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Degradation of Protein in the Rumen of the Sheep

2. THE ACTION OF RUMEN MICRO-ORGANISMS ON AMINO-ACIDS

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In the previous paper (el-Shazly, 1952) evidence that ammonia is formed from protein in the rumen was reviewed and it was suggested that some of the volatile fatty acids (VFA's) found in the rumen originate mainly from the breakdown of protein. To investigate more closely the character of this breakdown the action of rumen micro-organisms on free amino-acids has now been studied. Only low concentrations of free amino-acids exist in the rumen liquor, but there is evidence (Sym, 1938) of protease activity by the micro-organisms; McDonald (1948) has measured the disappearance of zein from the rumen and Chalmers & Synge (to be published) have observed the dissolution of lumps of denatured casein. The nature of this proteolytic action, which is likely to prove of great importance for the protein economy of the animal, must await more detailed study. The present work has been concerned solely with the fate of the free amino-acids which it is reasonable to assume are liberated in the proteolysis.

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The best course would have been to study the fate of amino-acids added in low concentrations to whole rumen liquor within a very short time of its removal from the animal, but this was not technically feasible. It was, however, possible to observe the disappearance of somewhat high concentrations of amino-acids added to freshly removed rumen liquor during anaerobic incubation for a few hours; the high concentrations of ammonia and VFA's already present prevented detailed study of new formation of these products from the added amino-acids. However, following recommendations of Dr S. R. Elsdon, conditions were found for using washed suspensions of mixed rumen micro-organisms which attacked amino-acids present in mixtures at much the same relative rates as these were attacked in whole rumen liquor. Under these circumstances it was relatively easy to determine the end products of the fermentation and to construct a rough balance sheet for the reactions. Although the reactions may take a different course under physiological conditions (where the amino-acid concentrations are lower, other essential nutrients for the micro-

organisms are present in greater variety and other fermentations, e.g. of carbohydrate, may be proceeding) the results with washed suspensions probably give a fair idea of the physiological fate of part of the protein destroyed in the rumen.

Many workers have studied the attack by micro-organisms on amino-acids administered singly. For the present purpose this did not seem to be a good approach, since under physiological conditions many different amino-acids must become available to the micro-organisms simultaneously. Moreover, as Stickland (1934) first showed, interactions between amino-acids may completely modify the course of fermentation. Such interaction was, in fact, demonstrated in the course of the present work; accordingly, for most of the experiments an acid hydrolysate of casein was used to supply the mixed amino-acids. This protein was chosen because much of the work of my colleagues Dr M. I. Chalmers, Dr D. P. Cuthbertson and Dr R. L. M. Syngé has involved the administration of casein supplements to sheep.

EXPERIMENTAL

Materials and general methods

The mixture of amino-acids usually employed was a casein hydrolysate (vitamin-free; Ashe Laboratories Ltd., London). Filter-paper chromatography of amino-acids was as described by Syngé (1951*b*) using phenol/collidine but omitting H_2O_2 unless otherwise specified. NH_3 was determined as in the previous paper (el-Shazly, 1952).

Behaviour of free amino-acids on incubation with rumen contents

Rumen-liquor samples, collected from sheep on a diet of casein, concentrates and hay (el-Shazly, 1952; Table 1, Exp. 2) in the morning before feeding, were strained through muslin and saturated with CO_2 . Four 20 ml. portions were then taken in boiling tubes *A*, *B*, *C* and *D*. Tubes *C* and *D* contained casein hydrolysate (8.1 mg. N) previously evaporated to dryness. After mixing, a layer of liquid paraffin B.P. was pipetted on to the contents of all four tubes; tubes *B* and *D* were incubated for 3 hr. at 40°. Tubes *A* and *C* were analysed forthwith. Analysis of the aqueous phase from all four tubes was as follows:

After cooling to 0°, 10 ml. portions were dialysed in washed cellophan tubing for 24 hr. at 0° against 30 ml. of water. NH_3 was determined on portions of the diffusates. Other portions were acidified to pH 1 with H_2SO_4 , steam-distilled to remove VFA's and desalted according to Consden, Gordon & Martin (1947); two-dimensional filter-paper chromatograms of the amino-acids present were then prepared.

