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Sheep Blowfly Research III.—Studies on the Olfactory Reactions of Sheep Blowflies.*

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The Blowfly Problem.

THE Joint Blowfly Committee of Australia in their report No. 2, 1940 (The Prevention and Treatment of Blowfly Strike in Sheep) deal comprehensively with this subject. They discuss sheep blowfly control measures under three groups:—

(*a*) measures to reduce inherent predisposition;
(*b*) measures to reduce immediate susceptibility;
(*c*) measures to reduce fly abundance.

In the third group would fall the trapping of flies, the disposal of carcasses as sources of blowflies and the treatment of strike. Experience in the Union of South Africa has shown that the most important breeding place of *L. cuprina* is the live sheep. This appears to be true under Australian conditions as well. In the control of sheep blowflies, therefore, efficient treatment of strike is of primary importance. This phase of the investigation is dealt with in article VI of this series.

An investigation on the part played by carcasses in the blowfly complex was undertaken at Onderstepoort, a report on it appearing in article V of this series.

I. M. Mackerras and Fuller (1936) have shown that extensive use of traps will reduce the incidence of fly strike by over 50 per cent. In these tests trapping was done on a very intensive scale. The average distribution of traps was one to about twenty-five acres. The bait used was meat. To bait on a comparable scale on most sheep farms in the Karroo would require eighty traps to a paddock one thousand morgen in extent. And when it is considered that many farms comprise five thousand morgen and more, the practical difficulties of maintaining such a large number of traps may readily be appreciated. The cost both in initial outlay and maintenance would be extremely great. Whether trapping on such a scale would be necessary to obtain similar results under our conditions is not known, but it is safe to say that, even if it could be reduced to 75 per cent., the costs would be too great for the average farmer.

On the other hand, if an efficient inexpensive bait could be found, trapping, combined with all the other suggested measures, might be considered an economic proposition.

* See footnote to article I of this series.

With the object of trying to find a bait which would meet all requirements, this phase of the investigation was commenced in April, 1940, by the writers, and carried on until September, 1942.

The properties of an ideal bait may be stated thus:

(*a*) It should be very attractive to *Lucilia cuprina.*

(*b*) It must be attractive for a reasonably long period, thus obviating its frequent renewal.

(*c*) It must be cheap.

(*d*) It must be readily procurable and easy to prepare.

Meat bait and in some cases meat treated with calcium or sodium sulphide is the only bait that has been used for trapping blowflies. It lures *L. cuprina,* but in addition it attracts, especially when old, *Ch. albiceps, Ch. chloropyga, Sarcophaga* spp. and *Musca* spp. It does not long remain attractive to *L. cuprina,* hence it requires renewal every week in summer; furthermore, it is not cheap. From the above requirements it will readily be conceded that meat does not make a satisfactory bait. However, of hundreds of substances tested by many workers in this field of research, nothing has been found to equal it in attractiveness to blowflies.

It was with some misgivings, therefore, that a search for a suitable bait was undertaken. In the following account the various aspects of the investigation are described. The investigation is very incomplete, but it is felt that although the results are largely negative in the broad sense, publication of the data may be useful to other investigators.

Freney (1937) suggested that studies on olfactory responses of blowflies should be conducted under laboratory controlled conditions rather than in the field, and the writers being of the same opinion, the major portion of this work was carried out in the laboratory.

To perform any work on olfactory responses of insects, suitable apparatus is a prime essential. Several different olfactometers have been devised for use with blowflies, but the ideal has not yet been attained. Dr. H. O. Mönnig of this Institution designed an olfactometer which was under construction prior to the commencement of this investigation and which was a modification of the olfactometer described by Lee (1937). It was completed shortly after commencement of these studies and the early work with blowflies was carried out by means of this apparatus. Although it was shown that this olfactometer could work, its great drawback was its unwieldiness. Much time was consumed in testing the apparatus and making alterations to its construction. Probably, after certain more adjustments are made, it will be found to give good service. But as the investigations proceeded it was felt that simpler apparatus of a type more easily handled was required. So, after many months, work with this instrument was stopped and attentions were transferred to another kind of apparatus. This apparatus, known as a cage-olfactometer, has been described elsewhere in this journal, Hepburn (1943). While this apparatus is not without certain disadvantages, its ability to indicate attractiveness or otherwise of baits in short exposures has been well demonstrated. It has the advantage too of being very simple and cheap to construct, so that ultimately four of these cages were made, thus allowing several experiments to be run concurrently.

These cage-olfactometers were set up in a small dark room which was kept at a constant temperature of 27° C. and a relative humidity of 40-50 per cent. Plans had been drawn up for air-conditioning this dark room, but as the investigation was rather suddenly terminated, these were not carried out. Although the room was not ventilated, no serious ill-effects on experiments were observed, though the desirability was recognized at the commencement of the investigation.

Methods.

The blowflies were reared in an insectary and sufficient numbers were collected daily in cages and removed to an aquarium room, where they remained for one or two days before being used in experiments. While in the insectary and in the aquarium room, they were fed on sugar and raw meat, and given a liberal supply of water. Eighteen to twenty hours before they were used in an experiment their meat was removed from the cages. The temperature of the aquarium rom was maintained at 23-25° C., while the minimum relative humidity was 30 per cent. Flies ranging in age from three to fourteen days were used in the experiments, though at times, perhaps, older ones only were available. Under ideal arrangements it may be an advantage to work with flies of the same age, but this would necessitate a special organization for breeding flies in quantities sufficient for the purpose. Under present circumstances this was impossible and the writers had to accommodate themselves accordingly.

It would be desirable also for studies on chemotropic behaviour to be confined to one sex at a time, but it was not possible to do this. The separation of sufficient numbers of females and males for daily experiments in four olfactometers could have been achieved with an adequate staff. In these experiments, therefore, mixed sexes were used, but in the counts of trapped flies the sex was noted. Thus, any sexual response to an odour would have been detected.

Method of Testing.

For each experiment about one hundred flies are used. The baits to be tested are put into small Petri-dishes, screened, and placed within glass traps. In some cases the test substance is run *versus* water, a blank, or an attractive beef bait. Care must be taken where test substances are in solution to compare equal volumes of them and their controls, and to have their surface areas of exposure the same. Attractiveness of a substance depends on many factors, but that of concentration is very important, especially in experiments where two odours are being compared.

