CREWTER, W. G. & McQUADE, A. B. (1955). *J. gen. Microbiol.* 12, 311-313

The Intestinal Microflora of the Clothes Moth Larva *Tineola bisselliella* in relation to Wool Digestion

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SUMMARY: The intestines of clothes moth larvae, *Tineola bisselliella*, examined usually contained very few micro-organisms; this may be related to the high pH values of the intestinal contents. It was concluded that bacteria play no part in the digestion of wool by these larvae.

It was shown by Linderstrøm-Lang & Duspiva (1936) and by Waterhouse (1952) that the mid-gut of the clothes moth larva, *Tineola bisselliella*, has a reduction potential of *c.* -250 mV. at pH values near 10. These reducing conditions, thought to assist the digestion of keratin, might result from the metabolic processes of micro-organisms within the intestine. We therefore examined the intestinal microflora of the moth larvae.

METHODS

Larvae were obtained from laboratory cultures growing on unsterilized lactic casein powder (90-mesh) containing 10% dried yeast powder, and from naturally occurring colonies on greasy wool. Laboratory cultures on wool were obtained by transference of larvae or eggs from casein medium to greasy wool and incubation at the optimal temperature of 28°. Larval intestines were withdrawn aseptically after circular incision of the skin near the head and amputation of the tail tip.

Emulsions of four to six intestines in 0.05 ml. peptone water were equally distributed on six plates of nutrient agar containing 1% (w/v) glucose and 0.5% (w/v) yeast extract adjusted to pH values 7.5, 8.5 and 9.5. Plates were incubated aerobically and anaerobically at 28°.

RESULTS

Table 1 shows that with few exceptions the bacterial population in the gut, particularly in the mid- and hind-gut, was very small. This was confirmed by microscopic examination of smears of the gut contents which in general appeared to be free from bacteria. Smears of gut contents from larvae grown on wool consisted largely of partly degraded cortical cells and of other wool debris.

The larvae with high intestinal counts of bacteria (Table 1) were all derived from a single culture of larvae on casein medium and were shown by plating and by direct microscopic examination to be heavily infected with Gram-positive cocci. Other larval colonies on casein, however, were not infected in

this way. On transferring infected larvae to greasy wool the infection persisted indefinitely. In pure culture the coccus grew optimally at 28°, formed white colonies aerobically on nutrient agar, and anaerobically, slowly, a translucent growth. This coccus was catalase-positive, coagulase-negative (rabbit plasma), gave an acid clot in litmus milk, acid but no gas from glucose, produced no acetoin, and according to the classification of Shaw, Stitt & Cowan (1951) belonged to the *Staphylococcus lactis* group of organisms which are often found in dairy products.

Table 1. *Micro-organisms in the gut of Tineola larvae*

Source of larvae	Growth of micro-organisms	pH value of medium	Total count/gut
Casein culture (C.S.I.R.O. Geelong)	Aerobic	8.5	Many staphylococci
	Anaerobic	8.5	Many staphylococci
Casein culture (C.S.I.R.O. Canberra)	Aerobic	7.5	1
	Aerobic	8.5	120
	Aerobic	9.5	12
	Anaerobic	7.5	Spreader
	Anaerobic	8.5	0
	Anaerobic	9.5	1
Casein culture (Melbourne)	Aerobic	7.5	3
	Aerobic	8.5	1
	Aerobic	9.5	7
	Anaerobic	7.5	0
	Anaerobic	8.5	0
	Anaerobic	9.5	0
Wool culture (C.S.I.R.O. Canberra)	Aerobic	7.5	5
	Aerobic	8.5	7
	Aerobic	9.5	5
	Anaerobic	8.5	0
Wool culture (Melbourne)	Aerobic	7.5	Overgrown with fungi
	Aerobic	8.5	Overgrown with fungi
	Aerobic	9.5	Overgrown with fungi
	Anaerobic	7.5	0
	Anaerobic	8.5	1
	Anaerobic	9.5	40
Wild culture (greasy wool)	Aerobic	7.5	0
	Anaerobic	7.5	0

Only one colony developed (aerobically) on six plates inoculated with an emulsion of mid/lower guts from larvae on casein medium.

As the mid-gut has a pH value of about 10.0 (Duspiva, 1936; Linderstrøm-Lang & Duspiva, 1936; Waterhouse, 1952) the growth of the isolated coccus over a range of different initial pH values was compared with that of a culture having the same physiological properties but which was derived from a different source (supplied by the Department of Bacteriology, University of Melbourne). The organisms were incubated at 28° in a CO₂-free atmosphere in a medium containing: 1.0 % (w/v) peptone (Difco); 0.1 % (w/v) yeast extract (Difco); mineral salts; 0.15 % Na₂HPO₄ (w/v) buffer. Both strains grew well in 24 hr. at initial pH values in the range 7.0–10.0; neither grew at pH 10.5. These results contrast with those of Dernby (1921), who recorded

a pH range of 5.6–8.1 for the growth of an organism called *Staphylococcus blanc* at an unspecified temperature. Beveridge (1940) showed that certain haemolytic staphylococci from milk were alkali tolerant.

CONCLUSION

The bacterial population in the gut of most of the *Tineola bisselliella* larvae studied was too small to influence the oxidation-reduction potential of the gut contents or to assist in the digestion of wool; it is concluded that bacteria play no essential part in this process. The high intestinal pH value is probably an important factor in the suppression of bacterial growth in the gut.

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(Received 21 October 1954)