STUDIES OF THE BACTERIAL CELL WALL

I. ELECTRON MICROSCOPICAL OBSERVATIONS ON HEATED BACTERIA

by

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INTRODUCTION

The existence of an extremely thin outer cell wall enclosing the cytoplasm of the bacterial cell has been demonstrated in many studies[1–4]. With the development of the electron microscope and the shadowcasting technique, the cell wall has been observed repeatedly in studies of bacterial cytology and phage action[5–8]. Indeed, with the aid of the electron microscope DAWSON[9] has estimated the thickness of the cell wall of *Staphylococcus aureus*. In a recent study of the electron microscopy of heated bacteria by HEDEN AND WYCKOFF[10], thin collapsed cell walls could again be clearly seen in the preparations. It was evident that heating had resulted in rupture of the cell walls, although this fact was not commented upon by these workers.

This paper describes the effects on the cell walls, of heating suspensions of several bacterial species at various temperatures.

METHODS

The following organisms were used in this investigation: *Escherichia coli* H (kindly given by Dr E. F. GALE), *Salmonella pullorum* (kindly given by Dr R. COOMBS), *Streptococcus faecalis* ST (N.C.T.C. No. 6782), together with strains of *Pseudomonas fluorescens* and *Pseudomonas pyocyanea*.

The bacteria were grown in a medium consisting of 3% tryptic digest of casein, 0.1% Marmite and 1% glucose. Growth took place in Roux bottles containing 150 ml of medium incubated for 16 h at 37° C. The two Pseudomonas species were incubated for 16 h at 30° C. The cells were harvested from the liquid medium by centrifugation, washed three times with distilled water on the centrifuge and finally suspended in distilled water. The Pseudomonas species were washed once only before suspension in distilled water. Suspensions generally contained approximately 20 mg dry weight bacteria/ml.

Heat-treatment procedure

Bacterial suspensions were squirted by means of a pipette into volumes of distilled water in flasks held at various temperatures in water baths. One volume of suspension was squirted into about 10 volumes of distilled water at temperatures ranging from 55–100° C. The contents of each flask were agitated during the addition of the suspension and each flask was then held for 5 minutes at the bath temperature. Flasks of heated suspensions were then cooled by plunging into ice-cold water.

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Heated suspensions prepared in this way were suitably diluted with distilled water. Micro-drops of these suspensions were placed on specimen grids covered with nitrocellulose film. In some instances cell material deposited by centrifugation at 3,000 and 10,000 r.p.m. was examined, the deposited material being re-suspended in distilled water, diluted and mounted as previously described. Mounted specimens were dried in a desiccator and shadowed with gold-palladium alloy (60%–40%). The shadowing was at an angle of 45 degrees from the plane of the supporting film. Observations were made in the Siemens electron microscope, usually at direct magnifications of 8,000–12,000.

RESULTS

The typical appearance of untreated cells of *Esch. coli* is shown in Fig. 1. If suspensions of *Esch. coli* are heated for 5 min at 55, 60 and 65° C there is a tendency for the cytoplasm to become more granular and to shrink away from the outer cell wall which remains intact. These effects are shown in Figs. 2–4. Examination of suspensions held at 70, 75 and 100° C for 5 min revealed rupture of the cell walls, leaving naked cytoplasmic bodies. Not all cells showed complete rupture at temperatures of 70 and 75° C, but at 100° C all cells appeared to have ruptured cell walls. Figs. 5–7 show the thin collapsed cell walls together with the cytoplasmic bodies which cast appreciably more shadow and possess increased density with respect to the electron beam.

When suspensions of *Esch. coli* previously heated at 75° C are centrifuged at approximately 3,000 r.p.m. for 15 min the cytoplasmic bodies are deposited, whilst most of the ruptured cell walls remain behind in the supernatant fluid. Fig. 8 shows the cytoplasmic bodies. On centrifugation of the supernatant at 10,000 r.p.m. for 10 min the cell walls are deposited and Fig. 9 shows a concentration of the cell walls, together with some cytoplasmic bodies and debris.

Salmonella pullorum was investigated in a similar fashion and again rupture of the cell wall occurred when the suspensions were heated at temperatures of 70–75° C; below these temperatures no cell wall rupture was observed, but cytoplasmic shrinkage and granulation occurred as with *Esch. coli*. Fig. 10 shows the appearance of untreated cells of this organism, whilst Fig. 11 shows ruptured cell walls adhering to cytoplasmic bodies deposited by centrifugation at 10,000 r.p.m. after prior removal of most of the cell bodies. Fig. 12 shows separated circular fragments of the cell wall from *Salmonella pullorum*.