The time required for each experiment depends upon the activity of the flies, and the olfactory quality of the test substance. In general it was found that exposures of one to two hours were long enough for the flies to react. At the end of each exposure the apertures in the cages are closed and the two traps removed for the counting and sexing of the trapped flies. While this is being done in another room, the lights in the dark room are switched off and the flies left to rest until the experiment is continued. The flies in the traps are anaesthetised with ether or chloroform, removed, sexed and counted. The glass traps, wire cones and screens are then ventilated in bright sunlight, and the Petri-dishes are cleaned out and fresh test substances put in, prior to continuing the experiment. When the experiment is continued, the test substances are replaced in their respective traps, but these are now reversed in position, the former left hand one being placed on the right hand side and *vice versa*. When a reaction has taken place the experiment is ended and the flies removed for counting and sexing in the manner just indicated. The figures obtained give four totals, one for the test substance on the left side, one for that on the right side, and the two catches of the control, left and right. The relative attractiveness of the test substances to that of its control can be calculated by using the formula given in a publication by Ripley and Hepburn (1929a). The reason for reversing the baited traps is to eliminate, as far as possible, errors due to position factors.

The procedure adopted in this investigation was to run tests in the following manner. Water-soluble substances were tested against water to ascertain whether they were attractive or otherwise. A substance catching consistently more than water was then tested against a standard bait described on page 31. If the substance caught fewer flies than water it was regarded as non-attractive or even repellent. Substances which, when tested against blanks, caught less than the latter, were regarded as repellents. To test obscurant properties, each trap was baited with a standard attractant and the test substance added in a separate container to one trap. A comparison of the relative catches gives a measure of the obscurant value of the substance.

Experiments which yielded doubtful or conflicting results were repeated until enough data were accumulated to permit conclusions to be drawn.

The Search for Attractants.

In conducting an organized search for substances which will attract blowflies, several lines of approach may be adopted. Freney (1937) outlines a scheme which gave valuable results. He implied that his investigation could have been carried out to more advantage perhaps, had it been done under laboratory controlled conditions. His scheme was largely used here as a basis for these tests on olfactory responses of sheep blowflies.

Apart from testing substances which are known or assumed to occur in decomposing protein, it is not unreasonable to carry the search among substances which are not found in the environment of the flies. Working with fruitflies, Ripley and Hepburn (1931) found certain attractive substances, e.g., terpinyl acetate, which is not regarded as being associated with odours likely to occur in their natural environment. Similarly for blowflies, it would be wise to test as many substances as possible in the hope that a suitable attractant may be discovered. This, of course, may entail years of laborious research with perhaps disappointing results.

In our scheme it was planned to test as many substances as possible, but under present world conditions the difficulties of obtaining them are almost insuperable. Some of the chemicals which Freney listed as of great promise as attractants were obtained, and others which were considered might be suitable, were ordered. Some of these were tested, but the majority were unobtainable. Results obtained with pure chemicals will be given later and discussed (page 37).

As *L. cuprina* is greatly attracted to damp wool in the breech of certain sheep, special study was given to substances occurring in suint and this will be dealt with on page 32. A chemical analysis of suint was undertaken and reported on in article II of this series.

Particularly in the drier parts of the Union of South Africa there occur certain interesting xerophytic plants of the genus *Stapelia*, which produce remarkable flowers emitting an odour strongly resembling that of decomposing carrion. Blowflies are greatly attracted to these flowers and they freely oviposit on them. It was felt that if the attractive substances produced in these flowers could be isolated and identified, a valuable clue would be obtained to the solution of the problem of successful blowfly attractants. This phase of the investigation will be discussed on page 42.

The treatment of meat with various chemicals also received considerable attention. Attempts to make extracts of attractive beef baits were made using different solvents, e.g., petroleum-ether, ethyl ether, ethyl acetate, ethyl alcohol, maize oil and liquid paraffin (i.e., medicinal paraffin). It was hoped that well-defined differences in attractiveness of these extracts might be shown. Having thus found the most suitable solvent, further chemical treatment should eventually lead to the identification of some of the active constituents of the bait.

Finally, some attention was devoted to testing fermenting mixtures of fish meal, pancreatin and eggs; gelatin and blood serum; and hydrolysed casein. In some instances the bait was inoculated with mixed cultures of bacteria obtained originally from the intestinal flora of sheep.

A chronological survey of the work is not favoured, for, as the investigation proceeded, recapitulations of experiments were necessary and new ideas were obtained. Details of technique too, were improved, as the studies were furthered.

In the beginning it was considered desirable to have a standard attractant with which to compare test substances. No pure chemical having the requirements was known. Ethyl mercaptan and bromoform were found by other workers to attract blowflies to field traps. At the time the former could not be procured and the latter was found to be unattractive when used in dark room tests. The writers, therefore, were forced to use decomposing meat as the bait. In the beginning minced bovine liver and water were used, but later a change was made to minced beef. It was found that the bovine livers were very variable in quality. The reasons for this were numerous, but one of the main causes was due to their storage in refrigeration for varying periods. A fresh liver allowed to decompose in water appeared to produce different odours from that of a liver, say, kept previously for weeks in cold storage. As the supply of beef was more constant, it was decided to make the change. An attractive bait was made by mixing equal quantities of minced beef and water and allowing this to decompose for several days before use in the olfactory tests. But even in this great variation was encountered, and it was suspected that meat from different parts of the same carcass would produce differences in attractiveness. The work was often handicapped by the bait turning sour, or by contamination by moulds and fungi. Finally, although not entirely satisfactory, meat bait was prepared thus: Beef was obtained from cold storage and then minced; equal quantities of this and water were placed in a Mason jar, inoculated with a mixed bacterial culture and incubated at 37° C. for 40 hours. The bait container was kept closed, but not sealed. The fermenting mixture was then centrifuged at 3,000 r.p.m. for about twenty minutes and the liquid portion thus separated was decanted and transferred to stoppered bottles. This portion was used as the "standard attractant". In many experiments this "beef soup" was diluted instead of being used in concentrated form.

The attractiveness of beef bait depends, apart from the other factors, on its age. In the preparation of our "standard attractant" it was found that it was more attractive after being incubated for 48 hours than for 24 hours, although the latter was also attractive. Incubation for 72 hours caused a reduction in attractiveness. The attractiveness of the bait was enhanced by the inoculation of bacteria, and, judged by human senses, it appeared to produce more penetrating odours than the non-inoculated one.

Tests with Suint Preparations.

The suint extracts described in contribution no. II of this series was subjected to a number of olfactory tests during the course of this investigation. With the Mönnig olfactometer, in which the first tests were run, results obtained with steam distillates of acidified suint concentrates were disappointing, although there was just an indication of slight attractiveness. These distillates had all been neutralized with potassium hydroxide after distillation. A slight excess of the alkali was added and the solution evaporated to a small volume before olfactory tests were commenced. In the light of later results obtained with potassium and sodium hydroxides, an indication of attractiveness with these distillates may be misleading. Any such indication of attractiveness may not be due so much to the distillate than to the alkali present in excess. Further tests in this older type of olfactometer could not throw any more light on this problem. The crude benzoic acid derived from the steam distillate of acidified raw wool appeared to be very slightly attractive. The weak response by the flies and variation in individual trap catches, point to its doubtful olfactory value. Further repetitions of tests with suint distillates were even more unsatisfactory. It was not clear whether the poor results were due to the condition of the blowflies, the quality of the distillates or to defects in the apparatus.

