Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 18, *Numbers* 1 *and* 2, *July and October*, 1943.

Printed in the Union of South Africa by the Government Printer, Pretoria.

Sheep Blowfly Research II.—Suint Investigations.*

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Introduction.

According to the majority of the investigators, the olfactory oviposition stimulus supplied to the blowfly by the living sheep has its origin in the fleece of the sheep. Although it is realized that products of bacterial decomposition from moist parts of the skin probably are responsible for this stimulus, it has often been suggested that a correlation exists between amount and/or quality of wool suint and attractiveness to blowflies. Suint, being very hygroscopic, retains or attracts moisture, so providing the prerequisite for bacterial action in the epithelial débris and dried body exudates on the skin. Mönnig (1940) has given a concise description of the conditions that lead to blowfly strike, so that further details will be superfluous here.

Quantity of suint secreted in relation to blowfly strike has been the subject of studies by a number of research workers. The evidence has not been conclusive on the whole [*cf.* Holdaway and Mulhearn (1934) and Hobson 1936)]. Another variable that could conceivably operate in the problem of susceptibility is the composition of the suint itself, which may give rise to variable products of decomposition. These and similar considerations led to a tentative programme of suint analysis at this Institute in May, 1940, in conjunction with an extensive blowfly research programme. This suint investigation embraced two distinct projects, i.e. (1) basic work on suint in general, to discover which constituents of suint are attractive to blowflies, and (2) a qualitative and quantitative analysis of a series of fleece samples from selected examples of susceptible and non-susceptible sheep. The result of project No. (1) was to be used in No. (2), and the results of No. (2) could serve as a confirmation or otherwise of No. (1), while the information so obtained might be useful in the preparation of artificial baits for blowfiies.

Suint Constituents and Problems of Suint Analysis.

As regards the composition of wool suint, this complex problem has been submitted to systematic study by only a few investigators, notably Freney (1934). In a comprehensive survey Freney (1940) has reviewed present knowledge. Only a few salient points need, therefore, be mentioned here. It is known that the major portion of suint consists of potassium carbonate and organic compounds. The inorganic constituents have often been determined, and do not appear to vary outside certain narrow limits. This knowledge, naturally, does not enable one to indicate which inorganic compounds were excreted as such, but this is probably not of great consequence. A source of variation of inorganic constituents of wool suint that

* See footnote to article I of this series.

should always be kept in mind, is the nature of the soil of the sheep's environment. The fleece is always contaminated with dust, sand and vegetable material, which then contribute their part to the soluble alkaline solution of suint. The variation thus introduced into the chemical composition may even overshadow that due to variation between individuals.

Of the organic part of suint only a comparatively small percentage has been identified. The reason for this is not far to seek. Not only are we dealing here with a very complex mixture, the known constituents of which are mainly present in very low percentages, but probably this mixture exists in a kind of chemical equilibrium. Chemical manipulation would disturb this equilibrium, with a resultant formation or disappearance of some of the constituents, so confusing the analytical results. Furthermore, the suint solution has to be concentrated by boiling or by evaporation at an elevated temperature, a process that continues for hours and even days. This is a relatively drastic treatment where one is dealing with volatile substances present in very low concentrations. The probability that at this stage the suint is still identical in composition with that existing in the fleece, appears to be slight. In addition, it is quite possible that the water-soluble ingredients of fleece samples that are to be stored in not too dry an atmosphere will not remain constant over extended periods. The products of possible bacterial and oxidative changes may affect the problem one way or another [*cf.* Freney (1940)].

A further important consideration is that a large number of the known organic constituents of suint are homologous or otherwise inter-related compounds (e.g., the fatty acids), all of which together constitute only a small fraction of the whole mixture. Granted a successful isolation of this complex, further separation of it into its individual constituents is a most formidable task with the quantities likely to be available after isolation. If, further, one considers what this implies when analysing individual fleece samples (of about 10 to 15 grammes), for some definite suint constituent, the nature of the problem confronting one is perhaps better appreciated.

Investigation of Suint.

