also initiates the decrease of lactase along the entire small intestine (figure 2). However, thyroxine appears to be more effective in initiating the decrease of lactase than is cortisone, as the decreased activity can be observed for 48 h following the single injection. This conclusion is in accordance with that of Yeh and Moog[6]. The temporary depression of lactase observed after a single injection of hormone could be related to the inadequacy of the hormonal stimulus, even though a single injection of cortisone is able to provoke a precocious appearance of sucrase and an increase of maltase activity[3]. In order to verify this, the response of lactase has been studied after 3 injections of hormone. An opposite effect of cortisone is noted (figure 3). Indeed, a significant increase of lactase activity is recorded in the middle and distal thirds, cortisone being without effect in the proximal third. 3 injections of thyroxine (figure 3) also stimulate the lactase activity, even in the proximal third. The opposite effects of 1 and 3 injections of hormone on intestinal lactase activity in intact suckling mice is surprising. Lactase seems to be the only brush border enzyme to respond in that way. Indeed, both 1 or 3 injections of cortisone induce a premature increase of the other brush border enzymes[2, 3, 10]. All the factors possibly involved in the regulation of intestinal maturation are not known. In hypophysectomized or thyroidectomized suckling rats, repeated injections of cortisone or thyroxine do decrease lactase activity. It appears that hypophysectomy or thyroidectomy may affect the development of at least one unknown factor, and the administrated hormone is then able to provoke the decrease of lactase activity. The mechanism which regulates the postnatal development of lactase appears to be different from that of the other brush border enzymes and more complex than expected.

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Degradation of bacterial lipopolysaccharide by gut juice of the snail *Helix pomatia*

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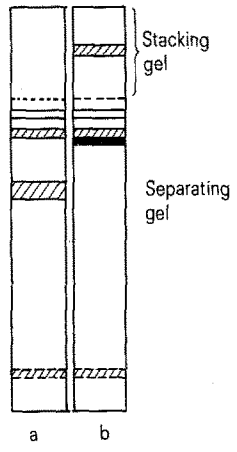
Summary. Lipopolysaccharides from several bacteria were selectively degraded by gut juice of the snail *Helix pomatia* with extensive loss of anticomplementary activity and changes in the electrophoretic pattern in polyacrylamide gels. The gut juice had little effect on ketodeoxyoctonate content or immunodominant sugars. The lipid A moiety of the lipopolysaccharide appeared to be the main site of attack.

In contrast to the voluminous literature on the alteration or degradation of bacterial lipopolysaccharides by chemical methods, there are only a few reports on biological systems which degrade lipopolysaccharides. These include phages[1,2], bacterial isolates[3,4], the amoebae of the cellular slime mould *Dictyostelium discoideum*[5] and mammalian tissue[6]. As part of a survey on the biodegradation of lipopolysaccharides in nature we have examined the gut juice of the snail *Helix pomatia* which is known to contain many degradative enzyme activities[7].

Methods. snail gut juice was obtained from Sigma Chemical Co., St. Louis, USA (β-glucuronidase from *Helix pomatia* crude soln. No. G-0876). Lipopolysaccharides (extracted by the Westphal procedure) from *Escherichia coli* strain $O_{55}B_5$, *E. coli* strain $O_{111}B_4$ and *Shigella flexneri* were purchased from Difco Laboratories, London, England. Lipopolysaccharide from *Salmonella minnesota* strain 9700 was prepared in this laboratory by the method of Westphal et al.[8]. This procedure was also applied to mixtures of snail gut juice plus lipopolysaccharide to analyze degradation of the lipopolysaccharide. Ketodeoxyoctonate was determined by a modification[9] of the thiobarbituric acid method[10] with ketodeoxyoctonate-1,4-lactone (from British Drug Houses, Poole, England) as standard. The lactone was found to yield an absorbance value equivalent to that given by ketodeoxyoctonate when allowance was made for the difference in mol.wt. For convenience, analytical results were expressed as ketodeoxyoctonate. Slab gel electrophoresis with a discontinuous SDS buffer system was carried out by the method described elsewhere[11] using lipopolysaccharide in place of protein. The bands were made visible by the periodic acid-Schiff procedure[12]. For haemagglutination-inhibition tests, serial dilutions of antigen (i.e. potentially



Line drawing of the band patterns observed after SDS-polyacrylamide gel electrophoresis of *E. coli* strain $O_{55}B_5$ lipopolysaccharide, *a* alone and *b* after incubation with snail gut juice as described in the table.