When the cage-olfactometers were used, a few further tests with suint products were made. A highly concentrated suint extract (a filtered and concentrated water extract of lox) was not attractive when tested against water or beef bait. Similar results were obtained with a concentrated solution of the potassium salts of the steam distilled acids of acidified suint solution, again in the presence of slight excess of potassium hydroxide.

Further tests were made with two extracts derived thus: the steam distillate of acidified merino lox was made slightly alkaline with potassium hydroxide, concentrated by evaporation, acidified with sulphuric acid, and extracted in a separating funnel by means of either petroleum ether or ethyl ether. These two extracts were allowed to evaporate spontaneously after which they were tested in the cage-olfactometer. The three species of blowflies *L. cuprina*, *L. sericata* and *Ch. chloropyga* were used together in these tests, the results of which were negative.

Experiments with Meat Bait to which Chemicals had been Added.

In the first experiments bovine liver baits were used to which were added calcium carbonate, calcium sulphide and sodium sulphide at the rate of 3 per cent. of the total weight of meat and water, while in the later work done with the cage-olfactometer, we had changed over to a beef medium. Here the range of substances added to meat was extended and the following were tested: cystine, phenothiazine, sodium thiosulphate, and sodium carbonate.

An interesting result was obtained by comparing liver and water and the same plus cystine at 0·75 per cent. of the total weight. Both baits were incubated at 37° C. for fourteen days. Firstly, the control was run against water and found to be five times as attractive (*L. cuprina* was used in the test). Then the untreated and cystine treated baits were compared, four grams of each being used. Two complete experiments, each with reversals, were carried out, the cystine bait being superior, trapping 171 flies and the control 116. 70 per cent. of the former were females and 59 per cent. of the latter. Of the total number of flies used in the experiment (466) 62·8 per cent. were females.

In another experiment using beef and beef plus cystine at 0·66 per cent. concentration, both ten days old at room temperature, the latter was twice as attractive as the former to *L. cuprina* but equal in attractiveness to *Ch. chloropyga*. No significant difference in sexual attractiveness was demonstrated. In other tests using the latest technique, i.e., incubation at 37° C. and centrifuging, the addition of 1 per cent. cystine to beef enhanced its attractiveness. The experiment was run in triplicate and in each case the percentage of trapped females was higher in the treated than in the untreated bait. In this experiment the baits were incubated for nineteen hours. In further tests using baits incubated for forty hours the superior attractiveness of cystine-beef was again demonstrated in four replicates, but no significant difference in sexual attractiveness was shown. The addition of cystine to a meat bait would facilitate the evolution of sulphur compounds, e.g., mercaptans and sulphides (see Wohlgemuth, 1904), and these may account for the increase in attractiveness of this bait to *L. cuprina*. Furthermore, such chemicals may be more attractive to females but, owing to the greater volatility of some portion, much of this attractiveness in a nineteen hours incubated bait is lost on further incubation.

A comparison of beef bait inoculated with bacteria and beef bait plus 1 per cent. cystine, both incubated for forty-five hours, showed that the latter caught more flies than the former (nearly twice as many) but, during the first fifteen minutes of the experiment more flies were attracted to the inoculated beef than to the cystine-beef which finally showed superiority. Similar results were obtained from other experiments. The addition of cystine 0·67 per cent. to a beef bait containing 2·66 per cent. calcium carbonate in one instance and 0·8 per cent. sodium carbonate in another, failed to increase attractiveness to *L. cuprina* or *Ch. chloropyga*. In the former the two baits attracted *L. cuprina* equally, but the calcium carbonate meat bait was more attractive to *Ch. chloropyga* than the cystine. The sodium carbonate meat bait appeared to be slightly more attractive to *L. cuprina* and more markedly so to *Ch. chloropyga* than the cystine bait.

Calcium carbonate: The addition of calcium carbonate at a concentration of 2·7 per cent. of the total weight of the bait increases attractiveness. A comparative test with treated and untreated baits, five days old, shows that the treated is more attractive to *L. cuprina* and *Ch. chloropyga*, while for the latter species there is an indication that females are more attracted than males. Later tests with sixteen and thirty days old bait show that the treated bait is more than twice as attractive to *L. cuprina* and about four times as attractive to *Ch. chloropyga.* In other words, the addition of this substance at first enhances attractiveness and then maintains it more effectively than does an untreated bait.

Sodium carbonate: Meat bait containing 0·8 per cent. of sodium carbonate was tested against meat and calcium carbonate 2·7 per cent., both baits being six days old. They were equal in attractiveness to *L. cuprina* and *Ch. chloropyga.*

Sodium sulphide: The results obtained with this substance in the cage-olfactometer were not highly satisfactory. In the early stages of this investigation tests indicated that meat and sodium sulphide 3 per cent. was more attractive than meat plus calcium sulphide or meat plus calcium carbonate. The results were variable and no final conclusions on the effect of sodium sulphide could be drawn. Further experimentation is thus indicated.

Phenothiazine: At a concentration of 1·33 per cent. this compound raised the attractiveness of beef both to *L. cuprina* and *Ch. chloropyga.* A mixture of phenothiazine and sodium carbonate in meat is also attractive, but it is not clear to which substance this attractiveness is mainly to be attributed.

Sodium thiosulphate: 1 per cent. of this salt in a thirteen days old fish-meal bait increased its attractiveness for a day, but thereafter it apparently lost its effect.

When testing the above substances in meat baits the writers were handicapped by having only one cage-olfactometer. In one case seven meat baits with chemicals added and an untreated control were prepared together. It was impossible to test these baits individually at the same ages, but it was endeavoured to obtain as many different tests as possible. This work has been left incomplete but enough data were obtained to show the general effects produced by such chemical treatment of meat.

Field Experiments.

Although the difficulties surrounding trapping experiments under field conditions were fully appreciated, some tests were carried out with chemically treated meat baits.

In these experiments the baits were exposed in large oil drum traps which were so constructed as to prevent visiting or trapped flies from ovipositing on the bait. In the first experiment eight traps were used. Four baits, each one being duplicated, were set out in a circle in a paddock adjacent to sheep pens. The baits were alternated in position and after each recording of trapped flies each bait was moved in position to the adjacent trap, working in a clockwise manner. By this method errors due to position factor were somewhat reduced.

Eight kilograms of fresh minced bovine liver was equally divided into eight portions and to each was added one litre of water. Two of these were left untreated as controls while the remainder were each chemically treated. The chemicals used were calcium carbonate, calcium sulphide, and sodium sulphide, each at a concentration of 3 per cent. of the total weight of the bait plus water. The experiment was commenced on the 22nd April, 1941, and continued until May 26th.