The NH_3 determinations showed increases of NH_3 -N (differences between tubes *B* and *D*) on incubation of about 15–25 mg./100 ml. rumen liquor taken, i.e. 35–65% of the N of the casein hydrolysate appeared as NH_3 ; these figures are of necessity rough owing to the considerable amounts of NH_3 initially present and formed during incubation without added casein hydrolysate. The filter-paper chromatograms

from tubes *A* and *B* showed only faint traces of amino-acids. All those of the original casein hydrolysate were seen with *C*, while with *D* it was seen that phenylalanine, tyrosine, and proline had completely disappeared; alanine, threonine, glycine, serine, and aspartic acid were very weak or absent; glutamic acid was considerably weakened while the remaining spots were relatively strong, although obviously weaker than the corresponding spots from *C*.

A new spot always appeared, corresponding in position and colour on the phenol/collidine chromatograms with γ -aminobutyric acid, δ -aminovaleric acid, etc. Using phenol/*n*-butanol-acetic acid chromatograms (cf. Syngé, 1951*a*) this spot corresponded with δ -aminovaleric acid, and γ -aminobutyric acid, etc., were thus shown to be absent (mixed chromatograms with authentic materials). On incubating rumen liquor as above, but with only L-proline (7.4 mg.) added, there was no obvious disappearance of proline, nor was any δ -aminovaleric acid formed. On incubation with L-proline (7.4 mg.) and DL-alanine (8.3 mg.), the alanine spot was weakened, the proline spot almost disappeared and a strong spot corresponding with δ -aminovaleric acid was produced. On incubation with the same amount of DL-alanine alone, the alanine spot was weakened but no new spot appeared.

In the course of these experiments indications were obtained that some added material or reaction product inhibited formation of NH_3 , since if the amounts of casein hydrolysate added to the rumen liquor were increased on that stated above, the proportion of deamination on incubation was diminished. Control experiments showed that this effect was not due to NH_3 .

Isolation and identification of δ -aminovaleric acid formed by Stickland reaction (cf. Stickland, 1935; Woods, 1936). Rumen liquor from a sheep on a similar diet (el-Shazly, 1952; Table 1, Exp. 7), collected before feeding, was strained through muslin and 400 ml. were incubated with 127 mg. of L-proline and 140 mg. of DL-alanine at 40° for 3 hr. in a closed vessel; N_2 containing 5% (v/v) of CO_2 was bubbled through. The mixture was dialysed in cellophan tubing against 2 l. of water for 48 hr. at 0°. The diffusate was acidified to pH 1 with H_2SO_4 , concentrated to small volume *in vacuo* and then steam-distilled to complete removal of VFA's. $Ba(OH)_2$ was added to bring the residue to pH 4. $BaSO_4$ was removed by filtration and the filtrate was desalted in the apparatus of Consden *et al.* (1947). A filter-paper chromatogram showed that proline had completely disappeared, being replaced by a strong spot in the position of δ -aminovaleric acid. Small amounts of alanine, valine, leucine, phenylalanine, etc., were present. The preparation was evaporated to dryness *in vacuo* and fractionated on a cellulose-powder chromatogram (140 g., 5.4 cm. diam.) using the system *n*-butanol-water-acetic acid (4:5:1 by vol.) as for the isolation of γ -aminobutyric acid (Syngé, 1951*a*). The effluent fractions containing δ -aminovaleric acid were pooled and evaporated to dryness *in vacuo*; the residue was dissolved in water and on filtration and evaporation yielded 205 mg. of a clear gum. Two successive crystallizations from ethanol-ether yielded 85 mg. of pale-yellow crystals having m.p. 154° (uncorr.). The product did not, on admixture, depress the m.p. (156–158°) of authentic δ -aminovaleric acid prepared according to Wallach (1900) from cyclopentanone oxime (cf. Fox, Dunn & Stoddard, 1941). In two-dimensional filter-paper chromatograms (phenol/*n*-butanol-acetic acid) the material gave a single