Pseudomonas fluorescens and *Pseudomonas pyocyanea* were both heated at 75° C for 5 min and rupture of the cell wall was observed with both organisms. Untreated cells of *Ps. fluorescens* are shown in Fig. 13, whilst a typical field from the heated suspension is shown in Fig. 14. Fig. 15 shows a typical cell of *Ps. pyocyanea* and Figs. 16 and 17 illustrate the cell wall rupture on heating at 75° C.

The proportion of ruptured cells in *Strep. faecalis* preparations was observed to be much lower than in any of the other organisms studied, even when the suspensions were heated at 100° C. Fig. 18 shows untreated cells of this organism and the appearance of cells heated for 5 min at 100° C is shown in Figs. 19 and 20.

DISCUSSION

The effects observed in the present study of heating bacterial suspensions are in general agreement with the results obtained by HEDEN AND WYCKOFF[10]. These latter workers, however, used much younger cultures and cell wall rupture appeared to have taken place at lower temperatures. Subjecting suspensions of 16 h cultures of several

References p. 42. *(Text contined p. 41)*

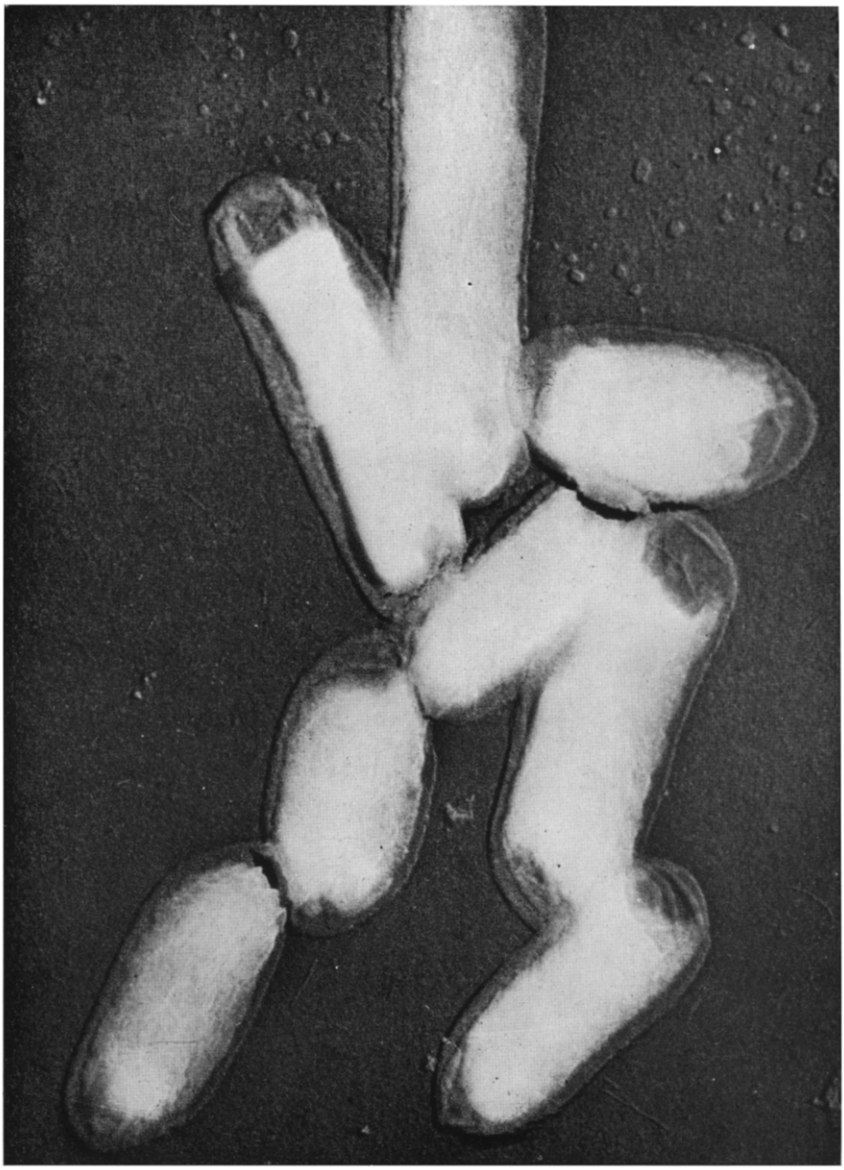

Fig. 1. *Escherichia coli.* Washed suspension of cells from 16 h culture. (25,500 ×)

