EFFECT OF ANAESTHETICS ON WORKERS OF THE ANT *MYRMICA*

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(*Received* 11 *April* 1957)

INTRODUCTION

During recent experiments on ant populations it has been found convenient to anaesthetize worker ants. Anaesthetics have been used in apiculture, and it has been shown that the oviposition of queens (Mackensen, 1947) and the behaviour of workers of *Apis* (Ribbands, 1950, 1954; Simpson, 1954) may be significantly altered as a result. The experiments described below were carried out to determine the effects on worker ants of the anaesthetics commonly used on bees. The ant used was *Myrmica rubra* L., taken from colonies in the west of Scotland.

METHODS AND RESULTS

For the experiment described below ants were selected by occupation and were either nurses (*N*), domestics (*D*), or foragers (*F*). Nurses are approximately 4–6 months old, domestics 10–15 months old, and foragers more than 15 months old (Weir, 1958).

Workers were cultured in special containers (Brian, 1954), which represent optimal laboratory conditions. The experiment was carried out on five batches each of four culture tubes, each containing ten workers. Workers were kept in hibernation at 10° C. immediately prior to the experiment. Of the four cultures in each batch one consisted of segregated foragers, two of domestics, and one of nurses.

Worker oviposition (Brian, 1953), and worker survival, were the criteria used in the assessment of the effect of the anaesthetics. Eggs were removed each week and discarded. Observations were made on the workers at the same time.

Four of the five batches of cultures were treated, each with one of four anaesthetics, the fifth being used as a control. Anaesthetics used were carbon dioxide, nitrogen, nitrous oxide, and compressed air saturated with ether. Control cultures were subjected to a stream of compressed air. In the case of carbon dioxide, nitrogen, and nitrous oxide, each culture was subjected to the anaesthetic for 5 min. each day, timed from the moment that all workers in the culture had been immobilized. In the case of ether, cultures were subjected to the anaesthetics for only 1 min. after the immobilization of the workers. This was sufficient to keep

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workers narcotized or partially narcotized for periods up to 20 min. after removal of the gas source. After observation cultures were then subjected to a stream of compressed air for 2–3 min. to remove any residual anaesthetic. There was a 5-day break in treatment during the fifth week of the experiment.

The weekly egg-output of each culture during the 7 weeks of the experiment is shown in Table 1. The results for the etherized cultures are omitted since no eggs were laid and all workers died within 7 days. Statistical analysis by the partitioning of variance shows that there are significant differences between the egg-outputs of the different kinds of workers. The egg-output of *N*-type workers is high, that of *D*-type workers moderate, and that of *F*-type workers low. Thus egg-output declines with age (Table 1). This change of egg-laying with age provides a basis for the detection of 'artificial ageing' (Janisch, 1924) of workers of *Myrmica*. With respect to the criteria used by Simpson (1954) for the detection of the effects of anaesthetics on *Apis* (glandular retrogression, worker disorientation on removal of the hive, and the inhibition of pollen collection), glandular retrogression was not investigated and the other two criteria are here not applicable.

Table 2 shows that except in the case of nitrous oxide, the total egg-output of

Table 1. *Worker egg-output*

	Anaesthetic used															
	Carbon dioxide				Nitrogen				Nitrous oxide				Control compressed air			
Worker type	*F*	*D*	*D*	*N*	*F*	*D*	*D*	*N*	*F*	*D*	*D*	*N*	*F*	*D*	*D*	*N*
Week 1	.	.	.	.	.	.	.	.	.	3	4	6	.	.	.	.
2	.	.	.	.	.	.	.	.	4	4	8	10	.	.	.	.
3	.	.	.	.	.	.	3	3	3	12	13	17	.	6	9	12
4	.	.	.	.	5	24	.*	29	2	14	12	27	25	32	33	33
5	.	17	13	.	7	23	.	20	.	21	17	34	13	27	23	30
6	.	9	16	.	6	13	.	21	5	9	14	30	17	16	9	21
7	.	.	.	.	2	.†	.	5	.	7	15	12	14	.	.	6
Total egg production	.	26	29	.	20	60	3	78	14	70	83	136	69	81	74	102
Worker survival	8	6	4	3	3	0	.	5	7	5	9	6	9	7	7	9

* Culture accidentally destroyed.
† No workers alive after this week.

cultures in all treated groups is significantly lower than that of the controls. 'Artificial ageing' as described by Janisch (1924) might then be assumed to have occurred in *Myrmica*. If this were so, then, as postulated by Ribbands (1950) in *Apis*, the expectation of life of the individual worker should be reduced. This, however, is no valid proof of ageing. Worker mortality is higher in all treated groups of cultures than in the controls (Table 3). Some of the differences in egg-output may be attributed to worker mortality, but no consistent relationship is demonstrable in this experiment.

Further experiments on the side effects of ether and chloroform as anaesthetics were undertaken. The time of exposure to the anaesthetic was reduced to 30 sec.

per day. Worker survival was still low and no workers laid eggs or survived for more than 7 days. Observations on the individuals made about 1 hr. after anaesthetization on the third and fourth days of the experiment showed that many were by that time abnormal.

Table 2. *Total egg-output during 7 weeks of experiment*

Anaesthetic	Ether	Carbon dioxide	Nitrogen	Nitrous oxide	Control
Total egg output	Nil	55	161	303	326

Table 3. *Worker mortality after 7 weeks*

Anaesthetic	Ether	Nitrogen	Carbon dioxide	Nitrous oxide	Control
Percentage worker mortality	100*	73†	47	33	20

* 100% mortality within seven days.
† Allowance has been made for the accidental destruction of one culture in this group.