spot with or without admixture of authentic δ -aminovaleric acid. $[\alpha]_D^{25.5} + 1^\circ$ in water (*l*, 0.5; *c*, 7.0). (Found: C, 50.4; H, 9.2; N (Kjeldahl), 11.7. Calc. for $C_8H_{11}O_3N$: C, 51.3; H, 9.4; N, 11.9%.) In the Van Slyke amino-N reaction (30 min. at 21°) 94% of the N reacted (for previous data see Syngé, 1951*a*). In the ninhydrin- CO_2 reaction according to Van Slyke, Dillon, MacFadyen & Hamilton (1941), using citrate buffer (pH 2.5), the result was completely negative. A very slight reaction in the amino-N determination according to Pope & Stevens (1939) was given equally by the synthetic material. The isolated material behaved in electrical migration experiments as an ω - rather than an α -amino-acid (cf. Syngé, 1951*a*).

with and without casein hydrolysate as described above except that N_2 (freed from O_2 by passing over hot Cu) rather than liquid paraffin was used for securing anaerobic conditions. NH_3 was determined before and after incubation. The results (Table 1) show close agreement in the amounts of NH_3 produced from the amino-acids added. Filter-paper chromatograms were likewise run on the incubated mixtures. With the washed suspensions phosphate was removed by precipitation in the presence of $CaSO_4$ and NaOH before desalting; the steam distillation was omitted as only small amounts of VFA's were present. The chromatograms likewise showed closely similar results in the extent to which the various amino-acids were attacked, the only noticeable

Table 1. *Formation of ammonia from amino-acids by rumen liquor and by washed suspensions derived therefrom*

(For details see text. Exps. 1 and 2 were with rumen liquor obtained on different days from a sheep on a casein-concentrate-hay diet (el-Shazly, 1952; Table 1, Exp. 2).)

	Ammonia N (mg./100 ml.)			Difference attributable to casein hydrolysate
	Before incubation	After incubation	Difference	
Exp. 1				
Rumen liquor	14.7	27.6	12.9	28.1
Rumen liquor + casein hydrolysate (81 mg. N/100 ml.)	16.4	57.4	41.0	
Washed suspension	2.8	6.2	3.4	23.7
Washed suspension + casein hydrolysate (81 mg. N/100 ml.)	2.6	29.7	27.1	
Exp. 2				
Rumen liquor	14.2	19.7	5.5	19.6
Rumen liquor + casein hydrolysate (40 mg. N/100 ml.)	15.8	40.9	25.1	
Washed suspension	2.0	4.7	2.7	14.5
Washed suspension + casein hydrolysate (40 mg. N/100 ml.)	3.5	20.7	17.2	

Experiments with washed suspensions of micro-organisms from rumen liquor

Preparation of washed suspensions. Use of simple buffer solutions during centrifugation gave preparations having very low deaminating power. The addition of Na_2S as recommended by Dr S. R. Elsdén (cf. Johns, 1951) gave, however, satisfactory preparations. Rumen-liquor samples, collected from the sheep before feeding in the morning, were cooled to 0° , strained through muslin, centrifuged very lightly to remove plant particles and Protozoa, and then centrifuged 15 min. at 17000 rev./min. on the high-speed attachment head (previously cooled to below 0°) of a 'MSE Major' centrifuge (Measuring and Scientific Equipment Ltd., London S.W. 1). The resulting precipitate was resuspended to the original volume with 0.033 M-potassium phosphate buffer (pH 6.5) containing 0.02% (w/v) $Na_2S \cdot 9H_2O$. Centrifugation was repeated and the precipitate was resuspended with the same potassium phosphate buffer (without added Na_2S), and used immediately for the incubation experiments as described below.

Comparison of attack on amino-acids by washed suspensions and by rumen liquor. Washed suspensions, and the rumen liquor from which they had been prepared, were incubated

Table 2. *Formation of ammonia from amino-acids by washed suspensions from the rumen of sheep on different diets*

(Portions (5 ml.) of washed suspension incubated with casein hydrolysate (1.8 mg. N) for 3 hr. at 40° under nitrogen.)

