Phenotypic Variability of the Envelope Proteins of *Klebsiella aerogenes*

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SUMMARY

The envelope proteins of *Klebsiella aerogenes* (syn. *Aerobacter aerogenes*) grown in glucose-, sulphate-, phosphate-, ammonia-, potassium- and magnesium-limited environments, in chemostats, have been isolated, and compared by SDS-polyacrylamide gel electrophoresis; marked differences were evident. The envelopes from glucose- and sulphate-limited organisms were examined further: protein content was growth-rate dependent, but sulphate-limited envelopes always contained less protein than glucose-limited envelopes and this protein had a lower sulphur content. The sulphate-limited envelopes contained one major protein component with a molecular weight of 30000 daltons whereas the glucose-limited envelopes contained three main protein components (molecular weights of 46000, 38000 and 28500 daltons).

Selective extraction of membrane proteins with Triton X-100 indicated that both wall and membrane proteins altered in response to changes in the growth environment. Similarly, the soluble proteins of the organisms varied, but the ribosomal proteins remained almost constant.

INTRODUCTION

Bacteria have a marked capacity to undergo substantial changes in structure and function in response to changes in the growth environment (Herbert, 1961; Neidhardt, 1963) and the controlled environments provided by the chemostat are of considerable value in analysing this adaptability (Tempest, 1970).

The walls of both Gram-positive and Gram-negative bacteria vary markedly in content and composition with changes in the growth environment (Ellwood & Tempest, 1972). When *Bacillus subtilis* var. *niger* was grown in a chemostat under conditions of either glucose-, ammonia-, sulphate-, potassium- or magnesium-limitation, the walls contained a teichoic acid component. However, this wall-bound teichoic acid was replaced completely by a teichuronic acid-type polymer when the organisms were grown in a phosphate-limited environment. Other wall components have been found to vary with the growth environment (Ellwood & Tempest, 1972; Johnson & Campbell, 1972) and the object of the present study was to determine whether this variability extended to the wall proteins. The envelope proteins of *Klebsiella aerogenes* (syn. *Aerobacter aerogenes*), grown in a chemostat under conditions of different nutrient limitation, have been isolated and compared, using polyacrylamide gel electrophoresis, and substantial differences observed. The effects of glucose- and sulphate-limitations on envelope protein content and composition have been examined in detail. The soluble and ribosomal proteins have also been examined.

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METHODS

Micro-organisms. *Klebsiella aerogenes* (syn. *Aerobacter aerogenes*) (NCTC418) was maintained by monthly subculture on tryptic–meat–digest agar slopes containing glucose (0.2 % w/v).

Growth conditions. Organisms were grown in 0.5 l chemostats (Herbert, Phipps & Tempest, 1965; Evans, Herbert & Tempest, 1970), in simple salts media containing, for glucose-limitation (per 20 l), 350 ml M-(NH₄)₂SO₄; 150 ml M-KH₂PO₄; 100 ml 0.5 M-K₂SO₄; 20 ml M-citric acid; 150 g glucose; and trace elements as described by Evans *et al.* (1970). For sulphur-limitation, the glucose concentration was raised to 50 g/l and the K₂SO₄ concentration lowered to 10 mM. The media for potassium-, phosphate-, magnesium- and ammonia-limitations were as above, with appropriate lowering of the concentration of the growth-limiting nutrient. The temperature was maintained at 35 °C and the pH controlled automatically at pH 6.9 (± 0.1) by the addition of 4 N-NH₄OH (or, in the case of ammonia-limited growth, 2 N-NaOH). The organisms were harvested by centrifugation at 17000g for 30 min, crushed in the Hughes (1951) press and stored at -20 °C until required for analysis.

Isolation of envelopes. To ensure complete disintegration of the organisms, the paste from the Hughes press was suspended in 0.9 % (w/v) NaCl (2 to 6 g paste: 35 ml saline) and subjected to 3 min treatment in a Braun MSK Homogenizer. After removal of Ballotini beads on a sintered glass filter, the envelopes were isolated by centrifugation at 74000g (30 min, 4 °C), washed twice with 0.9 % (w/v) NaCl and twice with water.