DISCUSSION

Simpson (1954) concludes that nitrous oxide, carbon dioxide, and ammonium nitrate fumes (as smoker fumes), may affect worker bees in the following ways:— (1) by lack of oxygen; (2) by the presence of excessive quantities of carbon dioxide; (3) by the presence of substances as impurities in the production of nitrous oxide from ammonium nitrate smoker fumes. The third conclusion might be revised in the light of recent work on the combined action on crustaceans of two or more physiological poisons; Barnes & Stanbury (1948) on *Nitocra* and Hunter (1949) on *Marinogammarus* showed that more-than-additive (i.e. synergic) effects occurred in poisoning by mixtures of copper and mercury salts (respiratory and protoplasmic poisons respectively). Simpson (1954) suggested that certain anaesthetics may cause anaesthesia by interference with respiratory enzymes in *Apis*.

In the present experiments, the effect of lack of oxygen is seen in the cultures treated with nitrogen, where high worker mortality is coupled with low egg-output. The difference between these results and those for nitrous oxide treatment shows that the results of both these treatments cannot be attributed solely to oxygen lack. It appears that nitrous oxide itself has an effect on the workers; it causes premature egg-accumulation. The effects of nitrous oxide treatment are unlikely to be due to impurities since the gas was supplied in cylinders and conformed to medical standards of purity.

The effect of excessive carbon dioxide can also be distinguished from those of oxygen shortage or nitrous oxide treatment. The most striking effect is the complete suppression of egg-output until treatment is stopped for 5 days during the fifth week. It is interesting to contrast this result with that of carbon dioxide on queen bees, where the effect of a small number of doses is to accelerate oviposition (Mackensen, 1947).

Ether and chloroform, in the doses used, kill all workers within 7 days; both

appear to have lasting and irreversible effects on the muscular co-ordination and nervous system of the workers.

It may be supposed that the effects of ether, carbon, dioxide, and nitrous oxide are here combined to varying extents with the effects of oxygen shortage. Nevertheless, each of the three anaesthetics has a separate and distinguishable effect on the workers.

If, as seems likely, anoxia effects are present when nitrous oxide is used, then the effect of the nitrous oxide may be partially to cancel the effect of oxygen lack, since both worker oviposition and longevity are better in the nitrous oxide-treated group than in the nitrogen-treated group. Nitrous oxide may even have a specific stimulatory effect on oviposition.

Janisch (1924) introduced the concept of 'artificial ageing' of insects following treatment with carbon dioxide. This concept was further investigated by Ribbands (1950), who showed that although the original concept was untenable with reference to *Apis*, normal changes in the behaviour of the worker might be accelerated by anoxial anaesthesia, whereas chloroform did not alter behaviour or longevity.

The experiments on workers of *Myrmica* described above allow measurement of any 'artificial ageing' by either egg-output or longevity. Ageing may be said to have occurred in cultures treated with nitrogen, carbon dioxide, and ether since egg-output in all cultures is reduced. In cultures treated with nitrous oxide, egg-accumulation starts 14 days earlier than in control cultures, so these also may be supposed to have 'aged' prematurely. In all groups, worker longevity was reduced after treatment. Therefore the requirements of the ageing hypothesis have again been fulfilled.

Nevertheless, these effects cannot represent true 'ageing' as postulated by Ribbands, since there is no detectable differential mortality between workers of different initial ages (*F*, *D* and *N*). Therefore the ageing hypothesis is untenable with respect to workers of *Myrmica*, for the same reason that Ribbands (1950) found it untenable in *Apis*.

It remains to consider the possible application to *Myrmica* of the hypothesis (Ribbands, 1950) that acceleration of changes in normal behaviour, caused by anoxia, would produce the observed results. All anaesthetics used show some effects on the workers, but these effects can be differentiated from that of anoxia. In respect of differences of egg-output one cannot differentiate between physiological effects (e.g. worker oviposition) and ethological effects (e.g. egg-eating by workers); worker egg-output is the resultant of all such factors. In this experiment longevity is not confused with environmental hazard, since the experiment was undertaken in optimal laboratory conditions. Thus, while different behaviour may lead to different metabolic rates under these conditions, changes in metabolic rate are unlikely to be sufficiently great to cause the differences here observed. These differential mortalities can then be attributed to the direct effect of the anaesthetic on the physiology of the insect.

As a result of these observations it is suggested that such effects as apparent ageing are the result of the direct action of anaesthetics on the physiology of workers.

This physiological interference may be reflected in behavioural changes. All anaesthetics used show this effect to varying extents, and none are completely satisfactory for experimental use.

The quantitative effects of these anaesthetics have not been investigated, but in the experiments described here, workers of *Myrmica* were exposed to repeated doses of anaesthetics, in contrast to previous work on *Apis* (Fyg, 1950; Gontarski, 1950; Ribbands, 1950; Simpson, 1954), where single doses or a few doses were given. This may well account for some of the differences observed.

SUMMARY

1. Experiments have shown that ether, nitrous oxide, and carbon dioxide all have significant and different post-anaesthesial physiological effects on workers of *Myrmica*. They reduce longevity and all except nitrous oxide reduce oviposition.

2. Nitrous oxide causes abnormally early accumulation of an egg mass.

3. The effects of these three anaesthetics can be distinguished from one another, and from the effects of nitrogen anaesthesia (presumed anoxia). Their effects may occur together with, and in addition to, those attributable to anoxia.

4. The effects of the anaesthetics on *Myrmica* longevity appear to be due to the direct physiological action of the anaesthetic and do not operate indirectly via ethological change as supposed in previous work on *Apis*.

5. These results are discussed in relation to previous work including the concept of artificial ageing which is shown to be untenable in this instance.

I am indebted to the Carnegie Trust for the Universities of Scotland, to Prof. C. M. Yonge of the Department of Zoology at Glasgow University for facilities, and to Mr M. V. Brian for his advice throughout.

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