	Ammonia N (mg./100 ml.)		
	Sheep 1	Sheep 2	Sheep 3
(a) Diet: casein, concentrates, hay:			
Before incubation	1.5	2.2	3.3
After incubation	17.6	35.4	30.2
Increase	16.1	33.2	26.9
(b) Diet: hay only:			
Before incubation	2.9	2.6	2.8
After incubation	6.2	5.2	4.0
Increase	3.3	2.6	1.2
(c) Diet: fresh grass only:			
Before incubation	—	3.1	3.3
After incubation	—	25.2	23.3
Increase	—	22.1	20.0

difference being that glutamic acid was not much attacked by the washed suspensions.

Deaminating power of washed suspensions from sheep on different diets. Three sheep received during successive 3-week periods diets as indicated by el-Shazly (1952; Table 1). (a) A concentrate-casein-hay diet (as in Exps. 7 and 8); (b) an all-hay diet (as in Exp. 1); (c) a fresh-grass diet (as in Exps. 5 and 6) and had rumen-ammonia curves similar to those recorded. At the end of each 3-week period deamination on incubation of casein hydrolysate with washed suspensions as described above was studied, with the results shown in Table 2.

Quantitative study of reaction products formed from casein hydrolysate by washed suspensions of rumen micro-organisms

Attempts were made to construct balance sheets for the fermentations studied. Determinations were made, before and after incubation, of: (a) ammonia, as described above; (b) α -amino-acid N of free amino-acids by the ninhydrin- CO_2 procedure (Van Slyke *et al.* 1941) using citrate buffer at pH 2.5; (c) ether-extractable acids and VFA's by the techniques used by el-Shazly (1952); (d) gaseous exchanges during incubation, in Warburg manometers as described below. Not all determinations were done in each experiment. Rumen-liquor samples were from animals on a casein-concentrate-hay diet as above, taken before feeding.

Exp. 1. Portions of washed suspension (7.5 ml.) were incubated with casein hydrolysate (2.83 mg. N) for 3 hr. at 40° under N_2 ; the micro-organisms were then centrifuged down at high speed and suitable portions of the clear supernatants analysed. Parallel analyses were done on unincubated mixture.

Exps. 2-6. These were done with 2.7 ml. portions of washed suspension incubated with casein hydrolysate (2.6 mg. N dissolved in 0.3 ml. of the same phosphate buffer) in Warburg manometers under N_2 or A. With KOH solution in the centre compartment to absorb CO_2 , the course of

Table 3. *Balance sheet of action of washed suspensions on casein hydrolysate*

(Casein hydrolysate incubated at 40° for times shown. For experimental details see text.)

	mg. atoms or m-moles/100 ml. suspension		
	Before incubation	After incubation	Change
Exp. 1 (incubation time 3 hr.):			
α -Amino-acid N	2.03	0.60	-1.43
NH_3	0.47	1.48	+1.01
VFA's	0.31	0.88	+0.57
Non-volatile ether-extractable acids (mg. equivs.)	0.09	0.07	-0.02
Exp. 2 (incubation time 1.75 hr.):			
α -Amino-acid N	1.99	1.68	-0.31
NH_3	0.11	0.43	+0.32
CO_2	—	—	+0.30
Exp. 3 (incubation time 1.75 hr.):			
α -Amino-acid N	1.98	1.54	-0.44
NH_3	0.09	0.56	+0.47
Total ether-extractable acid (mg. equivs.)	0.37	0.74	+0.37
CO_2	—	—	+0.39
Exp. 4 (incubation time 1.75 hr.):			
α -Amino-acid N	1.65	1.36	-0.29
NH_3	0.09	0.40	+0.31
Total ether-extractable acid (mg. equivs.)	0.07	0.25	+0.18
CO_2	—	—	+0.35
Exp. 5 (incubation time 2 hr.):			
NH_3	0.11	0.79	+0.68
VFA's	0.41	1.03	+0.62
CO_2	—	—	+0.56
Exp. 6 (incubation time 2 hr.):			
NH_3	0.13	0.59	+0.46
VFA's	0.30	0.73	+0.43
CO_2	—	—	+0.49

Table 4. *Volatile fatty acids formed on incubation of casein hydrolysate with washed suspensions of rumen micro-organisms*

(Figures in brackets indicate the percentage of total increase contributed by each acid.)

