Table II. Values of kt at varying values of n and C_N/a, as the result of computer-calculation

C_N/a	10^{-2}	10^{-4}	10^{-6}	10^{-8}	10^{-10}	10^{-12}
$n=1$	0.10×10^{-1}	0.10×10^{-3}	0.10×10^{-5}	0.10×10^{-7}	0.10×10^{-9}	0.10×10^{-11}
$n=2$	0.15×10^{0}	0.14×10^{-1}	0.14×10^{-2}	0.14×10^{-3}	0.14×10^{-4}	0.14×10^{-5}
$n=5$	0.13×10^{1}	0.44×10^{0}	0.17×10^{0}	0.66×10^{-1}	0.26×10^{-1}	0.10×10^{-1}
$n=10$	0.41×10^{1}	0.22×10^{1}	0.13×10^{1}	0.77×10^{0}	0.47×10^{0}	0.29×10^{0}
	kt	kt	kt	kt	kt	kt

decreasing values of k. From equation (2) a leakage-halftime (τ) can be calculated by setting C_N equal to $a/2$:

$$e^{-k\tau}\cdot\sum_{p=0}^{n-1}\frac{(k\tau)^p}{p!}=\frac{1}{2} \tag{3}$$

Because this equation is transcendental, no exact analytical solution can be given. Graphic solution, however, leads to the results presented in Table I. A less time-consuming and more generally useful solution can be attained by application of the Newton-Raphson procedure, with the assistance of an appropriate computer program (copies of this program are available upon request). Apparently the following generalization is allowed:

$$\tau_n\sim\{(n-1)+\ln 2\}/k. \tag{4}$$

It is also possible to calculate a value for the time-lag, mentioned before. By substituting chosen values of C_N/a and n, the same computer program provides the accessory values of kt (Table II). In accordance with expectations, there is a decrease of kt at decreasing values of C_N/a and constant n, and an increase of kt at increasing values of n and constant C_N/a. A remarkable result is that for increasing values of n the difference between the values of kt at the extrema of C_N/a (first and last column) diminishes. Replacement of a monovalently by a bivalently coupled ligand gives much more profit than, for example, the change from $n=5$ to $n=10$. From the experimental results of TESSER et al.[15], a value can be computed for the detachment rate constant. In their experiments at pH 8 and room temperature, this constant appears to be 0.25×10^{-4} min^{-1}. This value combined with data from Table II leads to the result that after 2–3 sec (!) the concentration of free ligand molecules will reach a value of 2 picomoles/ml wet gel, when originally 2 μmoles/ml wet gel were coupled, monovalently. This order of magnitude is in agreement with ligand-leakage rates found by the authors mentioned before[16–19]. At the same value of k the 2–3 sec, mentioned above, will change in 1 h ($n=2$), 5 days ($n=5$) and 5–6 weeks ($n=10$). This trend corresponds with the findings of WILCHEK[17].

In many cases, this rather minimal leakage will not be prohibitive for the isolation of biological macromolecules by affinity chromatography. However, it will be disastrous in the case of isolation of minute amounts of material out of large volumes, or in receptor localization[18]. In this context we feel that the given mathematical approach to the problem of ligand-leakage may be useful in the interpretation of experimental results and in the development of new coupling-procedures, which is the subject of our current investigations.

Zusammenfassung. Es wird ein theoretisches Modell beschrieben für die Abspaltung polyvalent gebundener Liganden von einem, wasserunlöslichen Träger. Die Annahme einer konsekutiven Reaktion führt zu einer Abschätzung der zu erwartenden Zeitverzögerung.

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PRO EXPERIMENTIS

Lanthanum Staining of the Intermediate Region of the Cell Wall in *Escherichia coli*

The cell wall of gram-negative bacteria basically consists of an outer double track membrane (wall membrane) and an intermediate region which contains the mucopeptide (peptidoglycan) layer responsible for wall rigidity[1]. This region is difficult to see by ordinary electron microscopy of thin sections, its preservation being greatly affected by the nature of the fixatives[2]. In order to improve its preservation and preferential staining, we tried several experiments with alcian bleu, lanthanum salts and simple fixatives in *Escherichia coli*.