Freney (1937) found slight indications of attractiveness to *Calliphora augur* of butyric and valeric acids, whereas Parman *et al* (1927) had found n-valeric acid to be slightly attractive to a different blowfly the screw-wormfly *Cochliomyia macellaria*, but n-caproic and n-caprylic acids were repellent to this fly. Laake *et al* (1931), on the other hand, found butyric aldehyde to be slightly attractive to *Lucilia* spp. Freney (1937), again, found a mixture of sodium caproate and sodium sulphide to attract *Lucilia cuprina*, whereas sodium sulphide alone was unattractive. These results do not appear very convincing when the actual figures are examined, but they seemed to justify further investigation. If it could be shown that butyric, valeric and caproic acids are present in suint to any significant extent, a correlation between suint composition and blowfly strike may be shown to exist. With this end in view it was decided to concentrate initially on the study of the organic acids in the fleece.

At the outset attention was devoted to the different methods of direct extraction of raw wool. It was desirable that in the course of the extraction and concentration of the wash liquors, the suint constituents should be subjected to conditions as mild as possible. High temperatures for continued periods as well as a long series of manipulations and treatments

appeared undesirable, for reasons set out above in the section on problems of suint analysis. Any direct extraction of the constituents concerned was, therefore, to be preferred. Experimentation with various methods of wool extraction soon demonstrated the chief difficulty to be encountered, viz., the bulkiness of wool and its absorbing powers for solvents. These properties entailed special measures for an efficient removal of any residual solvent after washing, otherwise an important percentage of the original quantity would not be recovered, so wasting solvent as well as dissolved substances. With water as the extraction medium the first-named factor is of no account, but the last-named is a very important consideration. As large quantities of wool had to be extracted for the present purpose, the washing of small samples at a time was impracticable. The use of larger quantities of solvent, as in repeated washing or rinsing, was also inadvisable because of the difficulties of filtration and concentration. A technique that appeared to suit the purpose admirably was that developed by Mr. S. D. Rossouw, of the Wool Section of this Institute, a modification of the procedure described by Rossouw (1938). This consists of consecutive Soxhlet extractions of absolutely dry wool with petroleum ether, absolute alcohol and water, all under reduced pressure. Any moisture in the wool would interfere with the desired extraction by removing some of the water-soluble constituents on being syphoned over with the petroleum ether. Alternatively, in the extraction with absolute alcohol, moisture would carry into solution some substances otherwise insoluble in absolute alcohol. This would result in misleading extractions, so that working with absolutely dry materials is imperative. Wool and suint are both extremely hygroscopic, therefore it calls for careful manipulation and special facilities to achieve satisfactory results.

This procedure would bring about the extraction of all the potassium salts of organic acids in the fleece by the alcohol. The desired separation would thus be effected by comparatively mild and simple means. Preliminary extractions according to this technique showed, however, that extractions would have to be performed with large quantities of wool. The available apparatus did not allow of this; moreover, owing to war-time conditions, suitable apparatus could not be procured. The alternative course, viz., direct extraction by means of water alone, precludes most of these difficulties, and for the present purpose it did not appear that serious objections could be raised in this respect. Freney (1940), in his discussion of various methods of fleece analysis, concluded in a similar comparison of two methods resembling the two under discussion, that neither of the two could be wholly rejected in favour of the other. The circumstances, as well as the purpose for which the investigations are undertaken, must determine the procedure to be adopted. Once the choice has fallen on a suint extraction by means of water, it also entails the concentration of the relatively dilute suint solutions for analytical purposes.

The suint used in the majority of these experiments was derived from merino lox, a soiled type of wool containing a relatively high percentage of water-soluble substances. This wool was sampled at random from a number of merino fleeces. A few extractions were also made of the whole fleece of individual sheep, but this practice was ultimately discontinued.

In the course of the extraction of suint from the wool, a number of difficulties had to be overcome. To collect as much suint as possible it was desirable to scour the maximum amount of wool in a limited quantity of

water. The water was kept at 50° C. The scouring liquor was of a fairly "thick" consistency and offered considerable difficulty in filtration (a more dilute scouring liquor does not filter with any greater ease). The time required for filtration was sufficient to allow bacterial changes to occur in the liquor. As this bacterial action was also liable to affect the acidic components in the liquor, there was every reason for accelerating the process as far as possible. The filtrate had to be concentrated by evaporation either by boiling or by heating on a hot water bath kept at 70° C. This was a time-consuming process. On cooling, the concentrate was subjected to further treatment by one of the four methods described below.