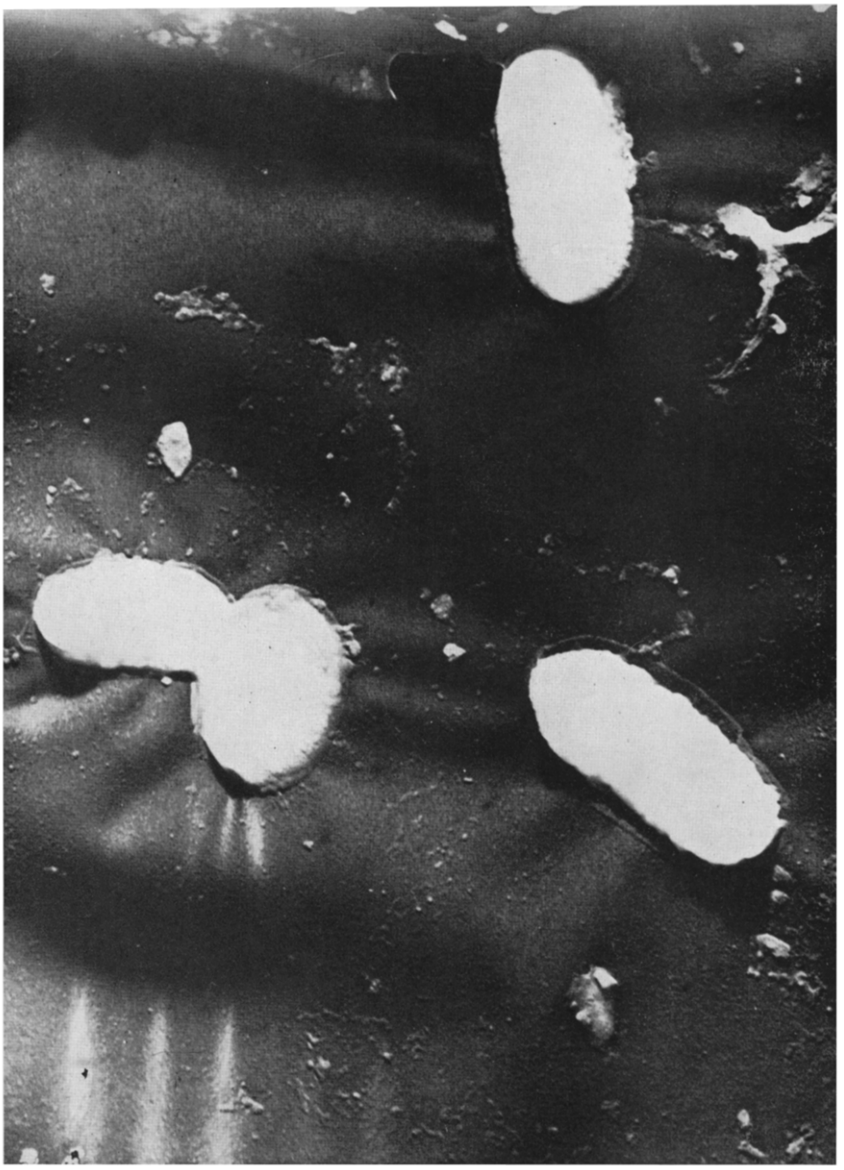

Fig. 2. *Escherichia coli* suspension heated for 5 minutes at 55° C. (22,000 ×)

