The Microbial Flora of the Gut of the Pouch-young and the Pouch of a Marsupial, *Setonix brachyurus*

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(Accepted for publication 9 December 1971)

SUMMARY

Bacteria were found in the alimentary tract of 40 pouch-young, ranging in age from 1 to 157 days, and within the marsupium of six adult females of *Setonix brachyurus*. Colonization of the neonate's intestine occurred soon after birth; *Escherichia coli* and *Streptococcus faecalis* were frequently isolated from the intestines of pouch-young of all ages. Other micro-organisms present, but infrequently encountered, were *Aerobacter aerogenes*, *Klebsiella* sp., *Salmonella newport*, *Pseudomonas aeruginosa* and *Staphylococcus albus*. Protozoan ciliates were present in the stomachs of pouch-young older than 150 days.

The pouch of non-lactating and lactating adults harboured a variety of bacteria, but in one female expecting a birth the pouch flora was absent.

INTRODUCTION

The natural colonization and development of the bacterial flora of the gastro-intestinal tract of young animals generally provide the host with its initial contact with a variety of antigens. These early associations with micro-organisms may result in immunization, tolerance and persistence of a 'normal' flora, or death due to infection. Since relatively few babies die in early life, it is clear that some balance between the baby and its microbial flora is achieved, but no detailed study of the complex interrelationships has been made (Donaldson, 1968). The complexity of the situation is exemplified by *Escherichia coli* which is a common symbiont in the intestinal tracts of young mice, but one strain can cause a lethal wasting disease in them (Miller, Sterzl, Kostka & Lang, 1967). It is not clear why one strain of *E. coli* should be enteropathogenic whereas another is harmless.

Study of the bacteria in the faeces of the young animal (Smith & Crabb, 1961) and the development of the intestinal flora (Smith, 1965) in a variety of domestic animals suggests that the first organisms to colonize the alimentary tract in most species of animals are *Escherichia coli*, *Clostridium welchii* and *Streptococcus* sp. and that they appear within a few hours after birth. These species are followed by lactobacilli and other bacteria, some of which are restricted to particular species. Yeasts are not found in animals whose diet consists solely of milk.

Marsupial young are born at a state equivalent in general development to a eutherian foetus which is immunologically incompetent (Block, 1964; Yadav & Papadimitriou, 1969). The newborn crawls into the pouch unaided, attaches firmly to one of the four or more teats and undergoes further development in conditions unlike those in the uterus. There is no published report on the microbial flora of the gut of pouch-young, or the pouch environment. There-

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fore, it is of some interest to record the microbial colonization of the gut in these animals so that we can begin to understand the mechanisms by which the neonate survives in the pouch which also harbours a variety of micro-organisms. The marsupial, *Setonix brachyurus*, was used for this work for two reasons: there is some information on the development of immunological maturity (Yadav, 1969) and on the mode of transmission of maternal immunoglobulins on this animal (Yadav, 1971; Yadav, Eadie & Stanley, 1972), and the pouch-young of *Setonix* whose thymus tissue has been totally removed by surgery do not waste (Yadav, 1969) as do neonatally thymectomized mice and some other mammals (Miller & Osoba, 1967). The runting syndrome following neonatal thymectomy of some mice strains is not seen in germ-free conditions (McIntyre, Sell & Miller, 1964; Wilson, Sjodin & Bealmear, 1964). This, and an analysis of the situation by Miller & Osoba (1967) and Keast (1968), suggests that runting in thymectomized animals is associated with the inability of the animal to cope with bacterial endotoxin derived from the gut flora. The extent and nature of colonization of the gut, particularly with Enterobacteriaceae, the Gram-negative rods normally present in the gut and also ubiquitous in nature, is therefore of some interest.

METHODS

Animals. *Setonix brachyurus* is a rare marsupial found on Rottnest Island, Bald Island and in some swamps, which are difficult of access, along the south-west coast in Western Australia. Except where specifically mentioned, all the animals used in this study were captured on Rottnest Island, transported to the Department and held in large outdoor pens. Food and water was supplied *ad libitum*. The animals put on weight and bred well under these conditions.

Setonix is monovular, has a gestation of 25 days, and a pouch term of 6 months. In the final month of pouch term the young often peers out over the lip of the pouch opening, which is normally closed, to nibble at vegetation. After vacating the pouch the young is suckled from outside the pouch for varying times ranging from 6 months to 1 year. Sexual maturity is reached in about two years. Many aspects of the physiology of *Setonix* have been previously summarized by Waring, Moir & Tyndale Biscoe (1966).

Bacteriological technique. Pouch-young were killed with ether or by decapitation at selected times after birth. Under aseptic conditions, the contents of the whole alimentary tract were thoroughly mixed and then samples of it plated out on selective solid media in Petri dishes. Culture media comprised nutrient agar, blood agar and MacConkey agar plates; nutrient broth and cooked meat medium. The plates and broth were incubated overnight, under aerobic conditions at 37°. The bacteria were subsequently isolated and identified by standard methods.