Acid	mg. equiv./100 ml.								
	Exp. 1 (Table 3) (Elsden technique)			Exp. 5 (Table 3) (James & Martin technique)			Exp. 6 (Table 3) (James & Martin technique)		
	Before incuba- tion	After incuba- tion	Increase	Before incuba- tion	After incuba- tion	Increase	Before incuba- tion	After incuba- tion	Increase
Acetic	0.263*	0.680*	0.417* (73.4%)	0.278	0.461	0.183 (29.5%)	0.117	0.450	0.333 (78.5%)
Propionic	0.018	0.080	0.062 (10.9%)	0.053	0.356	0.303 (48.9%)	0.097	0.143	0.046 (10.8%)
isoButyric } n-Butyric }	0.010	0.055	0.045 (7.9%)	{0.000 0.033	{0.030 0.100	{0.030 (4.8%) 0.067 (10.8%)	0.000	0.007	0.007 (1.6%)
Branched C ₅ }	0.022	0.066	0.044 (7.7%)	{0.020 0.025	{0.050 0.045	{0.030 (4.8%) 0.020 (3.2%)	0.018	0.034	0.016 (3.8%)
n-Valeric							0.022	0.018	—
Total	0.313	0.881	0.568	0.410	1.030	0.620	0.300	0.725	0.425 —

* By difference.

the reaction (judged by amino-acid consumption and NH_3 production) was no different from that when CO_2 was allowed to accumulate. No significant output of H_2 or CH_4 or uptake of N_2 or A was observed. CO_2 output was determined by measuring the difference in gas output in manometers (not having KOH) on tipping in 0.2 ml. 10N- H_2SO_4 from the side arms at the beginning and end of incubation respectively.

The results are given in Table 3. In general the rough equivalence of moles ammonia, carbon dioxide and VFA's produced to moles amino-acid destroyed is striking.

Distribution of volatile fatty acids. In Exps. 1, 5 and 6 the distribution of VFA's was ascertained chromatographically (for techniques see el-Shazly, 1952), and is given in Table 4. It is seen that, while acetic and propionic acids predominate, the proportions of C_6 isomers present tend to exceed those usually found in rumen liquor.

DISCUSSION

No information has yet been gained as to which of the micro-organisms are responsible for bringing about the decompositions observed. The present experiments show, however, that the power of mixed rumen organisms to decompose amino-acids to ammonia and other products is strikingly increased by increasing the content of soluble protein in the diet. On microscopic examination of the different populations Miss Marjorie Masson found no correspondingly striking changes in the morphology or staining properties of the micro-organisms present. The very slight deamination obtained with washed suspensions from hay-fed animals serves as a control, suggesting that selective growth of deaminating organisms during the incubations probably did not intervene to alter greatly the proportions in which the organisms occurred from those existing in the rumen.

The chemical changes resulted mainly in the formation of VFA's, ammonia and carbon dioxide. The virtual absence of hydrogen, gaseous hydrocarbons and other ether-extractable organic acids was demonstrated. Formation of alcohols and neutral carboxyl compounds was not excluded. The only other nitrogenous fermentation product detected was δ -aminovaleric acid, which was shown experimentally to arise from proline (cf. Stickland, 1934, 1935) and may also have arisen from arginine via ornithine (Woods, 1936). The reaction products observed were, in fact, strikingly similar to those reported by previous workers for the anaerobic decomposition of individual amino-acids by a variety of micro-organisms. Cohen (1949) has given an excellent review of this subject, in which the experimental evidence of the importance of mutual oxidation-reduction reactions of the Stick-