Unfortunately there was not an abundance of flies, and despite the changing of bait positions there were big individual variations. After the first day the two control baits did not catch. Calcium carbonate bait attracted more blowflies than the other two treated baits, especially *Ch. albiceps*, a secondary fly. Calcium sulphide was the least attractive of the treated baits. As far as *L. cuprina* was concerned the calcium carbonate bait was somewhat more attractive than the sodium sulphide one.

TABLE 1.

Species of Blowfly.	Number of Flies trapped by baits.			
	Control.	Sodium Sulphide.	Calcium Sulphide.	Calcium Carbonate.
L. cuprina	1	64	47	106
L. sericata	1	4	2	92
Ch. chloropyga	2	81	9	208
Ch. albiceps	5	335	34	2,683
Ch. marginalis	15	713	217	602
TOTAL	24	1,199	309	3,691

Calcium carbonate meat bait trapped nearly as many *L. sericata* as *L. cuprina*, while it was also attractive to *Ch. chloropyga*, a not unimportant primary fly. The results are shown in Table 1. With these field experiments one of the most disconcerting factors is that of variation. This may be offset very largely perhaps by having four or five replicates of each bait per experiment.

From the experiments listed it appears that the addition of unstable sulphur compounds to meat or carrion baits produces substances which increase the attractiveness. A critical survey of the results from all these different chemical treatments of meat and carrion failed to indicate any specific compound responsible for this attractiveness.

In addition to these various treatments of meat bait, experiments with small carcasses chemically treated were run under field conditions. It was thought at the time that results obtained by the addition of chemicals to minced meat and water might not be parallel to those obtained by similar chemical treatment of whole carcasses. Furthermore, from a practical viewpoint any chemical treatment of carcasses to render them more attractive, and, at the same time toxic to flies, should be investigated. With these objects in view some preliminary tests were made. It has been found more convenient to discuss them and also the subject of hydrogen ion concentration of meat and carrion baits chemically treated, in the following paper, No. IV of this series.

FERMENTING BAITS.

Apart from the systematic method adopted in the search for attractants, the writers experimented at random with various fermenting mixtures in the hope of meeting something of a promising nature. Some years ago Dr. M. Sterne, one of the bacteriologists at this Institution, noted that while he was working with some accidentally contaminated *botulinus* cultures, offensive odours were given off which apparently attracted swarms of flies to his laboratory. Unfortunately the species of flies invading the laboratory were not identified and the nuisance created by these insects was so great that the bacterial cultures were destroyed. Subsequently, on request, attempts by this bacteriologist were made to rediscover these contaminative organisms, but with no success. Many cultures were made and exposed to the different species of blowflies, and while some were weakly attractive the majority appeared not to be greatly promising. It is intersting to note, however, that one of these cultures when put in a cage containing *L. cuprina*, immediately stimulated a few flies to copulate. Further investigations on cultures of this type might lead to the discovery of a valuable sexual attractant to blowflies. This phase of the investigation had to be abandoned because there was no bacteriologist available to continue the work.

The following baits all inoculated with bacteria were tested under field conditions: unsterilized meat plus unboiled culture media, unsterilized meat plus boiled culture media, sterile meat plus boiled culture media, and sterile meat plus unboiled media. These were supplied by Dr. E. M. Robinson of Onderstepoort. They were exposed simultaneously in ordinary field traps, the first two for five days and the latter two for seven days. The first-mentioned was the most attractive, catching 34 *L. cuprina*, 200 *L. sericata*, 360 *Ch. chloropyga*, 53 *Ch. albiceps* and 53 *Ch. marginalis*. The attractiveness of the baits lasted only for the few days they were exposed.

In another experiment the following baits were exposed in traps for fifteen days during November, 1940: minced bovine liver and water, 6 *L. sericata*; minced liver and water plus bacteria from sheep intestines, 5 *L. cuprina*, 25 *L. sericata*, 2 *Ch. albiceps*, 30 *Sarcophaga*, 180 *Musca spp.*; blood meal and water, 16 *L. sericata*, 2 *Ch. albiceps* and approximately 700 *Musca* spp.; blood meal and water inoculated with bateria, 2 *L. cuprina*, 10 *L. sericata*, 1 *Ch. chloropyga*, 1 *Ch. albiceps*, 40 *Sarcophaga* and approximately 700 *Musca* spp.; fish meal and water, 56 *L. cuprina*, 56 *L. sericata*, 72 *Ch. albiceps*, 50 *Sarcophaga* and over 3,000 *Musca* spp.; fish meal and water, inoculated, gave no catch. The fish meal bait was promising and further tests were made later.

Laboratory Experiments.

Fish meal baits.—Variable results were obtained with these baits. The following mixtures were tested: Fish meal 50 gm., pancreatin 0·5 gm., 1 egg, and water 200 c.c., and the same plus 0·5 gm. arsenic trisulphide. A comparison of these two at three days old and then at five days, showed the former to be more attractive, but it was not so attractive as a beef bait twenty days old. However, when the former fish mixture was six days old it was apparently more attractive than beef bait eight days and twenty-three days old. A fish meal bait of the same formula but without the addition of an egg was found to be attractive when ten days old and superior to an eleven days old beef bait. This bait was kept in a closed jar in a room at 25° C. When repeated later the bait was kept in an open jar and, compared with similarly treated beef baits, it failed to attract so well as beef, when two and fifteen days old. Finally, another mixture including an egg, was found when twelve days old, to become more attractive than a five day old beef bait. Further work in this direction is indicated in order to answer the question concerning fish meal as a suitable medium for an attractive bait.

The following preparations were also tested: (*a*) gelatin 12·5 gm., egg yolk 42·5 gm., egg albumen 50 gm., cystine 2·5 gm. and water 200 c.c.; (*b*) blood serum-gelatin media. Both these baits were made in duplicate, one of each being inoculated with bacterial cultures. The blood serum mixture with and without bacteria gave unpromising results, but the egg gelatin and cystine bait, inoculated and non-inoculated, was appreciably attractive, but not better than beef baits. Freney obtained promising results with casein hydrolysed with sodium sulphide, but the writers did not: 50 gm. of casein were mixed in 200 c.c. of water containing five gm. of sodium sulphide, and kept in a stoppered jar at room temperature. Tests on the third and sixth days showed that no attractive products had been formed.

Field Experiment.

Finally, a field test with addled eggs was run. Small holes were drilled through the egg shells and a water infusion of horse manure inoculated into each egg. The holes were then sealed by means of wax. After incubating these eggs at 26° C. for seven days they were beaten up in a dish and put in a field trap. A similar lot of eggs incubated for nine days was also placed in a field trap. Both lots attracted all the species of blowflies. The peak of attractiveness was reached about three days after the traps were put out and flies were still attracted on the fourteenth day, but not in large numbers.

