only the microbasic mastigophore of *Porites compressa* dissolved within 3 min, whereas those of *Cyphastrea* required 9–10 min to dissolve. The other coral nematocysts examined remained undissolved after 10–30 min exposure to DTE. In all cases, the spirocysts remained unaffected by the DTE, thus providing evidence for the chemical differences between spirocysts and nematocysts proper[4]. Electron micrographs by one of us (R.N.M.) have revealed the distinct ultrastructural differences between anemone spirocysts and nematocysts.

The unfired nematocysts of the various coelenterates tested, with one notable exception, remained unaffected by the DTE in the present study. The single exception was the microbasic amastigophore of the swimming sea anemone *Boloceroides*; both the discharged and undischarged microbasic amastigophores dissolved in 1–2 min.

The fact that only 1 nematocyst type, the microbasic amastigophore of *Boloceroides*, dissolved in an undischarged state deserves further study in regard to the osmotic theory of nematocyst discharge. All the other undischarged and partially discharged nematocysts appeared to be totally unaffected by the DTE.

That some of the nematocysts examined required 9–10 min to dissolve, or did not dissolve at all during the period under examination, remains enigmatic. This was especially true for the coral nematocysts. One obvious possibility is that some coral nematocysts may differ chemically. However, it is also possible that the relatively large amounts of mucus liberated by the corals (compared to the other coelenterates examined) protected the nematocysts from dissolution by DTE in spite of steps taken to minimize this problem[5].

Zusammenfassung. Während sich die Mehrzahl der entladenen Nesselkapseln mehrerer hawaiischer Coelenteraten in Dithioerythritol (DTE) auflöst, sind die Spirozysten und die meisten unentladenen Nesselkapseln in diesem die Disulfidbindungen reduzierenden Reagens unlöslich.

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[4] L. HYMAN, *The Invertebrates: Protozoa through Ctenophora* (McGraw-Hill, New York 1940), vol. 1.

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The Role of the Bacterial Cell Wall in the Development of the Lethal Effect of Colicin E1

The basic biosynthetical processes of sensitive bacterial cells – and consequently their growth and division – are blocked by colicins immediately after the contact[1,2]. In spite of that, this blockade is temporarily reversible; in a part of the cells, it is possible to abolish the inhibitive action of colicins for a limited time interval by trypsin[3] or a specific anti-colicin serum[4]. The first, reversible phase of the effect of colicins can thus be regarded as the bacteriostatic one; this gradually passes on to the irreversible phase representing the bactericidal effect.

Some previous results, with colicins E2 and G, suggested that their receptors could not be situated in the cell wall of a sensitive bacterium (as had been supposed), but in its cytoplasmic membrane[5]. In the present paper we thus tried to find out the role of the bacterial cell wall in the development of the effect of colicin E1 (produced by the strain *Shigella dispar* P 14); we followed the chronological dependence of the onset of the bactericidal effect on the degree of structural damage of the cell walls of sensitive bacteria.

Firstly, we compared the outset of the bactericidal effect of colicin E1 on intact bacterial cells and spheroplasts. Sensitive rods of the strain *Escherichia coli* B1 from the stationary phase of growth or glycine spheroplasts[6] of the same strain, were incubated in broth at 37 °C with an excess of colicin E1. After 1, $2^1/_2$ or 5 min, trypsin (0.25 mg/ml) was added, reactivating those elements, the inhibition of which had not yet reached the irreversible phase. (Neither the cells nor the spheroplasts themselves were influenced by trypsin.) A drop of each suspension was transferred to the surface of a little agar block mounted on a slide, covered with a coverslip and framed with paraffin; this preparation was then photographed in a phase-contrast microscope in 30 min intervals for 5–6 h. From the photographs the percentages of dividing rods or of dividing and regenerating spheroplasts, that is of elements exhibiting their viability, were calculated.

In control preparations without colicin, nearly 100% of rods and over 63% of spheroplasts divide, while only some 10^{-4}% of rods and still less spheroplasts survive the action of colicin E1 (without succeeding trypsin addition). The effect of trypsin reactivation is clearly visible from Table I: after all 3 exposures to colicin used, it is possible to reactivate the rods in a remarkably higher proportion than is the case with spheroplasts. So the bactericidal step of colicin E1 effect starts more readily in an absolute majority of spheroplasts, lacking the rigid murein cell wall layer, than in intact rods of the same strain; this layer evidently delays the onset of its irreversible, bactericidal effect.

[1] F. JACOB, L. SIMINOVITCH and É. WOLLMAN, Annls Inst. Pasteur, Paris *83*, 295 (1952).

[2] J. ŠMARDA, Nature *194*, 792 (1962).

[3] M. NOMURA and M. NAKAMURA, Biochem. biophys. Res. Commun. *7*, 306 (1962).

[4] J. ŠMARDA, Antimicrob. Ag. Chemother. 345 (1965).

[5] J. ŠMARDA and U. TAUBENECK, J. gen. Microbiol. *52*, 161 (1968).