Extraction of envelope proteins. (i) Sodium dodecyl sulphate, SDS. Envelope proteins were extracted into SDS by dispersing the envelope pellet (about 0.5 to 2.0 g wet weight), at room temperature, into 5 to 10 ml 0.01 M-sodium phosphate buffer (pH 7.2) containing 1 % (w/v) SDS, 1 % (v/v) 2-mercaptoethanol (Koch-Light Laboratories, Colnbrook, Buckinghamshire) and 10 % (v/v) glycerol (Inouye & Guthrie, 1969). The mixture was allowed to stand at room temperature for 30 to 60 min and then overnight at 4 °C before centrifuging at 48000g for 45 min at 15 °C. The pellet was washed once in the above solvent and the supernatant fluids pooled. The final solution contained about 5 mg protein/ml. (ii) Triton X-100 extraction. Attempts to isolate the membrane proteins using Triton X-100 were made as follows: the envelopes were dispersed in 10 mM-HEPES buffer (pH 7.4) containing 0.5 or 2 % Triton X-100 (Calbiochem, La Jolla, California, U.S.A.) as described by Schnaitman (1971). After standing for 15 min at room temperature, the preparations were cooled to 4 °C and centrifuged at 74000g for 1 h at 4 °C. Solid SDS was added to the supernatant (Triton-soluble proteins) to a final concentration of 1 % (w/v), 2-mercaptoethanol to 1 % (v/v) and glycerol to 10 % (v/v). The preparation was then dialysed overnight at room temperature against 0.01 M-sodium phosphate buffer (pH 7.2) containing 1 % (w/v) SDS, 1 % (v/v) 2-mercaptoethanol and 10 % (v/v) glycerol. The pellet (Triton-insoluble proteins) was extracted into SDS as described for total envelope proteins.

Isolation of soluble-plus-ribosomal proteins. The supernatant, after the initial centrifugation step to remove envelopes, contained both soluble and ribosomal proteins. Solid SDS was added to the solution to a final concentration of 1 % (w/v), 2-mercaptoethanol to 1 % (v/v) and glycerol to 10 % (v/v). The preparation was dialysed, overnight at room temperature, against 0.01 M-sodium phosphate buffer (pH 7.2) containing 1 % (w/v) SDS, 1 % (v/v) 2-mercaptoethanol and 10 % (v/v) glycerol.

Isolation of ribosomal proteins. Ribosomes (70-S) were prepared by dispersing crushed cells (Hughes press) in 4 vol. 0.01 M-tris, 0.02 M-MgCl₂, 0.03 M-NH₄Cl and 6 mM-2-mercaptoethanol (pH 7.6) (Traub & Nomura, 1968) containing 10 µg/ml deoxyribonuclease (Koch-

Light). The envelopes and unbroken organisms were removed by centrifugation at 50000g for 45 min, at 4 °C. The ribosomes were sedimented by centrifugation at 74000g for 2 h, at 4 °C, and washed once in the above solvent. The ribosomal proteins were then isolated by either (i) dispersing the ribosome pellet in 0.01 M-sodium phosphate buffer (pH 7.2), containing 1 % (w/v) SDS, 1 % (v/v) 2-mercaptoethanol and 10 % (v/v) glycerol, as described for envelope proteins, or (ii) by the addition of LiCl-urea as described by Traub & Nomura (1968).

Polyacrylamide gel electrophoresis. The method of SDS-polyacrylamide gel electrophoresis employed was essentially that of Maizel (1966) using 0.5 cm × 10 cm, 7.5 % polyacrylamide gels in 0.1 M-sodium phosphate buffer (pH 7.2) plus 0.1 % (w/v) SDS and 0.5 M-urea (Schnaitman, 1969). Samples (about 150 µg protein) in 0.01 M-sodium phosphate buffer (pH 7.2) containing 1 % (w/v) SDS, 1 % (v/v) 2-mercaptoethanol and 10 % (v/v) glycerol, were layered directly on to the gels and electrophoresis carried out at room temperature (5 h at 10 mA/gel). The gels were stained for 90 min in 0.25 % (w/v) Amido Black in water, methanol and glacial acetic acid (5:4:1, by vol.). Similar results were obtained when the stain used was Coomassie Brilliant Blue. The gels were destained by soaking (with shaking) in the water-methanol-acetic acid solvent and then scanned in the Joyce-Loebl Chromoscan. Standard proteins used to calibrate the system were cytochrome *c*, chymotrypsinogen A, carbonic anhydrase, aldolase, glutamic dehydrogenase and catalase (all samples obtained from Boehringer, London).

Ribosomal proteins isolated in LiCl-urea were analysed by the non-detergent-polyacrylamide gel electrophoresis system described by Spitnik-Elson & Atsmon (1969). These gels were stained and destained as above.