Tests with Pure Chemicals.

All the experiments with pure chemicals were carried out in the laboratory under conditions described elsewhere in this report. The intention was to test many of the chemicals known to be products of proteolytic decomposition, but unfortunately only some of those ordered could be procured. All the tests made are listed in Table 2.

The tests were qualitative, designed to discover attractants. With the exception of two or three of the chemicals all were tested in the cage-olfactometer. An important consideration in olfactometer tests is the dilution of the test substance. It is conceivable that a chemical may be attractive at one concentration and not attractive or even repellent at another. A further factor to consider is that of the solvent for the test substance. In this investigation most of the substances used were soluble in water, but some had to be dissolved in oil or alcohol. In choosing oil solvents care must be taken to use one which is olfactorily neutral to the flies. Maize oil and "liquid paraffin" meet this requirement. The use of alcohol as a solvent is not entirely satisfactory unless it can be used very dilute. In concentrations of more than 10 per cent. it should not be used for the vapour given off stuns and even kills the flies approaching the traps. On the other hand, solutions of the test substance in weak alcohol often are too easily thrown into suspension, with a portion of the suspended particles floating on the liquid which virtually amounts to the chemical being exposed undiluted on the surface. The solvents and the dilutions at which the tests were made have been specified in our list, for it is our opinion that the publication of data without this information would lose much of its value to other workers engaged in the same or similar lines of research.

TABLE 2.

Chemicals Tested in Cage-olfactometers.

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Bromoform	Very weak	Water	Beef bait	Not attractive.
Bromoform	Very weak	Water	Water	Not attractive.
Ethyl alcohol	10 per cent	Water	Water	Repellent.
Linalool	10 per cent	Maize oil	Maize oil	Repellent.
Valeric aldehyde	20 drops	50 ml. of water	Water	Weak attractant.
Sulphaldehyde	0·03 per cent	Water	Water	Weak repellent.
Sulphaldehyde	0·03 per cent	Water	Beef bait	Not attractive.
Carvone	5 per cent	Maize oil	Maize oil	Repellent.
Carvone	10 per cent	Maize oil	Maize oil	Repellent.
Carvone plus beef bait	5 per cent	Maize oil	Beef bait	Obscurant.
Musk ketone	Pure	—	Blank	Weak attract. to *L. cuprina*. No reaction with *Ch. chloropyga*.
Phenyl acetic acid	0·8 per cent	Water	Water	Indefinite.
Phenyl acetic acid	0·8 per cent	Water	Beef bait	Not attractive.
n—Butyric acid	2 per cent	Water	Water	Not attractive.
n—Valeric acid	0·1 per cent	Water	Water	No reaction.
n—Valeric acid	0·1 per cent	Water	Beef bait	Not attractive.
iso—Valeric acid	0·1 per cent	Water	Water	Neutral.
iso—Valeric acid	0·1 per cent	Water	Water	Not attractive to *L. cuprina*, *L. sericata* or *Ch. chloropyga*.
n—Caproic acid	0·2 per cent	Water	Water	Not attractive.
n—Caproic acid	0·1 per cent	Water	Water	Repellent.
n—Caproic acid	0·2 per cent	Water	Beef bait	Not attractive.
n—Caproic acid plus Sodium sulphide	0·8 per cent and 1 per cent	Water	Sodium sulphide 1 per cent. in water	Equally attractive.
n—Caprylic acid	·02 ml. in 100 ml. of water; solution drawn off at bottom	—	Water	Neutral.
Erucic acid	Pure	—	Blank	Indefinite to both *L. cuprina* and *Ch. chloropyga*.
Thioacetic acid	0·1 per cent	Water	Water	Neutral to *L. cuprina*, *L. sericata* and *Ch. chloropyga*.
Thiopropionic acid	0·05 per cent	Water	Water	Conflicting, probably not attractive to *L. cuprina* and *Ch. chloropyga*.
Thiobutyric acid	1 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·1 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·01 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Ethyl alcohol, 10 per cent. in water	Slightly attractive.

TABLE 2.—(*continued*).

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Thiobutyric acid plus beef bait	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Ethyl alcohol, 10 per cent. in water (plus beef)	Obscurant.
Thiobutyric acid	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Beef bait	Not attractive.
Ammonium salt of n—Butyric acid	1·7 per cent	Water	Water	Slightly repellent to *L. cuprina, L. sericata* and *Ch. chloropyga.*
Ammonium salt of iso-valeric acid	1·7 per cent	Water	Water	Slightly repellent to *L. cuprina, L. sericata* and *Ch. chloropyga.*
Ethyl caprylate	4 drops on water	—	Water	Probably repellent.
Ethyl caprylate	4 drops on water	—	Beef bait	Not attractive.
Amyl caprylate	4 drops on water	—	Beef bait	Not attractive.
Amyl caprylate	4 drops on water	—	Water	Not attractive.
Linalyl acetate	10 per cent	—	Maize oil	Neutral.
Linalyl acetate	Pure	—	Blank	Slightly repellent.
Phenyl glycol diacetate	5 drops on water	—	Water	Neutral.
Phenyl glycol diacetate	5 drops on water	—	Beef bait	Not attractive.
Ethyl mercaptan	1 : 50,000	Water	Water	Not attractive.
	1 : 100,000	Water	Water plus beef bait	Not attractive.
	1 : 200,000	Water	Water	Not attractive.
	1 : 500,000	Water	Water	Not attractive.
Ethyl mercaptan and Indole	1 : 200,000 and 0·05 per cent	Water	Water	Not attractive.
Ethyl sulphide	0·125 per cent	Water	Ethyl sulphide 0·05 per cent.	More attractive than control.
	0·05 per cent	Water	Water	Not attractive.
	0·05 per cent	Water	Beef bait	Not attractive.
	0·1 per cent	Water	Water	Not attractive.
	0·25 per cent	Water	Water	Not attractive.
	1 : 100,000	Water	Water	Neutral.
	1 : 100,000	Water	Beef bait	Not attractive.
	0·04 per cent	*Liq. paraf	*Liq. paraf	Neutral.
	0·04 per cent	*Liq. paraf	Beef bait	Not attractive.
Ethyl disulphide	0·025 per cent	Water	—	Not attractive.
	0·1 per cent	*Liq. paraf	—	Weak attractant.
Ethyl disulphide plus a dish of water	0·1 per cent	*Liq. paraf	Beef bait	Not attractive.
Triethyl amine	1 per cent	Water	Water	Weak repellent to *Ch. chloropyga, L. cuprina.*
	1 per cent. exposed in sun 40 minutes	Water	Water	No reaction from *Ch. chloropyga, L. cuprina.*
	0·25 per cent	Water	Water	No reaction from *Ch. chloropyga, L. cuprina*

* Medicinal paraffin.