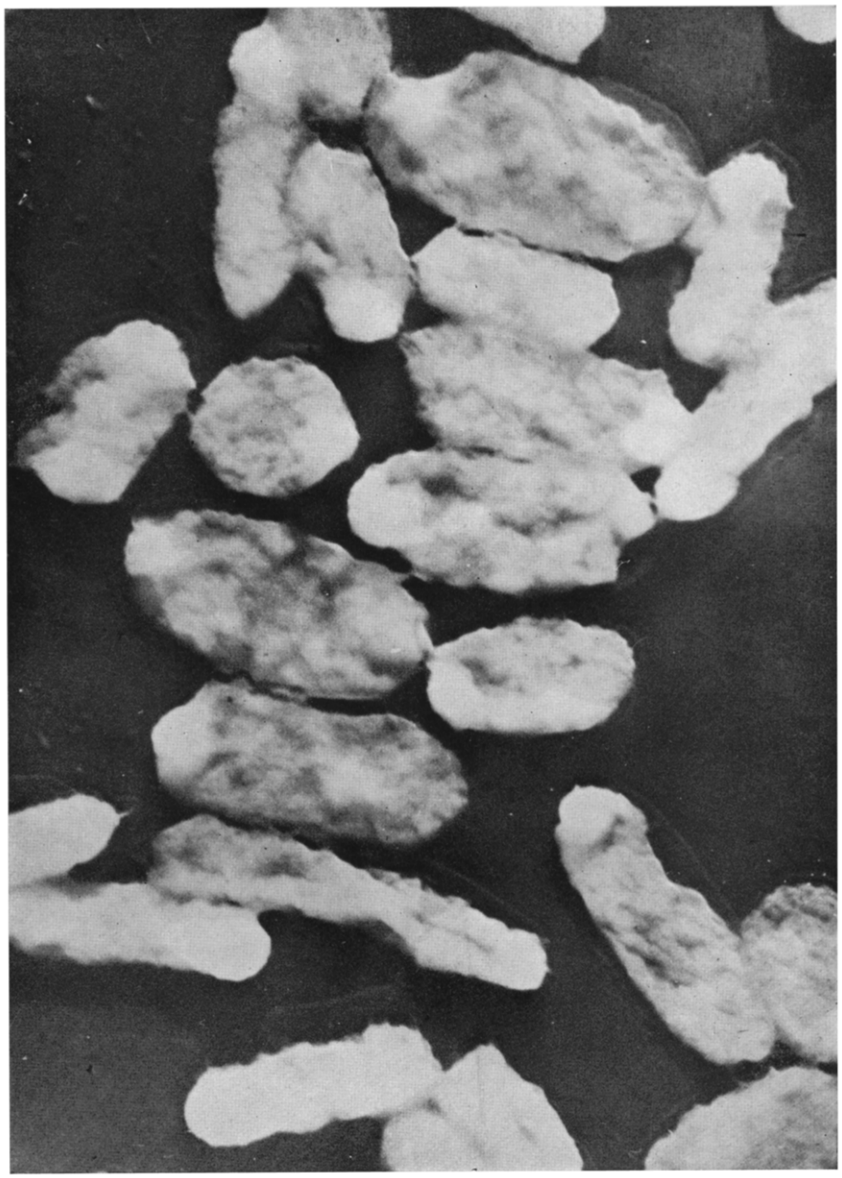

Fig. 3. *Escherichia coli* suspension heated for 5 minutes at 60° C. (19,500 ×)