The extraction by means of organic solvents was usually complicated by the formation of relatively stable emulsions. This was overcome by centrifuging, if possible, although the small capacity of the available centrifuge limited its use considerably.

The following methods of isolation of the organic acids were investigated.

1. That described by Rimington and Stewart (1932), where an ethyl alcohol solution of the acids liberated by acidification of the suint is salted out by means of ammonium sulphate.

2. That of Lassar-Cohn (1923), where an ethyl alcohol solution of the potassium salts of the organic acids is salted out with potassium carbonate.

3. Extraction of the acidified suint by means of solvents like ethyl ether.

4. Steam distillation of acidified suint for the recovery of the volatile acids in the distillate.

These different methods all yielded suprisingly small amounts of organic acids. In the case of numbers 1 and 2 the alcohol extracts naturally contained other organic material as well; in particular was that evident in Lassar-Cohn's method, where wax-like substances were soon precipitated on concentration of the original alcohol solution. In every case the amount of total acids derived from the extracts was equivalent to less than 20 ml. tenth-normal potassium hydroxide. The weight of raw wool concerned was roughly 500 grammes in each case. A few details concerning the results with each method follow below.

Method No. 1.

Although this method appeared to yield the best proportion of acids, these acids could not be shown to be mainly of low molecular weight. Even steam distillation of part of the extract did not yield volatile acids in any appreciable quantities, and this distillate still gave a precipitate with zinc acetate, indicating the presence of fatty acids higher than valeric acid. The major part of the acid complex appeared, from acid value determinations, to consist chiefly of oleic acid.

Method No. 2.

This method of extraction yielded an alcoholic solution of potassium salts of various organic acids. On evaporation of the alcohol the reddish-brown residue was once more taken up in absolute alcohol, but a considerable portion would not re-dissolve. The alcohol-soluble portion was again freed of alcohol, and the residue taken up in water. This solution gave a reddish-brown, gelatinous precipitate with barium nitrate solution; on filtering what

appeared to be a voluminous precipitate it was found that it amounted to hardly more than a trace. Acidification of this precipitate and subsequent extraction with ether gave an ultimate small residue having a strong, " rancid " odour.

A portion of the aqueous solution above was acidified with sulphuric acid, and shaken out, successively, with petroleum ether and ethyl ether. The residues from these last two extracts were weighed, and the acid values determined, viz., 126 for the petroleum ether and 183 for the ether extract. These values showed that we were dealing with mixtures of higher fatty acids with non-acid substances; there was no evidence of the presence of any of the lower fatty acids, oleic acid being probably the chief constituent. When these petroleum ether and ethyl ether extracts were neutralized with KOH, evaporated to dryness, washed with benzene and afterwards taken up in water acidified with sulphuric acid, steam distillation did not effect any separation of volatile fatty acids. Traces of a white, waxy solid floating on the distillate of the ethyl ether extract was identified as benzoic acid (cf. below). This acid was obviously derived from the hippuric acid known to be in suint. It is possible that traces of some of the intermediate fatty acids, e.g., capric and lauric, also occurred in the distillate, but that could not be demonstrated.

Method No. 3.

The extraction of acidified suint solutions with ethyl ether or petroleum ether was not accomplished without difficulty, the chief obstacle being the ease with which the liquids were emulsified. When dealing with dilute suint extracts these emulsions could be broken by appropriate manipulation, but stronger suint extracts defied any ordinary treatment. Ethyl ether was more prone to this tendency than petroleum ether, but, on the other hand, ethyl ether appeared to be much more desirable for the efficient extraction of lower fatty acids.

The extracts thus derived from suint did not provide sufficient material for further investigation. Accordingly, attention was turned to the possibility of obtaining more promising extracts by collecting bigger quantities of suint in an absolutely dry state. This was successively extracted with petroleum ether, absolute alcohol and water. These extracts were then worked up for acid constituents, but in no case could an appreciable amount of acids be separated. The present facilities for the rapid handling of large quantities of suint extracts (scouring liquors) were not available at the time, so that this method was eventually abandoned for the one about to be described.