TABLE 2.—(*continued*).

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Butyronitrile	0·5 per cent	Water	Water	Not attractive to *L. cuprina and Ch. chloropyga.*
	0·5 per cent	Water	Beef bait	Not attractive to *Ch. chloropyga and L. cuprina.*
Indole	0·05 per cent	Water	—	Weak attractant.
	0·05 per cent	Water plus 5 per cent alcohol	—	Indefinite.
	1 per cent	Water plus 40 per cent. alcohol	—	Flies stupefied.
Carbazole	Powder floating on water	—	Water	Neutral to *L. cuprina, L. sericata, Ch. chloropyga.*
Maize oil	Undiluted	—	*Liq. paraf	Both neutral.
Natural civet	Undiluted	—	Blank	Slightly attractive.
Dippel's oil	Undiluted	—	Blank	Strong repellent.
	1 per cent	Maize oil	Maize oil	Repellent.
Potassium hydroxide	24 per cent	Water	Water	Attractive.
	0·72 per cent	Water	Water	Attractive.
	0·72 per cent	Water	Beef bait	Not attractive.
Potassium hydroxide, boiled solution, high grade quality	20 per cent	Water	Potassium hydroxide 5 per cent. boiled solution, high grade	Equal in attractiveness.
Potassium hydroxide, purest grade, boiled solution	20 per cent	Water	Potassium hydroxide high grade, 20 per cent. boiled solution	Both attractive, little difference between them.
Sodium hydroxide	20 per cent	Water	Water	Attractive.
	20 per cent	Water	Beef bait	Slightly attractive.
	20 per cent	Boiled water solution	Water	Attractive.
Potassium carbonate	20 per cent	Water	Water	Slightly attractive.
	20 per cent	Water	Beef bait	Not attractive.
Calcium hydroxide	Saturated water solution	—	Water	Slightly attractive.
Sodium thiosulphate	20 per cent	Water	Water	Slightly repellent.
Sodium bicarbonate	9 per cent	Water	Water	Repellent.
Sodium sulphide	1 per cent	Water	Water	Slightly attractive.
Sodium sulphide and Ammonium carbonate	1·9 per cent and 2·2 per cent	Water	Ammonium carbonate 2·2 per cent. in water	Not attractive.

Ethyl mercaptan and other organic sulphur compounds found to be promising by Freney were given extensive tests. The former was tested at concentrations in water varying from 1:50,000 to 1:500,000 and also in combination with indole, with negative results. A 0·05 per cent. solution of indole was only slightly attractive. 1 per cent. sodium sulphide in water was slightly attractive and a mixture of sodium sulphide 1 per cent. and caproic acid 0·8 per cent. was slightly attractive, but caproic acid 1 per cent. alone was somewhat repellent. It seems likely, therefore, that the attractiveness of the mixture may be due to the sulphide. Thiobutyric acid 0·11 per cent. in 10 per cent. ethyl alcohol is slightly attractive, but when tested against a beef bait this attractiveness is not demonstrated. Concentrated valeric aldehyde is slightly attractive to *L. cuprina*. Undiluted natural civet and musk ketone* are also slightly attractive. Of repellent substances found carvone, Dippel's oil and linalool were the most outstanding.

It will be shown later in the discussion on experiments with various chemically treated extracts that certain apparently non-volatile substances, e.g., potassium hydroxide, were found to be attractive to *L. cuprina*. Even boiling the solution was found to reduce its attractiveness only to a small degree. Minute traces of volatile impurities must apparently be responsible for this attraction for the reaction was olfactory and not a visual one. That the blowfly is remarkably sensitive to certain odours is well-known; the results quoted above with tests on potassium hydroxide indicate a very delicate olfactory mechanism of the fly. In the course of the experiments in which meat bait controls were used, attempts were made to determine the threshold of attractiveness with beef baits. Beef bait was prepared in the manner already described and tests were run with this at various dilutions. Five drops of "beef soup", i.e., 0·13 c.c. diluted in 2·37 c.c. water were found to attract *L. cuprina* readily, while a solution twice as strong showed no greater attractiveness. A solution five times the strength of that of the first-mentioned was appreciably more attractive and the optimum concentration was that of the undiluted "soup". In all these tests the volume of bait used in each trap was 2·5 c.c. For most tests a 50 per cent. solution of soup and water was found to be satisfactory. Any chemical showing consistent superiority to such a control would be marked down for future field experiments.

It is interesting to recall Freney's experience with ethyl mercaptan which, he said, attracted many *L. cuprina* to the vicinity of field traps but did not induce them to enter. He put forward the theory that perhaps one set of odours attracts flies from a distance while another lures them into the traps.† In the tests with ethyl mercaptan at dilutions ranging from 1:50,000 to 1:500,000 no response was shown by the flies. This might be due to a matter of dilution or one of olfactory quality. It was hoped to test methyl mercaptan but this was unobtainable. It may be that a mixture of mercaptans or organic sulphides with chemicals like indole or skatole may be found to be attractive. The possibility of a single chemical attracting

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* Musk ketone is a dinitro derivative of tert. butyl acetophenone or a similar ketone.

† Hobson (1938), working with *L. sericata*, states that the oviposition response consists of two phases: (1) attraction from a distance, and (2) stimulation to oviposit. Perhaps the attraction to ethyl mercaptan as observed by Freney falls within the scope of the former and does not supply a stimulus for oviposition.

the flies has not been ruled out but, from our work and judging by that of other workers, it is our opinion that a mixture of odoriferous substances is responsible for this attractiveness.

Experiments with Stapelia Flowers.

Mention has been made on page 31 of the attractiveness to blowflies of *Stapelia* flowers. There are hundreds of species of this genus and a comparative study would probably reveal specific differences of attractiveness to blowflies. It was found that one species, *Stapelia flavirostris*, was very attractive to *L. cuprina*. A day-old bloom was placed inside a large cage (2 by 2 by 2 feet) containing several hundred flies and immediately the insects swarmed over the flower to such an extent that no portion of it was visible. Subsequently, in cage-olfactometer tests, a flower attracted more flies than two cubic centimeters of an attractive bait.

It was felt that in this plant there was something which might well repay a chemical investigation. Accordingly, collections of *S. flavirostris* were made at Grootfontein College of Agriculture in the Karroo by Mr. George Gill, botanist, and Mr. Sutton, Government Veterinary Officer. These flowers were preserved in a weak solution of mercuric chloride and despatched to Onderstepoort.