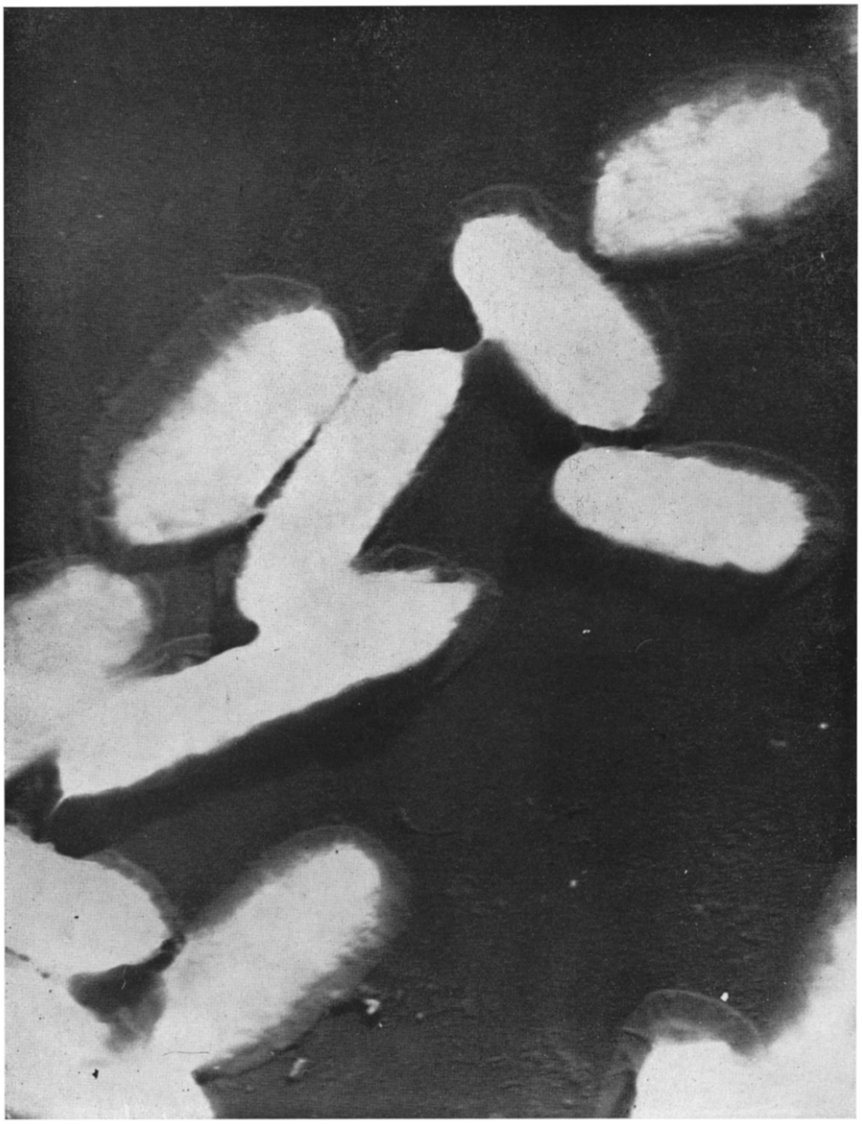

Fig. 4. *Escherichia coli* suspension heated for 5 minutes at 65° C. (25,000 ×)

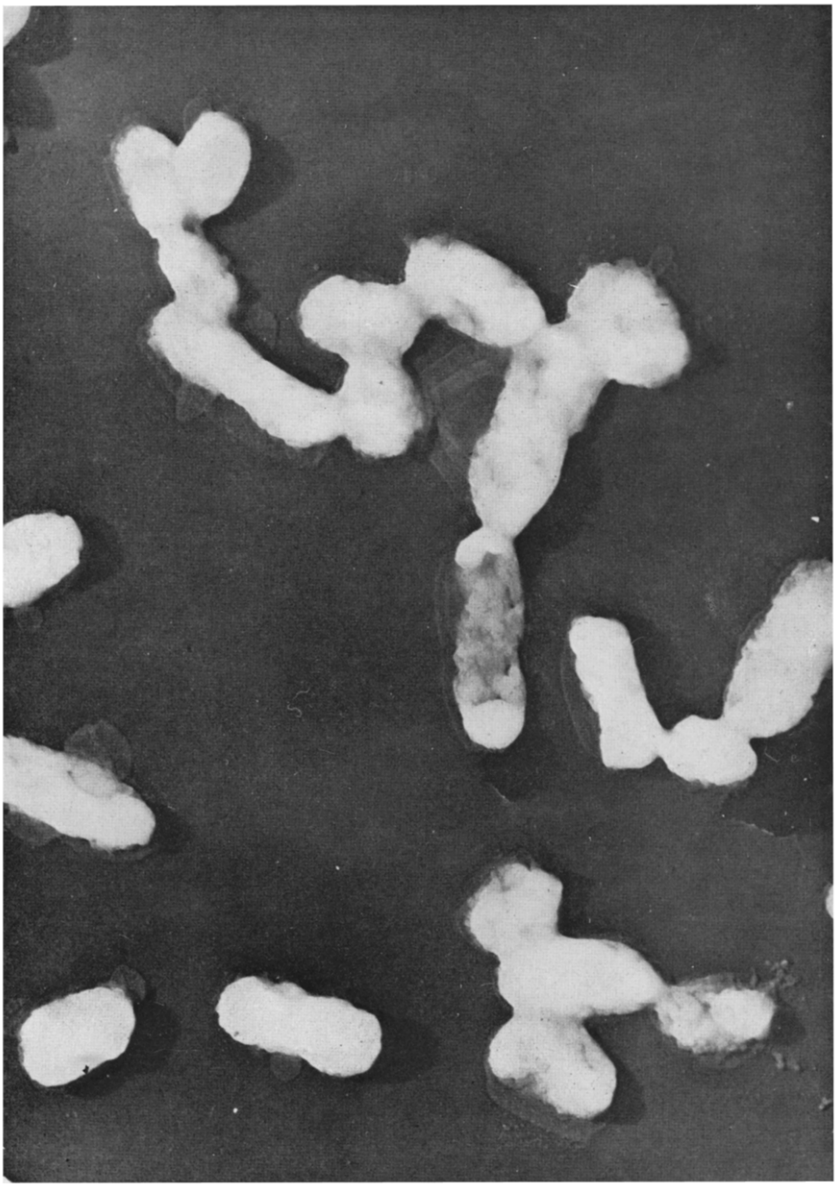

Fig. 5. *Escherichia coli* suspension heated for 5 minutes at 70° C. Shows rupture of some cell walls. (19,000 ×)