Chemical estimations. Protein was estimated by the biuret method described by Herbert, Phipps & Strange (1971) using crystalline bovine plasma albumin as standard. 'Total carbohydrate' was estimated with the anthrone reagent (Herbert *et al.* 1971).

RESULTS

Preliminary examination of the envelope protein composition of Klebsiella aerogenes

Klebsiella aerogenes was grown in the chemostat under conditions of either glucose-, sulphate-, potassium-, magnesium-, phosphate-, or ammonia-limitation at a $D = 0.2 \text{ h}^{-1}$. The envelope proteins of the bacteria were solubilized with SDS (approximately 90 % of the biuret-reacting material being solubilized using the procedures described in Methods) and examined by SDS-polyacrylamide gel electrophoresis. The patterns obtained were distinct for each type of nutrient limitation (Fig. 1), although sulphate- and phosphate-limited envelopes were quite similar. The protein content and composition of envelopes from glucose- and sulphate-limited organisms were then examined in more detail.

Protein content of the isolated envelopes from glucose- and sulphate-limited organisms

The protein contents of glucose- and sulphate-limited envelopes of *Klebsiella aerogenes*, grown at various dilution rates, were different (Table 1). The values for the percentage protein in the sulphate-limited envelopes were more variable than those for the glucose-limited envelopes (due probably to the presence of contaminating particulate carbohydrate material in the sulphate-limited envelope fraction). Nevertheless, the envelopes of sulphate-limited organisms consistently had a lower percentage of protein than did those of glucose-limited organisms even after taking into account the glycogen content. Envelopes from phosphate-

Fig. 1. SDS-polyacrylamide gels of the envelope proteins of *Klebsiella aerogenes* grown in a chemostat at 35 °C, pH 6.9, $D = 0.2 \text{ h}^{-1}$ under conditions of (a) glucose-, (b) sulphate-, (c) phosphate-, (d) magnesium-, (e) potassium-, and (f) ammonia-limitation. The electrophoresis was carried out as described in Methods.

Table 1. *Influence of growth rate and growth limitation on the protein content of Klebsiella aerogenes envelopes*

The organisms were grown as described. The protein concentrations of homogenates were estimated and then the envelopes isolated in water. The protein concentrations of the envelope fractions were estimated and the dry weights measured by drying 1.0 ml samples (105 °C, 24 h).

Growth-limiting nutrient	Dilution rate (h^{-1})	g protein/100 g dried envelopes	g protein
			in envelopes/100 g total cellular protein
Sulphate	0.1	38.4	21.1
	0.2	42.5	17.5
	0.6	45.0	18.6
Glucose	0.1	56.0	31.7
	0.2	55.1	29.3
	0.6	50.7	20.0

Table 2. Influence of growth rate and growth limitation on the sulphur content of envelope, ribosomal, and soluble proteins of *Klebsiella aerogenes*

Organisms were grown in media containing $^{35}\text{SO}_4^{2-}$, and disrupted in the Hughes (1951) press at -20°C . The disintegrated organisms were dispersed in 10 mM-tris-HCl buffer (pH 7.6) containing 1 mM- MgCl_2 and centrifuged at 20000g for 1 h to remove the envelope material. The supernatant fraction was further centrifuged (100000g, 3 h) to separate the ribosomal and soluble components. Each component was dispersed in tris-HCl buffer and analysed for protein and ^{35}S contents.

Growth limitation	Dilution rate (h^{-1})	^{35}S content of total protein	^{35}S content (g/100 g protein) in		
			Envelopes	Ribosomes	Soluble fraction
Glucose	0.2	0.74	0.79	0.68	0.74
Sulphate	0.2	0.61	0.64	0.66	0.60
	0.4	0.60	0.67	0.66	0.58
	0.8	0.60	0.62	0.60	0.58

limited organisms also had a low protein content. As the dilution rate was increased, and consequently the concentration of the growth-limiting nutrient approached a cell-saturation level, the values for glucose- and sulphate-limited envelopes became similar. The sulphate-limited organisms also contained a lower percentage of their total cellular protein in the envelope fraction, compared with the glucose-limited organisms (Table 1). But again, these values were more similar at the higher growth rates.

The sulphur contents of envelope, ribosomal and soluble proteins of *Klebsiella aerogenes* were determined using organisms that had been grown in a medium containing $^{35}\text{SO}_4^{2-}$. The envelope and soluble proteins from sulphate-limited organisms contained a lower ^{35}S content than those fractions from glucose-limited organisms (Table 2). But the ^{35}S content of the ribosomal protein fraction was constant (see later).