Method No. 4.

It was hoped that by distillation a quicker separation, of the volatile acids at least, would be effected than by other methods. A large amount of very concentrated suint solution (rather more like a thin paste) was therefore steam-distilled after acidification with sulphuric acid. For various reasons a modification was soon introduced, i.e., the distillation was carried out at reduced pressure, so that it actually was not a steam distillation any more. This arrangement appeared more satisfactory, as the escaping gases or vapours were drawn through an alkaline solution to absorb any acidic vapours. A further modification of this technique was finally adopted when

raw wool was used directly for the distillation, instead of a suint extract. The wool was soaked thoroughly in water in a large distillation vessel, the pH was adjusted to about 3·0 by means of sulphuric acid, and distillation commenced. In this way the long and tedious process of suint extraction was eliminated.

The various distillates obtained by this method were tested for acidity. Some were extracted by means of ether, after an initial concentration by evaporation in alkaline medium. The odour of these distillates strongly reminded one of the smells of a sheep's kraal. The only acid, however, that was present in appreciable quantities, was a white waxy solid floating on the distillate. This acid appeared responsible for the peculiar odour of the distillates. Further purification, however, revealed it to be benzoic acid. No other acid could be identified in any of the distillates.

Some of the concentrates of the acids from suint extracts were tested by Mr. G. A. Hepburn in a special olfactometer (see paper No. 3 of this series), in order to determine the olfactory value for the sheep blowfly. The benzoic acid collected from the distillates above was also given a preliminary test in an olfactometer. This preparation was still in a crude state, with some of the " sheep " odour still adhering to it. In none of these cases was a promising indication found. The absence of any response on the part of the flies did not stimulate the belief in the presence of one or more attractive substances in sheep's suint.

Discussion and Conclusions.

The appearance of benzoic acid in the distillates of suint was confirmed by more than one method of separation. In every case the distillate was derived from a strongly acidified solution of suint products. Hippuric acid has been identified in suint by various workers, so that the appearance of benzoic acid in the distillate is probably the result of hydrolysis of hippuric acid in a boiling acid medium, with a subsequent volatilization in steam.

With regard to the olfactory tests carried out with some of these suint products, it should be borne in mind that there was a gradual evolution of the olfactometer as used at present (see contribution No. 3 of this series). Any tests undertaken, therefore, had to depend on the concurrently used apparatus. Thus it is possible that some of the results of tests with the earlier type of olfactometer are misleading. Re-testing of some of the suint products may, therefore, be desirable. Owing to the fact that the writer has also been implicated in other concurrent projects pertaining to the blowfly problem, it has not been possible to do any re-testing up to the present. With the sudden termination of this research programme it has now been found impossible to repeat this work.

It was stated in the introduction that fundamental work on suint would be followed up by a systematic analysis of individual wool samples from susceptible and non-susceptible sheep. This aspect of the investigation naturally depended on the first part. In collaboration with this Institute, Mr. A. H. de Vries, entomologist at the Grootfontein College of Agriculture, Middelburg, C.P., regularly collected a large number of fleece samples from specially selected groups of sheep, with this object in view, i.e., of investigating the problem of susceptibility in sheep in relation to the amount of suint, or to the amount of some attractive constituent of suint. As no advance was made with the search for an attractive suint ingredient, the

investigation of the fleece samples was delayed. At the termination of this work, therefore, nothing has been attempted in that respect. A quantitaive analysis of these fleece samples for total suint alone, may perhaps be profitable. The technique employed by Freney (1940) should be well suited to this purpose.

In conclusion the following may be said:—

1. Attempts have been made to isolate and estimate some of the organic acids, chiefly lower fatty acids, said to be present in small quantities in suint. Apart from the identification of benzoic acid as a decomposition product of hippuric acid, a satisfactory separation of these acids could not be achieved, owing to the small quantities present.

2. In olfactory tests, suint preparations and extracts (e.g. acid fractions) failed to attract blowflies. No correlation between suint composition and blowfly strike could, therefore, be demonstrated.

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