We are indebted to Dr. H. L. de Waal, formerly of this Institution, for undertaking the chemical study of these flowers. He prepared steam distillates of the coronas and corollas, and also made extracts with various organic solvents in an effort to discover the most suitable method of extraction. At the time we did not have the cage-olfactometers, so the tests were carried out in the Mönnig olfactometer. All of the distillates were attractive to *L. cuprina*—even after prolonged distillation odoriferous material was carried over into the distillate. Some of the odours issuing from the receiver could be further absorbed in ice-cold water. Acidification with tartaric acid does not accelerate the distillation of the active ingredients, and addition of potassium hydroxide to the distilling flask does not appear to have any effect. Various organic solvents were also used for extraction. Chloroform seemed promising in the beginning but subsequently failed, as did all other solvents. Even a six-days extraction with ethyl ether in a bubble-extractor failed to remove any attractive ingredient from an attractive steam distillate of *Stapelia* coronas. Unfortunately there was insufficient material available for further work.

Several hundred plants were planted at Onderstepoort but these did not flower well the following season. From the blooms available from this source it was shown that (*a*) there is great variation in attractiveness of individual blooms, (*b*) attractiveness is correlated with the age of the blooms, e.g., a bloom picked when one to two days old is more attractive than a freshly opened one, (*c*) attractiveness disappears quickly after the bloom begins to wither.

The flowering period for this species is not long so that it is probable that further investigations on this problem will have to be continued for several seasons.

Experiments with Extracts of Beef Baits.

Extraction of beef baits was attempted only towards the close of this investigation, the sudden termination of which prevented the following up of any promising indications. With the exception of a beef bait containing 1 per cent. cystine, all the extracts were made from standard beef bait inoculated with bacteria (*vide* page 31).

Preliminary tests indicated that the liquor or " soup " obtained from the bait by centrifuging at 3,000 r.p.m. contained as much as, if not more, of the active olfactory constituents than the solid proteinaceous material. It was decided, therefore, to concentrate on the bait liquor for the preparation of extracts.

Further tests showed that, on addition of an excess of 96 per cent. ethyl alcohol to the liquor, most of the active ingredients probably remained in solution. Repeated washing of the precipitate, i.e., the alcohol-insoluble protein in the liquor, apparently removed all attractive substances present. The residue of the alcohol-soluble portion after evaporation, on the other hand, proved only weakly attractive.

Trials with medicinal paraffin and maize oil as extractants proved disappointing when compared with other solvents. The original idea in using these non-volatile oils was to find a solvent which would be olfactorily neutral and serve as a satisfactory diluent, while the viscosity and vapour pressure of the solvent should be such as to allow only a slow volatilization of the odoriferous material dissolved in it. This principle has been recognised by Ripley and Hepburn (1931).

In this way it was hoped to prepare an extract from which the odour would emanate more slowly and uniformly than it would from ether extract residues. That would have been a useful advance, a step nearer the achievement of a " standard attractant ". Judging by the human sense, odoriferous material had decidedly been extracted from the beef " soup " by these two oils, but the flies did not respond to them. It may be that these oils fixed the odours too successfully.

A further trial was made to fix the odours of beef soup in an emulsion with lanoline. The object was to dilute the beef soup in a medium which would permit only a slow evolution of odours, thus stabilizing the solution or mixture to a standard rate of production of odours. This is an extension of the principle of Ripley and Hepburn mentioned above.

Ethyl ether was ultimately selected as the most suitable solvent after some preliminary trials had shown it to be promising. The ease with which it emulsified with beef soup was an obstacle which was best overcome by centrifuging for short periods. In cold weather this did not entail a big loss of solvent by evaporation. For testing purposes, measured volumes of ether extract were evaporated spontaneously from weighing bottles which could be tightly stoppered with ground glass lids. It appeared that heating of the ether extract for rapid evaporation resulted in a big loss of olfactory material; evaporation before a fan did not appear to affect it so adversely so that this procedure was finally adopted. The bottle was stoppered immediately the last traces of ether were on the point of disappearing. Only when the test in the cage-olfactometer was about to commence was the bottle unstoppered and both bottle and lid were placed in the glass trap. A good quality ether was used in these extractions; those brands leaving pungent acidic residues were unsuitable.

The results consistently showed that ether removes some constituents from the beef soup that are very attractive to *L. cuprina*. It was also found that washing of the ether extract with water before evaporating it will reduce its attractiveness but only to a slight extent. Further work was then undertaken to try if possible to isolate and identify some of the constituents present in the extract. Preliminary trials with the extraction of acidified or alkaline beef liquor were abandoned when the tendency to emulsification was found to have been enhanced. Instead, the ether extract itself was washed with sulphuric acid (12 per cent.) or with potassium hydroxide (20 per cent.) solutions. The residues of these washed extracts as well as the acid and alkaline washings were then tested in the cage-olfactometer. The results indicated that the acid did not reduce the attractiveness of the extract to any significant extent, whereas washing with the hydroxide reduced attractiveness appreciably. The acid washings of the extracts showed no signs of attractiveness; the alkaline washings, however, were attractive although acidification of these (with sulphuric acid) apparently destroyed every trace of attractiveness. This unexpected result was confusing and led to the olfactory testing of the reagents, i.e., sulphuric acid and potassium hydroxide.

Sulphuric acid proved to be unattractive but potassium hydroxide, of which a high grade product had been employed in all the tests, was found to be attractive and quite consistently so. Further tests with the highest grade of potassium hydroxide, i.e., the analytical reagent grade, confirmed these results. There seemed to be a slight difference in degree of attractiveness between the purest and the high grades of hydroxide, the latter being slightly more attractive. When the solutions of these two grades of potassium hydroxide had been boiled rapidly for periods varying from thirty minutes to an hour no significant reduction in attractiveness could be demonstrated. Should any volatile impurities (?), therefore, be responsible for this attractiveness, they must be of a moderately volatile nature and not be distilled off by ordinary boiling. Potassium hydroxide solutions of different concentrations did not show appreciable differences in attractiveness. Whereas 24, 20 and 5 per cent. solutions were about equally attractive, i.e., caught on the average three to five times as many flies as the water control, a 0·72 per cent. solution was found to catch twice as many flies as the control.

Having thus found potassium hydroxide to be attractive to *L. cuprina* it was only natural to test sodium hydroxide. The analytical reagent grade was employed, and it showed the same degree of attractiveness as the potassium compound. Continued boiling also failed to destroy the attractiveness. The only treatment which appeared to destroy this property was one of acidification with sulphuric acid. This would lead one to suppose that the pH has some bearing on the matter.

A few tests with potassium carbonate and calcium hydroxide showed them to be slightly attractive: *L. cuprina* reacted positively to these alkalis only very slowly. On the introduction of potassium hydroxide to the experiment with potassium carbonate the flies reacted well in a very short time to the hydroxide solution and few only were caught in the trap containing the carbonate. Further, some tentative experiments were run with a .9 per cent. solution of sodium bicarbonate in duplicate tests, the results of which were very consistent, showing this solution to be unattractive, water catching four times as many flies. The flies were not thirsty for they were given an abundant supply of water throughout and before the running of the experiment.