No major differences were evident in the envelope structures of glucose- and sulphate-limited organisms when examined by electron microscopy. The sulphate-limited organisms grown at a dilution rate of 0.1 or 0.2 h^{-1} were seen to be smaller and more round than the sulphate-limited organisms grown at $D = 0.6 \text{ h}^{-1}$, or organisms grown at corresponding dilution rates in a glucose-limited environment.

Protein composition of the isolated envelopes from glucose- and sulphate-limited organisms

Separation on polyacrylamide gels of the envelope proteins from glucose- and sulphate-limited organisms (grown at $D = 0.1 \text{ h}^{-1}$, 0.2 h^{-1} and 0.6 h^{-1}) revealed marked differences (Fig. 2, 3). At $D = 0.1 \text{ h}^{-1}$ and $D = 0.2 \text{ h}^{-1}$ the sulphate-limited envelopes were similar in protein composition (Fig. 2a, b, 3a); the molecular weight of the major component in these cases was estimated, by comparison with the mobilities of known protein standards (Fig. 2g; Weber & Osborn, 1969), to be 30000 daltons. At $D = 0.6 \text{ h}^{-1}$ the sulphate-limited envelopes contained a somewhat different spectrum of proteins (Fig. 2c) which was more similar to that of glucose-limited preparations. The molecular weights of the main components in the glucose-limited preparations were approximately 46000, 38000 and 28500. The 28500 dalton component was not identical to the 30000 dalton component of sulphate-limited organisms and migrated faster on mixed gels.

Similar spectra to those shown in Fig. 2 were obtained when the envelope proteins were extracted into SDS at 70°C for 20 min (Inouye & Guthrie, 1969). The various spectra were totally reproducible for separate series of growth experiments in the chemostat. Moreover the observed differences are thought not to be due to protease action during the isolation of

Fig. 2. SDS-polyacrylamide gels of envelope proteins of *Klebsiella aerogenes* grown in a chemostat at 35 °C, pH 6.9, under conditions of (a) sulphate-limitation, $D = 0.1 \text{ h}^{-1}$; (b) sulphate-limitation, $D = 0.2 \text{ h}^{-1}$; (c) sulphate-limitation, $D = 0.6 \text{ h}^{-1}$; (d) glucose-limitation, $D = 0.1 \text{ h}^{-1}$; (e) glucose-limitation, $D = 0.2 \text{ h}^{-1}$; and (f) glucose-limitation, $D = 0.6 \text{ h}^{-1}$. Gel (g) contains a mixture of six standard proteins (see Methods).

the (sulphate-limited) envelope proteins since (i) sulphate-limited and phosphate-limited envelope protein patterns are similar, (ii) envelope proteins isolated from preparations of glucose- and sulphate-limited organisms which had been heated (100 °C, 10 min) to destroy enzyme activity were also different, and (iii) the envelope protein pattern obtained from a mixed cell paste of 0.7 g glucose- and 0.3 g sulphate-limited organisms showed no obvious degradation of the glucose-limited proteins, and closely resembled the glucose-limited envelope protein pattern.

Extraction of glucose- and sulphate-limited envelope proteins with Triton X-100

The changes in envelope protein spectra with glucose- or sulphate-limited growth cannot definitely be assigned to either wall or membrane proteins. Attempts to solubilize selectively the membrane proteins using the non-ionic detergent Triton X-100 (Schnaitman, 1971) gave spectra essentially similar to those of total envelope proteins. However, differences in the proportions of some peaks were observed when the Triton-soluble (i.e. membrane) proteins were compared with the Triton-insoluble (i.e. wall) proteins solubilized in SDS (Fig. 4). Triton X-100 was found to solubilize about 30 to 40 % of the SDS-soluble biuret-reacting material. Fig. 4(a) is a scan of the sulphate-limited Triton-soluble protein and Fig. 4(b) is a scan of the remaining Triton-insoluble protein; thus the 30000 dalton com-

Fig. 3. Scans of SDS-polyacrylamide gels of *Klebsiella aerogenes* envelope proteins: (a) sulphate-limited, $D = 0.2 \text{ h}^{-1}$; (b) glucose-limited, $D = 0.2 \text{ h}^{-1}$.