land type is set out. Such a reaction between alanine and proline was demonstrated in the present work, and the resulting δ -aminovaleric acid was isolated and characterized. Further, from the direct evidence obtained by the procedure of James & Martin (1952) that branched-chain C_4 and C_5 VFA's are formed, it is reasonable to postulate that these arise from valine, leucine and isoleucine acting as hydrogen donors in reactions similar to those studied in detail by Cohen, Nisman and their colleagues at the Institut Pasteur (Cohen-Bazire, Cohen & Prévot, 1948*a, b*; Nisman, Raynaud & Cohen, 1948*a, b*; Nisman & Thouvenot, 1948; Nisman & Vinet, 1949*a, b*, 1950; Rosenberg & Nisman, 1949*a, b*; Saissac, Raynaud & Cohen, 1948).

It is only in relatively few cases that evolution of hydrogen has been detected in amino-acid fermentations (cf. Woods & Clifton, 1937, 1938; Woods & Trim, 1942; Cardon & Barker, 1947). In the present fermentations of amino-acid mixtures by mixed organisms the availability of readily reduced amino-acids such as glycine and proline may have encouraged reactions of the Stickland type and prevented the appearance of molecular hydrogen or methane as reaction products. Different reducible substances may well also operate when carbohydrate is being simultaneously fermented.

The rapid and complete disappearance of tyrosine and phenylalanine should be noted. The present data do not make it clear whether they were assimilated or fermented by the micro-organisms, but it is interesting that Cohen (1949) reports a complete absence of data on anaerobic fermentation of phenylalanine or lysine. The latter did not rapidly disappear under the conditions of the present work.

The predominance of acetic acid among the resulting VFA's is notable. *n*-Butyric acid results in lower proportion than is normally present in the VFA's of rumen liquor. Propionic acid and *n*-valeric acid (which could conceivably arise from arginine, ornithine, proline, δ -aminovaleric acid or lysine) seem to be variable, while the branched-chain C_4 and C_5 acids are in higher proportion. These, whose probable origin from valine, leucine and isoleucine has already been discussed, are formed in fair quantity despite the relatively slow attack by the micro-organisms on these amino-acids. It is these branched-chain acids which, as previously shown (el-Shazly, 1952), are characteristic products of the breakdown of protein in the rumen under physiological conditions. The relative amounts of these acids that are absorbed from the rumen may prove to be in excess of the proportion suggested by their relative concentration in rumen liquor (cf. Johnson, 1951; Pennington, 1951). In any case their absorption and metabolic fate seem worthy of study.

Their carbon skeletons, unlike those of the corresponding α -keto acids, are probably not available for resynthesis of the corresponding 'essential' amino-acids within the mammalian body; the loss of dietary nitrogen absorbed as ammonia is thus made more certain. The branched-chain VFA's are presumably oxidized in the animal body. Considerable evidence has accumulated that they may be ketogenic; whilst there is some controversy about isobutyric and α -methylbutyric acids, all workers seem to be agreed on the ketogenicity of isovaleric acid (see Cohen, 1937; Wick, 1941; Bloch, 1944; Valdiguié & Séguélas, 1946; Atchley, 1948; Grafflin & Green, 1948; Coon, 1950; Gray, Adams & Hauptmann, 1950; Zabin & Bloch, 1950). It does not therefore seem unreasonable to postulate that branched-chain VFA's may play a part in at least some of those forms of ketosis in ruminants that have been attributed to high-protein diets. Should this speculation be substantiated, a number of possible courses for the control of these conditions would suggest themselves.

Note

It is of interest that, since these papers were written, Hansen & Shorland (1951) have described the isolation from the higher fatty acids of butter fat of branched-chain C_{17} acids which they considered to be 14-methyl- and 15-methyl-hexadecanoic acids. These could arise by successive condensation of C_2 units on to the carboxyl ends of the branched-chain C_5 acids recognized in the present work (cf. Popják, French, Hunter & Martin, 1951).

SUMMARY

1. The amino-acids of an acid hydrolysate of casein were attacked under anaerobic conditions at similar relative rates by sheep rumen contents and by washed suspensions of micro-organisms therefrom.