RESULTS

In common with all macropod marsupials, *Setonix brachyurus* possesses a ruminant-like digestion and in adults the population of bacteria is quantitatively similar to that of eutherian ruminants, although the ciliate population is relatively larger (Moir, 1968). In order to determine the time when ciliates first became apparent in the stomach of *Setonix* in the wild population, pouch-young were killed immediately after capture of the adults at Rottnest Island and the stomach contents analysed. Table 1 shows that ciliates were present in young older than 150 days and that these young had plant material in their stomachs. Animals of all ages had Gram-positive cocci, ranging from one type in the very young to three types in the older animal, but bacilli were infrequently encountered.

Table 1. *Examination of stomach contents of pouch-young Setonix for ciliate protozoa and bacteria*

Range in age (days)	No. of animals	Plant food in stomach	Ciliates	Gram stain. No. of animals showing			
				Bacilli		Cocci	
				+	-	+	-
10 to 140	10	None	Absent	4	1	10 (few to moderate)	0
150 to 200	4	Present	Present (1 to 4 species)	0	0	4 (abundant)	0

The indigenous flora of the alimentary tract in 40 quokka pouch-young, ranging in age from 1 to 157 days are presented in Table 2. The bacteria occurring most frequently were *Escherichia coli* and *Streptococcus faecalis*. The alimentary tract flora of the pouch-young of four tammar (*Macropus eugenii*), another wallaby brought from Garden Island, contained *E. coli* and *S. faecalis* (Table 2).

The pouch flora of six adult female quokkas are listed in Table 3. There was a wide range of bacteria in the five adults which had no suckling young. In one case, no bacteria were isolated from the pouch of a female (6510) quokka expecting a newborn; visually the pouch was clean, and was noticeably damp from secretions from the pouch-skin epithelium. The pouch of one female with a newborn had *Staphylococcus albus* and *Streptococcus viridans* only.

DISCUSSION

The pouch of non-lactating adult females harboured a wide variety of microbial flora, but notably absent was *Staphylococcus aureus*, an organism plentiful on other parts of the adult *Setonix* body.

The complete lack of pouch flora in the one expectant female suggests that in expectant animals the secretions of the glandular marsupium skin may contain antibacterial substances. It is not known if the bactericidal property is due to immunoglobulins in the pouch secretions or to some other substances in it. Since, at birth the newborn *Setonix* lacks immune competence (Yadav, 1969; Yadav & Papadimitriou, 1969), and since bacteria were isolated from the gut in the first days of life, it appears that the chief source of immune protection against infection is through milk immunoglobulins. The pouch-young has the capacity to absorb from the gut maternal immunoglobulins in milk for the total period it occupies the pouch (Yadav, 1971; Yadav, Eadie & Stanley, 1972). On the other hand a simpler explanation would be that the organisms isolated are not pathogenic in *Setonix*.

The predominant organisms recovered from the alimentary tract of the quokka (and tammar) at various times after birth were *Escherichia coli* and *Streptococcus faecalis*. Colonization of the digestive and respiratory tracts of the neonate could be expected to begin on the initial journey across the fur to the pouch and in the pouch itself before attachment to the teat. Subsequently colonization could occur via the milk and via the anus and nose; entry of pouch pathogens through the mouth is probably precluded by the continuous and firm attachment to the teat during early development. The mother frequently licks the inside of the pouch to remove faeces and urine of the young and this behaviour could introduce new micro-organisms into the pouch. The presence of *Salmonella newport*,

Table 2. *The indigenous gastrointestinal microflora in Setonix brachyurus and Macropus eugenii*