Methods. E. coli strain B, was used throughout this study. It was grown in agar Biolife medium at 37 °C until the late logarithmic phase of growth. Various combinations of aldehyde fixatives, *tris*, 1-aziridynil phosphine oxide, Polysciences (TAPO) and osmium tetroxide were coupled with lanthanum nitrate, based on slight modifications of the procedures described elsewhere[3–5], the following one

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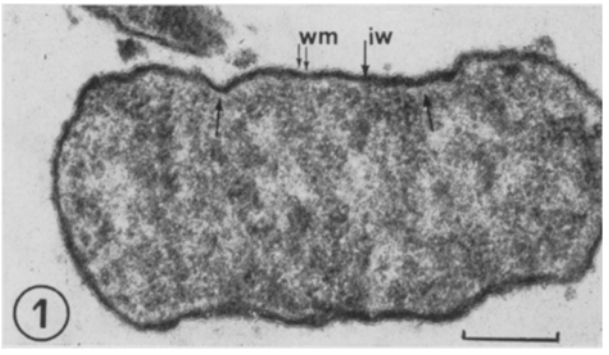

Fig. 1. An overall picture of *E. coli*, alcian bleu lanthanum en bloc stained. Sections counterstained with lead citrate and uranyl acetate according to conventional methods. Note the electron-dense outline of the intermediate wall region (iw), underlying the clearly resolved outer membrane (wm). The simple arrows point to the cytoplasmic membrane. Bar magnification = 200 nm.

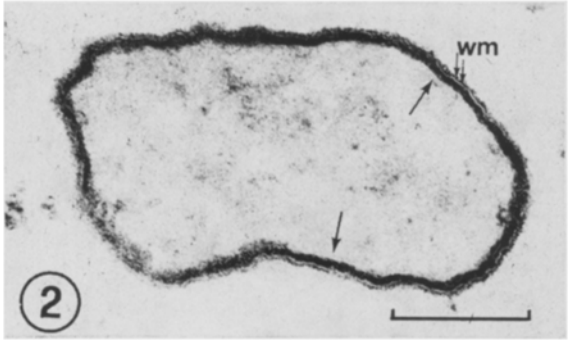

Fig. 2. As in Figure 1, but sections not counterstained. Note the flocculent material covering the outer membrane. Arrows point to low-contrast cytoplasmic membrane. Bar magnification = 200 nm.

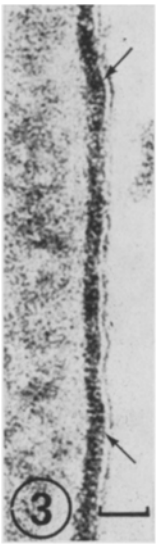

Fig. 3. High magnification picture of an unstained section, as in Figure 2. Note that primary stained region includes the inner track of the wall membrane. The cytoplasmic membrane (compare with Figure 2) is ill defined. Bar magnification = 25 nm.

being the most effective. The cells were prefixed (30 min over slant) with a mixture of glutaraldehyde, TAPO and alcian bleu (8 GX, Fisher) 0.3 (v/v), 0.05 (w/v) and 0.5 (w/v), respectively, in cacodylate buffer pH 6.8; 0.1 *M*. After several washings with the same buffer, the pellet was postfixed with osmium tetroxide 1% in S-collidine buffer (pH 7.5; 0.1 *M*) containing 1% (w/v) lanthanum nitrate, for 12 h at +4 °C, and subsequently with uranyl acetate 0.5% (w/v) in Michaelis buffer, pH 5.4 for 6 h at room temperature. The ensuing treatment was as described previously[5].

Results. The overall treatment of the cells for electron microscopy was reliable, giving no apparent artifacts. The preservation of the cytoplasm (Figure 1) was as good as with current techniques of chemical fixation for bacteria[6,7]. In lead-uranyl stained sections, cytoplasmic matrix was rich in dense granules, widespread with clear zones where nuclear fibrils appeared.

The cell wall had the basic structure of gram-negative wall but, at variance with ordinary reports, it showed an intensely stained intermediate region, 100 to 120 Å in width, including the inner track of the wall membrane and the outer leaflet of cytoplasmic membrane (Figure 1). This staining seems a primary consequence of lanthanum en bloc treatment, since the intermediate region appears highly contrasted also in unstained sections (Figures 2 and 3).

In numerous sections, especially in unstained ones, the region is not uniformly electron-dense but shows some separation of stained components. In cells undergoing plasmolysis, it is clearly separated from cytoplasmic membrane and is then visualized essentially as a dense layer, 25 to 35 Å thick, which retains a close linkage with the inner track of the outer membrane (Figure 4). This layer is clearly comparable to the mucopeptide layer already described[1], and evidenced also with simple fixation with TAPO, acrolein and osmium[8].