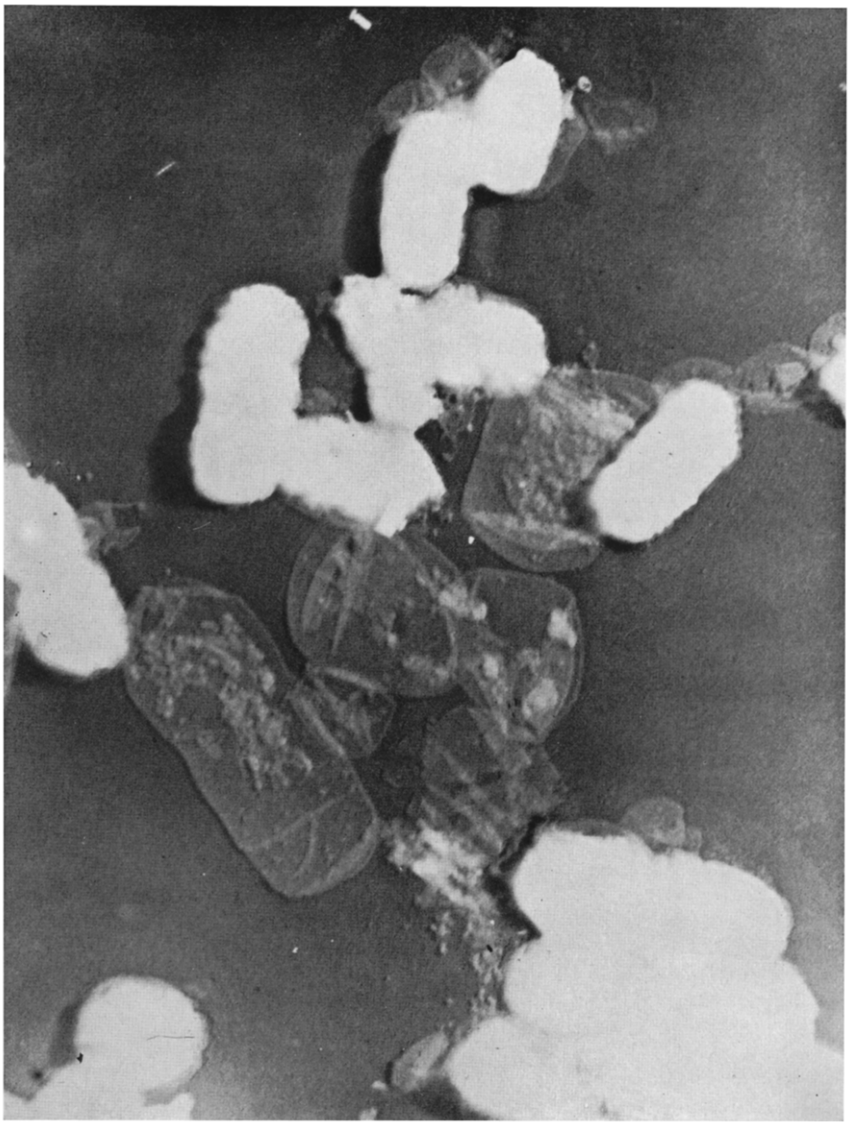

Fig. 6. *Escherichia coli* suspension heated for 5 minutes at 75° C showing many ruptured cell walls. (20,000 ×)