Mother tag no.	Pouch-young		<i>Escherichia coli</i>	<i>Streptococcus faecalis</i>	<i>Aerobacter aerogenes</i>	<i>Klebsiella</i> sp.	<i>Salmonella newport</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus albus</i>	<i>Proteus</i> sp.	<i>Strep. lactua</i>	<i>Clostridium</i> sp.	Yeasts
	Sex	Age (days)											
Quokka													
6513	—	I	+	—	—	—	—	—	—	—	—	—	—
6563	—	I to 2	—	—	—	—	—	—	+	—	—	—	—
6042	—	10	—	—	—	—	+	—	—	—	—	—	—
7340	O _A	21	++	++	—	—	—	—	—	—	—	—	—
6216	—	39	++	++	—	—	—	—	—	—	—	—	—
II	O _A	40	+++	+	—	—	—	—	—	—	—	—	—
6526	HO	46	++	—	—	—	—	—	—	—	—	—	—
6527	HO	48	++	++	—	+	—	—	—	—	—	—	—
6215	HO	59	++	++	—	—	—	—	—	—	—	—	—
6538	O _A	63	++	++	—	—	—	—	—	—	—	—	—
7371	HO	65	—	—	++	—	—	—	—	—	—	—	—
100	O _A	65	++	—	—	—	—	—	—	—	—	—	—
6593	O _A	68	++	—	++	—	—	—	—	—	—	—	—
6542	O _A	71	+++	—	—	—	—	—	—	—	—	—	—
6543	HO	71	+++	—	—	—	—	—	—	—	—	—	—
6515	O _A	71	—	—	+++	—	—	—	—	—	—	—	—
159	O _A	72	++	++	—	—	—	—	—	—	—	—	—
152	HO	73	++	++	—	—	—	—	—	—	—	—	—
7375	O _A	76	++	++	—	—	—	—	—	—	—	—	—
6531	HO	87	++	++	—	—	—	—	—	—	—	—	—
6533	HO	87	+++	—	—	—	+	—	—	—	—	—	—
6538	HO	96	+++	—	—	—	—	—	—	—	—	—	—
6570	HO	98	+++	—	—	—	—	—	—	—	—	—	—
6535	HO	98	+++	—	—	—	—	—	—	—	—	—	—
5996	HO	101	+++	—	—	—	—	—	—	—	—	—	—
6577	HO	101	+++	—	—	—	—	—	—	—	—	—	—
74	O _A	109	++	++	—	—	—	—	—	—	—	—	—
6553	HO	109	—	—	+++	—	—	—	—	—	—	—	—
34	O _A	110	++	++	—	—	—	—	—	—	—	—	—
20	HO	111	+++	—	—	—	—	—	—	—	—	—	—
28	O _A	111	++	++	++	—	—	—	—	—	—	—	—
6557	O _A	113	++	++	—	—	—	—	—	—	—	—	—
11	O _A	113	++	++	—	—	—	—	—	—	—	—	—
72	O _A	113	+++	—	—	—	—	—	—	—	—	—	—
49	O _A	113	+++	—	—	—	—	—	—	—	—	—	—
59	O _A	113	+++	+	—	—	—	—	—	—	—	—	—
6349	O _A	114	+	+++	—	—	—	—	—	—	—	—	—
6592	HO	114	++	+++	—	—	—	+	—	—	—	—	—
6573	HO	126	+++	—	+++	—	—	+	—	+	++	+	+
6553	HO	157	+	—	+++	—	—	—	—	—	++	—	+
Tammar													
182	O _A	50	++	++	—	—	—	—	—	—	—	—	—
183	HO	55	++	++	—	—	—	—	—	—	—	—	—
197	HO	60	++	++	—	—	—	—	—	—	—	—	—
198	O _A	70	++	++	—	—	—	—	—	—	—	—	—

+++ = Heavy growth; ++ = moderate growth; + = a few colonies; — = absent.

Table 3. *The indigenous pouch flora of Setonix*

Mother's tag	Presence of pouch-young (age days)	<i>Escherichia coli</i>	<i>Staphylococcus albus</i>	<i>Streptococcus viridans</i>	<i>Pseudomonas aeruginosa</i>	<i>Aerobacter aerogenes</i>	<i>Bacillus subtilis</i>	<i>Neisseria</i> sp.	Unidentified Gram-negative rod
6563*	I	—	++	++	—	—	—	—	—
6510	Nil but baby expected	—	—	—	—	—	—	—	—
3	Nil	—	—	—	+++	+	+	—	—
10	Nil	—	—	—	+++	—	—	—	—
22	Nil	+	—	—	—	—	++	—	+++
2	Nil	+	—	—	—	—	—	++	++

+++ = Heavy growth; ++ = moderate growth; + = a few colonies; — = absent.

* *Staphylococcus albus* was also present in the intestine of the pouch-young of this mother.

a rare organism, in one quokka pouch-young comes as a surprise, but suggests that, at birth, the exact colonization of the digestive tract may depend on the local presence of a particular organism. These organisms are later superseded by coliforms. Similar findings were reported by Smith (1965), who demonstrated some variability in colonization of the alimentary tract in the young of ox, sheep, pig, dog, cat, rabbit, guinea pig, rat and domestic fowl. The principal colonizers in these animals were *E. coli*, *Clostridium welchii* and streptococci; these were later followed by lactobacilli.

Milk forms the major diet of the quokka pouch-young until 150 days, after which the pouch-young starts to ingest plant materials from outside. The pouch-young leaves the marsupium at 180 days. With the change in the composition and complexity of the diet, the intestinal flora would be expected to change, and in fact yeasts and clostridia become apparent in the quokka at 126 days and ciliate protozoan at 150 days.

These observations show that the gut flora is complex and the lack of runting in thymectomized pouch-young *Setonix* (Yadav, Stanley & Waring, 1972) is clearly not due to absence of a gut flora. Further work is needed on the mechanisms of survival of the newborn in *Setonix* and other species and the lack of runting in thymectomized *Setonix* to explain these phenomena fully.

We are indebted to and thank Mr W. Evans, assisted by Mr L. Smallacombe for animal husbandry, to Mrs Margaret Eadie for skilled technical assistance, to Mr Lance Rowett for identification of the organisms and to the Director, State Department of Fisheries and Fauna for a permit to collect the specimens used.

The work was supported by grants from the Australian Research Grants Committee (to NFS), University of Western Australia Research Grants Committee, Commonwealth Scientific and Industrial Research Organization (to H.W.) and Nuffield Foundation (Post-doctoral Fellowship to M.Y.).

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