Fig. 4. Scans of SDS-polyacrylamide gels of *Klebsiella aerogenes* envelope proteins soluble and insoluble in Triton X-100: (a) sulphate-limited, Triton X-100 soluble; (b) sulphate-limited, Triton X-100 insoluble; (c) glucose-limited, Triton X-100 soluble; (d) glucose-limited, Triton X-100 insoluble. 'W' refers to components predominantly Triton X-100 insoluble and hence tentatively identified as wall proteins; 'M' refers to components predominantly Triton X-100 soluble and hence tentatively identified as membrane proteins.

ponent can be tentatively identified as a wall component. The glucose-limited Triton-soluble spectrum is shown in Fig. 4(c) and the Triton-insoluble spectrum in Fig. 4(d). The components labelled 'W' are probably wall components and those labelled 'M' probably membrane components. The 46000 dalton component cannot be assigned on these results as either a wall or membrane component. The selective isolation of membrane proteins was not however so convincing as that found by Schnaitman (1971) for *Escherichia coli* envelopes.

Examination of soluble and ribosomal proteins

Apart from the envelope proteins, the soluble proteins of *Klebsiella aerogenes* were found to differ between glucose- or sulphate-limited organisms. When gels containing soluble-plus-ribosomal proteins (i.e. the proteins remaining after envelopes have been centrifuged from homogenates) were compared, there were obvious quantitative differences between the two preparations. These were largely due to the soluble proteins because the ribosomal proteins from glucose- and sulphate-limited organisms, whether examined by SDS-polyacrylamide gel electrophoresis or detergent-free polyacrylamide gel electrophoresis, were practically identical. This agrees with the similar ^{35}S /protein ratios found in the glucose- and sulphate-limited ribosomes (Table 2).

DISCUSSION

The present study again demonstrates the marked adaptability of the bacterial wall and the value of using the chemostat to study patterns of phenotypic variation in micro-organisms. Simply by lowering the concentration of sulphate and increasing the concentration of glucose in the growth medium, not only do the envelopes of *Klebsiella aerogenes* contain less protein (which in itself contains less sulphur) but also the spectra of proteins in the envelope fractions are found to vary markedly. Other nutrients also alter the envelope protein pattern when their supply is growth-rate limiting. The selective extraction of membrane proteins into Triton X-100 indicates that both wall and membrane proteins alter in response to changes in the growth environment. The changes appear not to be due to protease action during the preparation of samples, although the possibility of a protease closely associated with the sulphate-limited envelopes, and thus unable to act on the glucose-limited envelopes, cannot be totally excluded. Complementary to the changes in the envelope proteins are the changes in the soluble proteins; in contrast, the ribosomal protein spectra remain virtually constant in the environments studied. This may be anticipated because in these environments the principal function of the ribosome will be the same, i.e. to provide the machinery for protein synthesis.

At present detailed knowledge of the wall proteins of bacteria is lacking. The general appearance of the *Klebsiella aerogenes* envelope protein spectra obtained here (especially the glucose-limited spectra with the major 46000 mol. wt component) is in agreement with the published spectra for other Gram-negative bacteria (see Schnaitman, 1970*b*). Other reports have shown changes in the envelope proteins of *Escherichia coli* with cessation of cell division, with mutants defective in DNA synthesis or on inhibition of DNA synthesis (Inouye & Guthrie, 1969; Inouye & Pardee, 1970; Siccaldi, Shapiro, Hirota & Jacob, 1971; Starka, 1971; Inouye, 1972; Siccaldi, Lazdunski & Shapiro, 1972). These relatively minor changes are unlikely to be related to the changes observed here because both glucose- and sulphate-limited organisms have been studied at identical growth rates. In contrast to the major changes in envelope proteins of chemostat cultures of *K. aerogenes* observed here, Schnaitman (1970*a*) found only minor changes in the envelope protein spectra of *E. coli* grown aerobically and anaerobically in batch cultures on different carbon sources. MacGregor &

Schnaitman (1971) also observed that the addition of nitrate to cultures of wild-type *E. coli* caused an increase in three membrane proteins, one of which has been identified as nitrate reductase. A functional analysis of the changes in the envelope proteins of *K. aerogenes* with sulphate-limitation has not as yet been made.

