future research will demonstrate the basis of the phenomenon of colony balance and the so-called 'hive-mind'.

The effects now recorded raise problems of practical importance as well as the theoretical ones so far discussed. There is a possibility that properly applied CO₂ treatments of whole colonies might prevent swarming, by converting the temporary excess of nurse bees into foragers, and also that they might be useful for increasing honey production in certain localities where brood rearing is said to be excessive, by reducing pollen collecting and brood rearing and increasing nectar collecting. Both the theoretical and the practical problems suggest interesting possibilities for future research.

SUMMARY

1. Chloroform anaesthesia did not impair the memory, change the foraging behaviour, or reduce the longevity of the treated bees; chloroform is therefore a satisfactory anaesthetic for use in experiments on bee behaviour.

2. Carbon dioxide anaesthesia did not impair the memory of treated bees, but it did induce a permanent change in their behaviour. Their pollen-collecting tendencies were either eliminated or suffered very marked reduction. Experiments with foraging bees of known age indicated that the carbon dioxide treatments had no direct effect on longevity. Treatment of recently emerged bees with carbon dioxide eliminated all or most of their brood-rearing and wax-secreting activities and caused them to forage at an early age. Foraging life is more hazardous than life within the
hive, and therefore the expectation of life of these carbon-dioxide treated bees was less than that of the controls.

3. The effects of nitrogen anaesthesia were similar to those obtained with carbon dioxide. The factor common to both treatments is oxygen lack.

4. The theoretical and practicable possibilities of these results are discussed.

REFERENCES