[6] M. H. JEYNES, Expl Cell Res. *24*, 255 (1961).

Secondly, we compared the possibility of reactivation of intact rods of the strain *Proteus mirabilis* D 52 with that of the cells of a stable L-form of the same strain[7]; this L-form is, in fact, of a protoplast type. Rods or stable L-form cells from the stationary phase of growth were incubated 1 min at 37 °C in broth with an excess of colicin E1, before trypsin (0.25 mg/ml) was added; here the viability of elements in question was checked according to their ability to form macroscopic colonies on agar plates. For cultivation of L-colonies, 10% (v/v) of beef serum and 300 units penicillin/ml were added to meat-peptone agar.

The results are summarized in Table II. After 1 min exposure to colicin E1, 49% of rods and about 39% of L-form cells are capable of forming colonies (which corresponds to the quantitative ratio of the adsorption capacity of both kinds of elements for colicin E1[8]). After a prolonged exposure, the proportion of colony formers is no more diminished. Following the addition of trypsin, all rod cells inhibited by colicin, but no L-form cells are reactivated. So colicin E1 after 1 min exposure exerts only a bacteriostatic effect on rods, while that on stable L-form cells reaches the bactericidal phase. With regard to the fact that the stable L-form used lacks any, even chemically provable remnants of the cell wall[9], this result shows again that the cell wall considerably reduces the bactericidal effect of colicin E1.

Table I. Reactivation of rods and glycine spheroplasts of *E. coli* B1, incubated with colicin E1, by trypsin (phase-microscopic observations)

	Time of incubation with colicin E1 (min)			
	0 (controls)	1	2.5	5
Rods				
No. of elements observed	170	337	432	348
No. of viable elements	168	319	374	259
% of viable elements	98.8	94.7	86.6	74.4
Spheroplasts				
No. of elements observed	327	359	262	420
No. of viable elements	207	27	7	2
% of viable elements	63.3	7.6	2.7	0.5

Table II. Survival and reactivation of rods and stable L-form cells of *P. mirabilis* D 52, incubated with colicin E1, by trypsin (No. of colony formers)

Sample	Rods		Stable L-form cells	
	No./ml	%	No./ml	%
Original suspension	8.00×10^8	100.0	8.00×10^8	100.0
Original suspension + colicin E1 (1 min)	3.92×10^8	49.0	3.13×10^8	39.1
Original suspension + colicin E1 (1 min) + trypsin	8.03×10^8	100.4	3.12×10^8	39.0

It is thus possible to conclude that the bactericidal effect of colicin E1 on a sensitive strain proceeds the more readily, the more the wall of its cells is decomposed. In cells lacking their walls completely, the bactericidal effect is instantaneous. Thus the normal cell wall of rods does not mediate the bactericidal action of this colicin, but, on the contrary, it reduces it. This conclusion is in accordance with previous experience, concerning the action of colicin Q on *E. coli* spheroplasts[10,11] and of colicin E2 and G on stable L-form cells of *P. mirabilis*[5].

However, this conclusion may not hold for the action of all colicins in general. So it is probable that a further investigation of the effect of individual colicins on stable L-forms – and especially comparative experiments with protoplast-like stable L-forms of *P. mirabilis* and *E. coli* – may produce interesting results on the problem of the mechanism of the action of colicins in general.

Zusammenfassung. Es konnte wahrscheinlich gemacht werden, dass Zellwände von Bakterien die Wirkung bestimmter Colicine hemmen.

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[7] U. Taubeneck, Z. allg. Mikrobiol. *2*, 132 (1962).
[8] V. Obdržálek, J. Šmarda, J. Adler and O. Čech, Zentbl. Bakt. Parasitkde, Abt. 1, in press (1968).
[9] H. H. Martin, J. gen. Microbiol. *36*, 441 (1964).
[10] J. Šmarda and M. Vrba, Folia microbiol., Praha *7*, 104 (1962).
[11] J. Šmarda, Zentbl. Bakt. Parasitkde, Abt. 1, *196*, 240 (1965).
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PRO EXPERIMENTIS

The Human Chorionic Gonadotrophin Bio-Assay by Seminal Vesicles in Mice

The quantitative determination of chorionic gonadotrophin (HCG) is of importance in diagnosis and prognosis of pregnancy, as also for the diagnosis of tumours excreting chorionic gonadotrophin. From 1934, when the first method was cited for the quantitative determination, a number of biological and recently immunological methods were presented. Since the values obtained by immunological assays in second and third trimester of pregnancy are higher than those obtained by immunoassays (for lack of specificity), it was considered that immunological methods could be employed only for qualitative tests but not for quantitative determinations[1–3].

We wish to describe the biological assay method for the quantitative determination of chorionic gonadotrophin in urine based on the response in weight of seminal vesicles in immature male mice. The specificity and sensitivity of the method allow the direct injection of urine without any extraction of chorionic gonadotrophin.