The significance of pH as a factor in attractiveness is not clear from the results obtained with these solutions for, except in the case of sodium bicarbonate, the pH falls within the range of about 12·3 to 13·4.

At this stage the investigation came to a sudden termination so that a number of ideas could not be followed further. The available evidence does not suffice to allow of any generalization. It does not appear likely that the potassium or sodium ions in solution are responsible for attractiveness, especially in view of the negative result obtained with sodium bicarbonate. On the other hand, according to orthodox ideas, the hydroxyl ions can hardly provide the attractive agents. In any case, these inorganic compounds are not supposed to be volatile, so that one is inclined to the view that this strange phenomenon must be attributed to volatile impurities. This, however, appears to be difficult to reconcile with the fact that a 0·72 per cent. solution of potassium hydroxide also showed definite signs of attractiveness. In this experiment the absolute amount of potassium hydroxide employed per trap was only 36 milligrams. An impurity could only have been present in traces in the original preparation (KOH), so that in the test extremely minute amounts only could have been involved.

Discussion.

In our introductory remarks reference was made to the experiments of I. M. Mackerras and others on the effect of intensive trapping on the incidence of strike, and the economic obstacles to any practical application of the method were stressed. Ordinary meat bait and also meat bait plus calcium sulphide was used, both of which caught great numbers of flies. Mary Fuller (1934) demonstrated by field tests the great attractiveness of meat bait treated with sodium sulphide. It has been shown that the addition of cystine to meat baits increases their attractiveness. From these and many other observations by various investigators it seems reasonable to associate attractiveness of baits to blowflies with the production of sulphur compounds.

No simple and cheap substances have yet been found to attract blowflies as do meat baits or chemically treated meat. Fermenting egg mixtures also supply attractive odours to blowflies but it is doubtful whether baits like these could compete with meat baits. Fish meal bait was found to produce attractive odours, but results showed it to be rather disappointing in general. Furthermore, an expensive chemical, pancreatin, was required to make this bait attractive. All these substances, while attracting blowflies in great numbers, fail to attract sufficient numbers of *L. cuprina*, the most important of sheep blowflies, to reduce the incidence of strike.

Although hundreds of pure chemicals and scores of chemically treated substances have been examined for possible use against blowflies, no suitable attractant has been found. Further studies in this field may eventually lead to the discovery of an ideal bait. It must be added, however, that the discovery of an ideal bait will not solve the blowfly problem, but it will provide a valuable control measure. In a search for the ideal bait investigators must be prepared to explore, perhaps for a very long time, a wide range of substances. It may not be out of place to offer some suggestions for future work in the light of our experiences.

Hobson (1938) has shown from field observations that attraction is twofold; one factor is supplied by the living sheep and the others by products of putrefaction. He points out that tests with repellents should be done on living sheep and stresses the importance of using gravid females.

From our results oviposition has been shown also to be stimulated by the odour emanating from *Stapelia* flowers. No sheep factor is present in this instance. The desirability of continuing chemical studies on these flowers has also been mentioned.

The advantages which may result from using the sexes separately in chemotropic tests have also been mentioned on page 29. In the use of mixed sexes in olfactometer tests it is possible that a female in a highly attractive condition to males might enter a trap and attract flies, thus giving a result which might easily be very misleading.

Further work on extracts of attractive meat baits may lead to an elucidation of the nature of the attractive substances, while more tests with inorganic alkalis should also be carried out.

Our studies on suint preparations are also incomplete. The chemical difficulties in work of this nature are very formidable and have been mentioned in contribution No. II of this series.

As regards suint as a factor in susceptibility, the results obtained do not permit of any generalization, except that thus far no indication have been found of the presence of any attractive constituent in suint. This does not exclude the possibilty that suint may provide a source for products of decomposition that are attractive. The idea that acids like caproic and caprylic may be specially attractive has not been substantiated either by tests with these acids by themselves, or with extracts of suint calculated to have retained (isolated) these acids. These extracts were tested in the acid state, or when made alkaline with potassium hydroxide, and in neither case was there any positive response from the blowflies. The remaining possibility is that the amount of suint secreted may be of significance in susceptibility. This question has received some attention in the past, but does not seem to have been conclusively disposed of [cf. Hobson (1936b) and Holdaway and Mulhearn (1934)]. No further evidence can be adduced at this stage.

The use of repellents on sheep to prevent oviposition does not, according to Hobson (1940), offer much encouragement. The requirements for a satisfactory repellent are so rigid that it is doubtful if any could be found. So far no repellents have been found which will keep off flies from sheep for more than a week. The most promising results so far appear to be obtained with the blowfly spray for the treatment of myiasis (see article VI of this series).

Although several pure chemicals, e.g., sodium and potassium hydroxide, have been shown here to be attractive under certain conditions, and other workers have mentioned the effects of bromoform and ethyl mercaptan, nothing superior to meat bait has been found. It has been demonstrated how meat bait could be improved by the addition of cystine and by inoculation with bacterial cultures. In both these instances the attractive odours produced are probably very mixed, and in the latter they were found to be very volatile. It is our opinion, and this seems to be shared by many other investigators that, while it is not impossible for a single chemical compound to supply the necessary stimulus to the flies, attractiveness is to be found in a mixture of odours.

Summary.

1. In a search for olfactory attractants to sheep blowflies, tests were conducted by means of an olfactometer in the laboratory, while some substances were tested under field conditions in traps.

2. Certain alcohols, aliphatic acids, esters, organic sulphides and inorganic salts were tested in the laboratory; boiled and unboiled solutions of sodium and potassium hydroxide were found to attract *L. cuprina,* while natural civet and musk ketone were weakly attractive. Sodium bicarbonate solution was repellent. Strong repellents are Dippel's oil, carvone, and linalool.

3. Preparations of suint were found in general to be unattractive.

4. The chemical treatment of meat bait by the addition of cystine, calcium carbonate, calcium sulphide, sodium carbonate, sodium sulphide and phenothiazine, enhanced its attractiveness.

5. Inoculation of meat bait with a mixed culture of bacteria from sheep's intestines increases attractiveness.

6. Fermenting baits, e.g., fish meal, pancreatin and egg, and addled eggs, proved to be attractive, but they were not so attractive as meat baits.

7. Some of the attractive substances of beef bait were extracted by ethyl ether, but these chemicals were not isolated or identified. A portion of these attractive substances were apparently removed from the ether solution by potassium hydroxide solution.

8. Flowers of *Stapelia flavirostris* are strongly attractive to, and stimulate oviposition by *Lucilia cuprina.* Distillates of these flowers were found to be attractive but no chemicals were isolated or identified. Further investigations on the chemistry of these flowers are recommended.

9. No blowfly attractant superior to chemically treated beef bait has been found.

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