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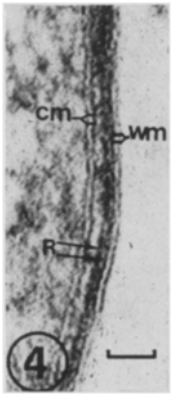

Fig. 4. High magnification picture of the periphery of a cell undergoing plasmolysis. Sections counterstained with lead citrate and uranyl acetate. Note overall wall layering, with the mucopeptide layer (R) in the intermediate wall region. Bar magnification = 25 nm.

Discussion. The intense staining of the intermediate region of gram-negative bacterial wall reported here can reasonably be attributed to the presence in this region of the mucopeptide components since, as emphasized recently by SHEA[4], the appropriate addition of alcian bleu-lanthanum to chemical fixatives provides specific, or at least preferential, staining of mucoproteins and acid polysaccharides. It is possible that other polysaccharides, probably present in this region[9,10], may contribute to the intensity of the staining and to the width of the stained region. It is also likely that the specificity of the reaction is not very strict and membranous structures in close linkage with the mucopeptide may consequently be stained.

The visualization of the mucopeptide layer in gram-negative bacteria has often been inconspicuous. This may be due, as recently shown by SILVA and SOUSA[2], to the use of uranyl ions. With the method described here, which also includes a normal postfixation with uranyl acetate, the intermediate region of the wall and the mucopeptide layer itself are constantly well preserved. Therefore it appears that the use of alcian bleu-lanthanum as described in this note may have two useful effects: 1. a more stable fixation of the wall components mentioned predominantly due to alcian bleu and TAPO[5,11]; 2. an increased contrast of these components depending on lanthanum-osmium staining properties.

Riassunto. Usando fissativi contenenti alcian bleu, TAPO e lantanio è risultata colorata preferenzialmente la regione intermedia della parete cellulare in *Escherichia coli*, con ottima preservazione dello strato mucopeptidico.

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23 January 1974.

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PRAEMIA

FRIEDRICH-MIESCHER-PREIS

Aus Anlass des 100 jährigen Jahrestages der Entdeckung der Nukleinsäuren hat die Schweizerische Gesellschaft für Biochemie einen Friedrich-Miescher-Preis für junge Biochemiker geschaffen. Der Preis wurde vom Friedrich-Miescher-Institut der Ciba-Geigy AG in Basel gestiftet.

Auszug aus den Statuten: 1. Der Friedrich-Miescher-Preis wird höchstens einmal jährlich einem jungen Forscher für eine oder mehrere hervorragende veröffentlichte oder zur Publikation angenommene Arbeiten aus dem Gebiet der Biochemie erteilt. 2. Kandidaten dürfen in dem Jahre, in dem sie den Preis erhalten, das 40. Lebensjahr nicht überschritten haben. 3. Die Preis-Arbeiten müssen entweder in der Schweiz oder von Schweizern im Ausland ausgeführt worden sein.

Bewerbungen und Vorschläge an den Sekretär der Gesellschaft (Dr. Cl. Bron, Institut de Biochimie, rue du Bugnon 21, CH–1011 Lausanne) bis 1. Januar 1975.

CONGRESSUS

Switzerland
10th EUCHEM Conference on Stereochemistry

at Bürgenstock, near Lucerne, 27 April–3 May 1975.

Inquiries and applications (no special forms are required) should be addressed before January 15, 1975 to the Chairman: Prof. J. Dale, Department of Chemistry, University of Oslo, Blindern, Oslo, Norway.

Canada
International Symposium on Flammability and Fire Retardants

in Montreal, 22–23 May 1975

Tentative titles and abstract with names of authors have to be sent to: Vijay Mohan Bhatnagar, Editor Advances in Fire Retardants, 209 Dover Road, Cornwall K6J 1T7, Ontario, Canada.

Italy
International Conference on Prostaglandins

in Florence, 26–30 May 1975

Information and inscription forms are available by the Secretary: Dr. G. C. Folco, Istituto die Farmacologia e Farmacognosia dell'Università, Via A. del Sarto 21, I–20129 Milano, Italia.

USA
3rd International Symposium on Detection and Prevention of Cancer

in New York City, 26 April to 1 May 1976

The Symposium includes 12 Conferences on general topics and 18 Panels on specific and organ-oriented subjects.

Approximately 22 Symposia will be arranged by groupings of proffered papers. There will be 14 Short Courses and 20 Practical Workshops.

Further information by H. E. Nieburgs, Secretary General, Mount Sinai School of Medicine of the City University of New York, Fifth Avenue and 100th Street, New York, N.Y. 10029 USA.