2. L-Proline was reduced when other amino-acids were present; the resulting δ -aminovaleric acid was isolated and characterized (cf. Stickland, 1934, 1935).

3. Using washed suspensions, the main reaction products from casein hydrolysate were ammonia, carbon dioxide and volatile fatty acids in roughly equimolecular proportions; there was no significant formation of hydrogen, methane or non-volatile organic acids. The volatile fatty acids were straight- and branched-chain C_2 to C_6 acids.

4. The power of washed suspensions of rumen micro-organisms to deaminate amino-acids depended on the diet of the animal.

5. The probable origin of the branched-chain acids by Stickland reactions from valine, leucine and isoleucine, and the nutritional significance of the deamination reactions for the animal are discussed.

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The Composition of the Honeydew Excreted by Australian Coccids of the Genus *Ceroplastes*

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The coccids included in the genus *Ceroplastes* are popularly known as 'wax scales' from the large amount of waxy secretion covering the adult female. Froggatt (1921) recognized three species as occurring in Australia, namely, *C. ceriferus* And., *C. rubens* Marshall and *C. rusci* L. A fourth species, *C. floridensis* Comst., is recorded in the catalogue of Fernald (1903) as occurring in Australia, and Froggatt remarks that if this is the case it is a rare and comparatively unknown coccid. Later (Zeck, 1932) it was shown that another species, *C. destructor* Newstead, is of wide distribution and that it was formerly confused with *C. ceriferus*. All these coccids are considered to be introduced species, although *C. rubens* was originally described from Australia (Froggatt, 1921).

These wax scales are pests and occur in tropical and subtropical regions. *C. ceriferus* (Indian white wax scale), although widely distributed, only infests a few garden plants and native shrubs, whereas *C. destructor* (African white wax scale) and *C. rubens* (pink or red wax scale) are pests of citrus trees and they also attack many other introduced and native trees and shrubs. *C. rusci* (a white wax scale), similar to *C. ceriferus* though smaller, has been reported as occurring on melaleucas growing on the far north coast of New South Wales and on the Queensland coast. Honeydew is produced by the insects, often in large quantities, and sooty moulds, which develop in association, may cover the leaves, stems and fruit upon which the secretion falls.

Little is known about the composition of the honeydew secreted by coccids, and nothing appears to have been published on the honeydew produced by the genus *Ceroplastes*, although Fraser (1937) has reported briefly on some results obtained with

material supplied from the early stages of this work. The coccid *Trabutina mannifera* feeding on tamarisk in Sinai produces a secretion (manna) which contains 55% sucrose, 25% invert sugar and 19.3% dextrin (Weber, 1930). Gontarski (1940) records the composition of the honeydew secreted by *Physokermes piceae* Schr. (*Lecanium hemicryphum*) as containing no fructose, 11.4% water, 28.1% dextrin, 14.3% glucose and 35.8% sucrose, and that secreted by *L. quercus* (L.) Burmeister as containing 16.3% water, 11.1% dextrin, 43.7% glucose and 39.9% sucrose. More information is available on the composition of the honeydew produced by another hemipterous family of the suborder Homoptera, namely, the family Aphididae (Michel, 1942; Molliard, 1931; Mawson, 1948). However, except for the paper by Molliard, no reference is made to the presence of sugar alcohols. Molliard reports that the excretion of *Aphis euonymi* Fabr. feeding on *Euonymus europaeus* L. contains quantities of dulcitol which are apparently obtained from the leaves of the host and pass through the insect unchanged; sugars are also present.

EXPERIMENTAL

Separation of the honeydew

From Ceroplastes destructor. A mixture of insects, wax and honeydew was collected from the branches of infested lemon trees and most of the honeydew separated by pressing and draining. The remainder of the honeydew was obtained after solution of the wax in CHCl_3 . Material collected from the Dural district of New South Wales yielded 80.6% honeydew, 7.0% insect bodies and 12.4% CHCl_3 -soluble material; that collected from the Gosford district of New South Wales yielded 85.8% honeydew, 1.7% insect bodies and 12.5% CHCl_3 -soluble material. While the material was