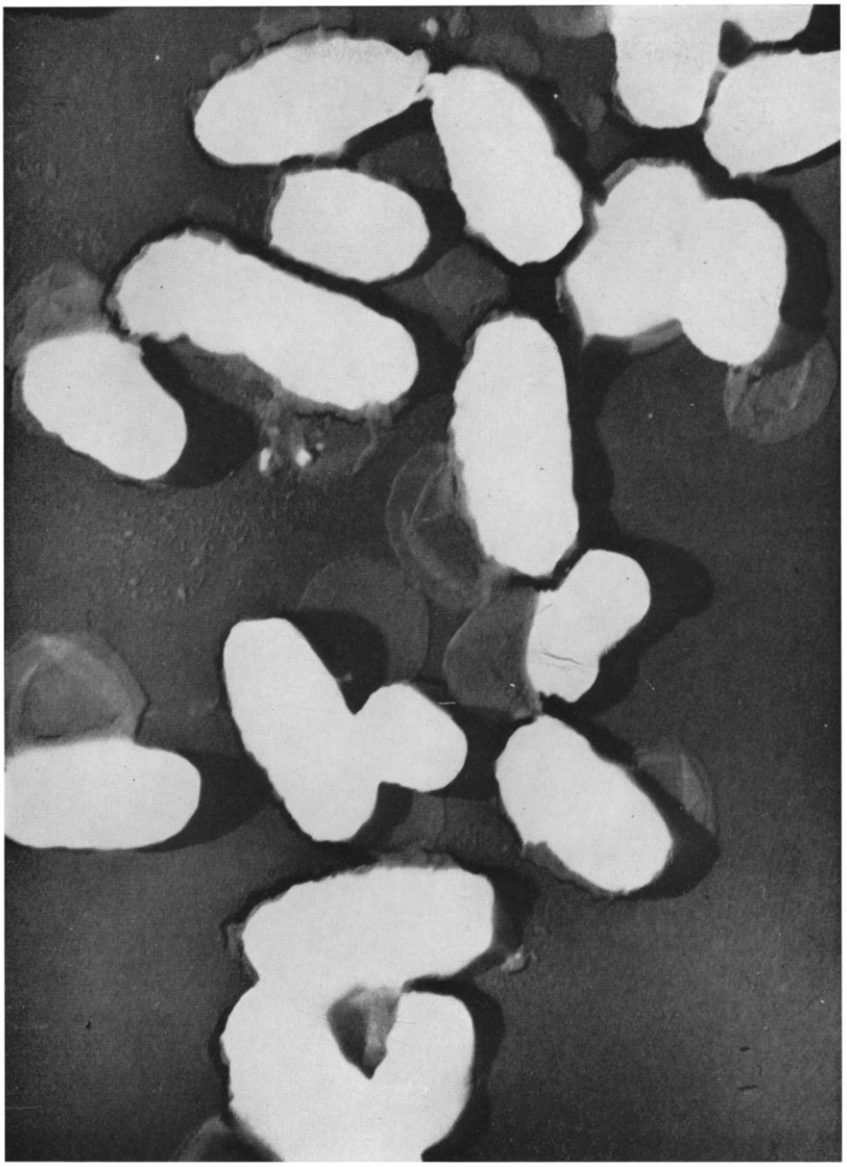

Fig. 7. *Escherichia coli* suspension heated for 5 minutes at 100° C. All cells appear to have ruptured walls. (22,000 ×)