Effect of snail gut juice on the anticomplementary activity and ketodeoxyoctonate content of lipopolysaccharides from various bacteria. Each reaction mixture contained 4 mg lipopolysaccharide with variable quantities of snail gut juice made up to final volume of 4 ml with 0.2 M citrate-phosphate buffer pH 6.

Reaction mixture		Residual quantity per mixture after incubation and extraction*		
Snail gut juice (ml)	Lipopoly-saccharide	Anti-complementary activity	Ketodeoxy-octonate (μg)	Haemagglu-tination inhibition (units)
0.0	*E. coli* strain $O_{55}B_5$	560	160	1024
0.1	*E. coli* strain $O_{55}B_5$	150	177	1024
0.025	*E. coli* strain $O_{55}B_5$	310	200	1024
0.01	*E. coli* strain $O_{55}B_5$	700	157	n.t.
0.0	*E. coli* strain $O_{111}B_4$	650	393	1024
0.025	*E. coli* strain $O_{111}B_4$	290	380	1024
0.0	*Sh. flexneri*	550	107	512
0.1	*Sh. flexneri*	85	106	512
0.025	*Sh. flexneri*	350	100	512
0.0	*S. minnesota* strain 9700	700	126	1024
0.025	*S. minnesota* strain 9700	550	98	1024
0.01	*S. minnesota* strain 9700	475	93	n.t.
0.1	–	10	12	–

*After incubation at 30 °C for 3 h the reaction mixtures were extracted by the Westphal method. n.t., not tested.

degraded lipopolysaccharide) were made in microtitre trays and 0.05 ml of a suitable dilution of antiserum containing 5 haemagglutinating units (5 times the titre previously determined) was added to each well. Mixtures were allowed to stand at room temperature for 1 h then 0.05 ml of lipopolysaccharide-sensitized erythrocytes was added. Results were read after 18 h at room temperature. The haemagglutination-inhibition titre was taken as the amount of sample giving definite inhibition of haemagglutination. Anticomplementary activity was assayed against human serum as a source of complement using the method described previously[13].

Results and discussion. Varying quantities of gut juice from *H. pomatia* were incubated with lipopolysaccharides from *E. coli, Sh. flexneri* and *S. minnesota* and the mixtures then subjected to phenol/water extraction, dialysis and freeze-drying. As shown in the table, the anticomplementary activity of each lipopolysaccharide sample was reduced, some very substantially, whereas the amount of ketodeoxyoctonate and the haemagglutination-inhibition titre of the extract were not significantly altered. Further evidence for production of changes in lipopolysaccharide by snail gut juice was obtained by examination of the electrophoretic profiles of control and treated samples of material from *E. coli*: Undegraded lipopolysaccharide showed a characteristic band pattern (figure a) while lipopolysaccharide treated with snail gut juice gave a different pattern (figure b). The major fast moving band had entirely disappeared and 2 new, slower moving bands were visible, 1 of which was in the stacking gel. When lower concentrations of snail gut juice were used, this fast moving band was reduced but did not disappear. A similar result was obtained with other lipopolysaccharides. A small amount of Schiff-positive material in the snail gut juice alone did not contribute significantly to the overall band pattern. Lipopolysaccharide preparations from different organisms have characteristic band patterns after SDS-polyacrylamide gel electrophoresis[14,15] and have electrophoretic mobilities of their different components proportional to their lipid A content[16]. One interpretation of the present results is that snail gut juice has removed some or all of the lipid A of the fast moving component thus forming 2 slower moving products. The marked loss of anticomplementary activity of lipopolysaccharides also points to an attack on the lipid A moiety since this component has been identified as being mainly responsible for non-specific activation of the complement system. In contrast to the apparent attack on the lipid A of lipopolysaccharide, there was little evidence of degradation of the polysaccharide component, although subtle changes might have escaped detection by the methods used. Thus the actions of snail gut juice on lipopolysaccharides appear to be very similar to that of the slime mould *Dictyostelium discoideum*[5] and bacterial isolates[3,4] which also degrade the lipid A moiety of lipopolysaccharides. It also resembles the action of the acellular slime mould *Physarum polycephalum*[17].

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