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REFERENCES

- ELLWOOD, D. C. & TEMPEST, D. W. (1972). Effects of environment on bacterial wall content and composition. *Advances in Microbial Physiology* **7**, 83-117.
- EVANS, C. G. T., HERBERT, D. & TEMPEST, D. W. (1970). The continuous cultivation of micro-organisms, II. Construction of a chemostat. *Methods in Microbiology* **2**, 275-327.
- HERBERT, D. (1961). The chemical composition of micro-organisms as a function of their environment. In *Microbial reaction to environment. Symposium of the Society for General Microbiology* **11**, 391-416.
- HERBERT, D., PHIPPS, P. J. & STRANGE, R. E. (1971). Chemical analysis of microbial cells. *Methods in Microbiology* **5 B**, 209-244.
- HERBERT, D., PHIPPS, P. J. & TEMPEST, D. W. (1965). The chemostat: design and instrumentation. *Laboratory Practice* **14**, 1150-1161.
- HUGHES, D. E. (1951). A press for disrupting bacteria and other micro-organisms. *British Journal of Experimental Pathology* **32**, 97-109.
- INOUE, M. (1972). Reversal by sodium chloride of envelope protein changes related to DNA replication and cell division of *Escherichia coli*. *Journal of Molecular Biology* **63**, 597-600.
- INOUE, M. & GUTHRIE, J. P. (1969). A mutation which changes a membrane protein of *E. coli*. *Proceedings of the National Academy of Sciences of the United States of America* **64**, 957-961.
- INOUE, M. & PARDEE, A. B. (1970). Changes of membrane proteins and their relation to deoxyribonucleic acid synthesis and cell division of *Escherichia coli*. *Journal of Biological Chemistry* **245**, 5813-5819.
- JOHNSON, K. G. & CAMPBELL, J. N. (1972). Effect of growth conditions on the peptidoglycan structure and susceptibility to lytic enzymes in cell walls of *Micrococcus sodonensis*. *Biochemistry* **11**, 277-286.
- MACGREGOR, C. H. & SCHNAITMAN, C. A. (1971). Alterations in the cytoplasmic membrane proteins of various chlorate-resistant mutants of *Escherichia coli*. *Journal of Bacteriology* **108**, 564-570.
- MAIZEL, J. V., JUN. (1966). Acrylamide-gel electrophorograms by mechanical fractionation: radioactive adenovirus proteins. *Science, New York* **151**, 988-990.
- NEIDHARDT, F. C. (1963). Effects of environment on the composition of bacterial cells. *Annual Review of Microbiology* **17**, 61-86.
- SCHNAITMAN, C. A. (1969). Comparison of rat liver mitochondrial and microsomal membrane proteins. *Proceedings of the National Academy of Sciences of the United States of America* **63**, 412-419.
- SCHNAITMAN, C. A. (1970a). Examination of the protein composition of the cell envelope of *Escherichia coli* by polyacrylamide gel electrophoresis. *Journal of Bacteriology* **104**, 882-889.
- SCHNAITMAN, C. A. (1970b). Comparison of envelope protein compositions of several Gram-negative bacteria. *Journal of Bacteriology* **104**, 1404-1405.
- SCHNAITMAN, C. A. (1971). Solubilization of the cytoplasmic membrane of *Escherichia coli* by Triton X-100. *Journal of Bacteriology* **108**, 545-552.
- SICCARDI, A. G., LAZDUNSKI, A. & SHAPIRO, B. M. (1972). Inter-relationship between membrane protein composition and deoxyribonucleic acid synthesis in *Escherichia coli*. *Biochemistry* **11**, 1573-1582.
- SICCARDI, A. G., SHAPIRO, B. M., HIROTA, Y. & JACOB, F. (1971). On the process of cellular division in *Escherichia coli*. IV. Altered protein composition and turnover of the membranes of thermosensitive mutants defective in chromosomal replication. *Journal of Molecular Biology* **56**, 475-490.
- SPITNIK-ELSON, P. & ATSMON, A. (1969). Detachment of ribosomal proteins by salt. I. Effect of conditions on the amount of protein detached. *Journal of Molecular Biology* **45**, 113-124.
- STARKA, J. (1971). Cell envelope proteins of dividing and non-dividing cells of *Escherichia coli*. *FEBS Letters* **16**, 223-225.

- TEMPEST, D. W. (1970). The place of continuous culture in microbiological research. *Advances in Microbial Physiology* **1**, 223-250.
- TRAUB, P. & NOMURA, M. (1968). Structure and function of *Escherichia coli* ribosomes. I. Partial fractionation of functionally active ribosomal proteins and reconstitution of artificial sub-ribosomal particles. *Journal of Molecular Biology* **34**, 575-593.
- WEBER, K. & OSBORN, M. (1969). The reliability of molecular weight determinations by dodecyl sulphate-polyacrylamide gel electrophoresis. *Journal of Biological Chemistry* **244**, 4406-4412.