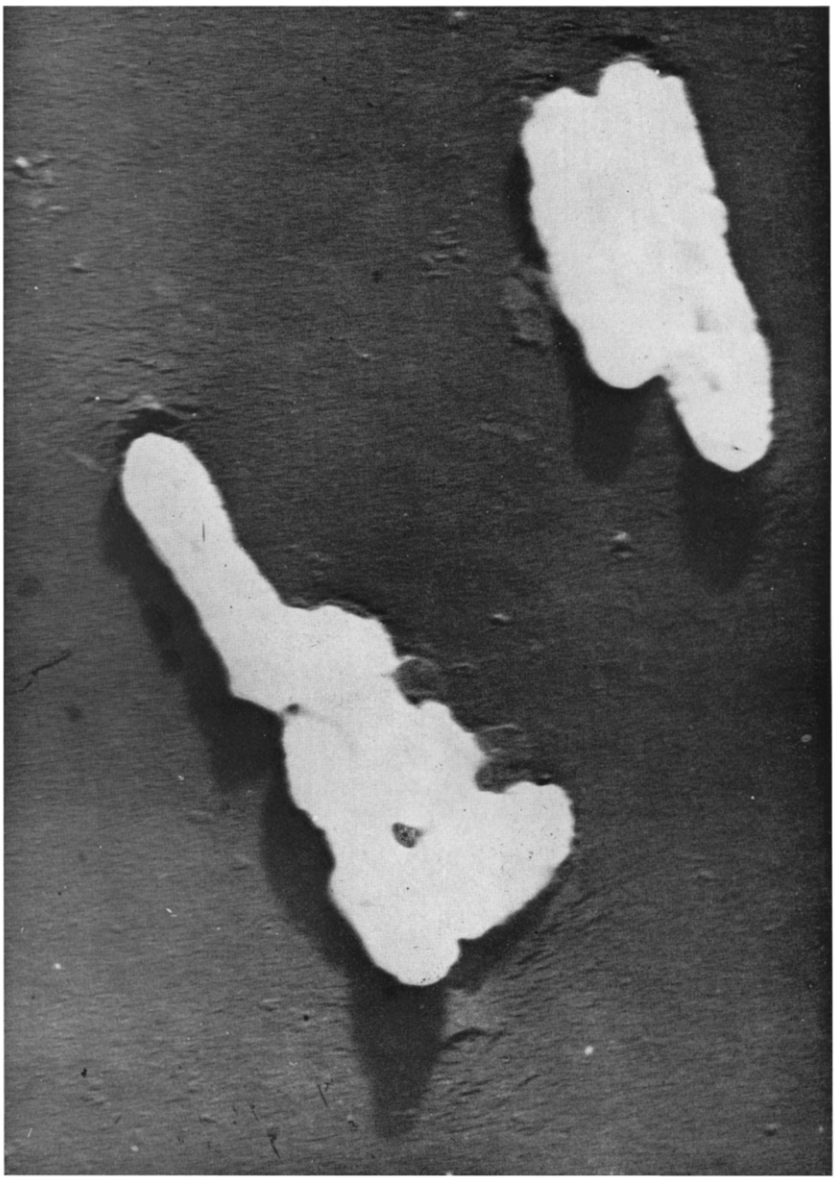

Fig. 8. *Escherichia coli* suspension heated for 5 minutes at 75° C. Electron micrograph shows cytoplasmic bodies deposited on centriftgation at 3,000 r.p.m. (22,500 ×)

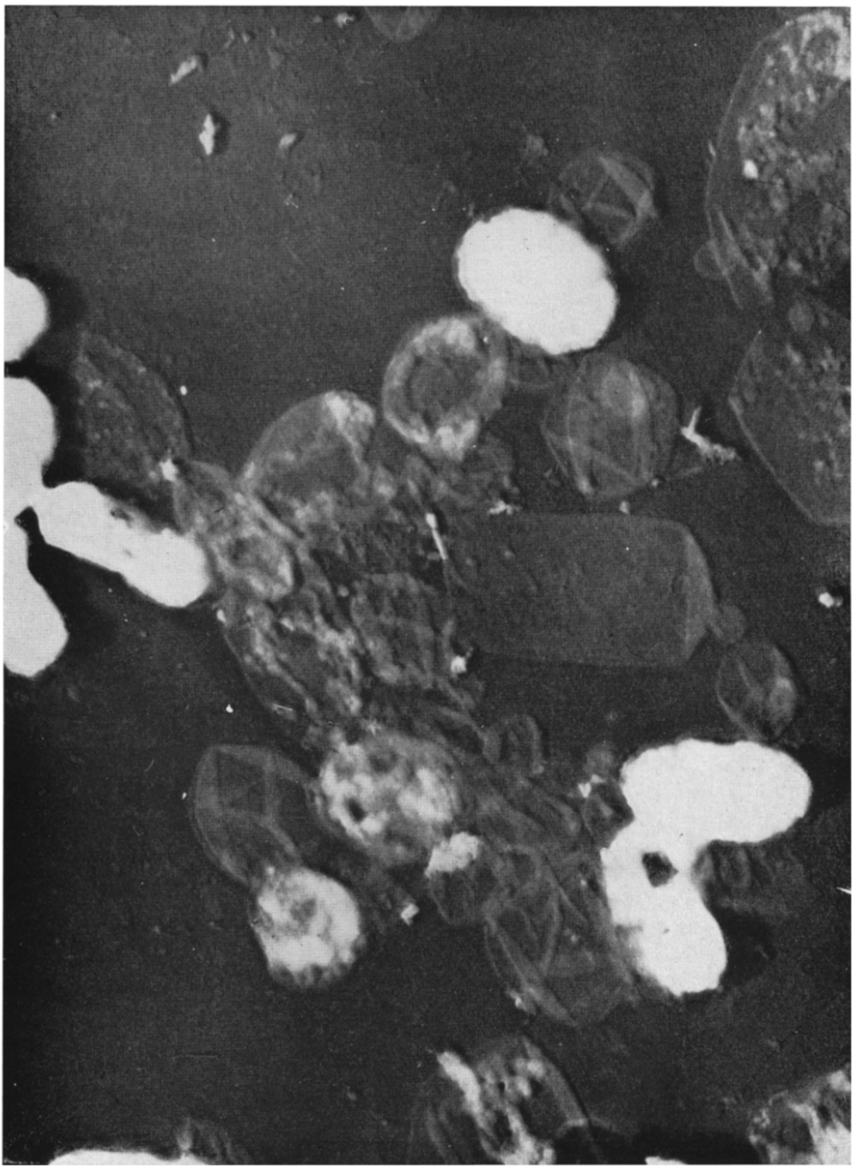

Fig. 9. *Escherichia coli* suspension heated for 5 minutes at 75° C. Shows cell walls deposited on centrifugation at 10,000 r.p.m. as described in text. (22,000 ×)

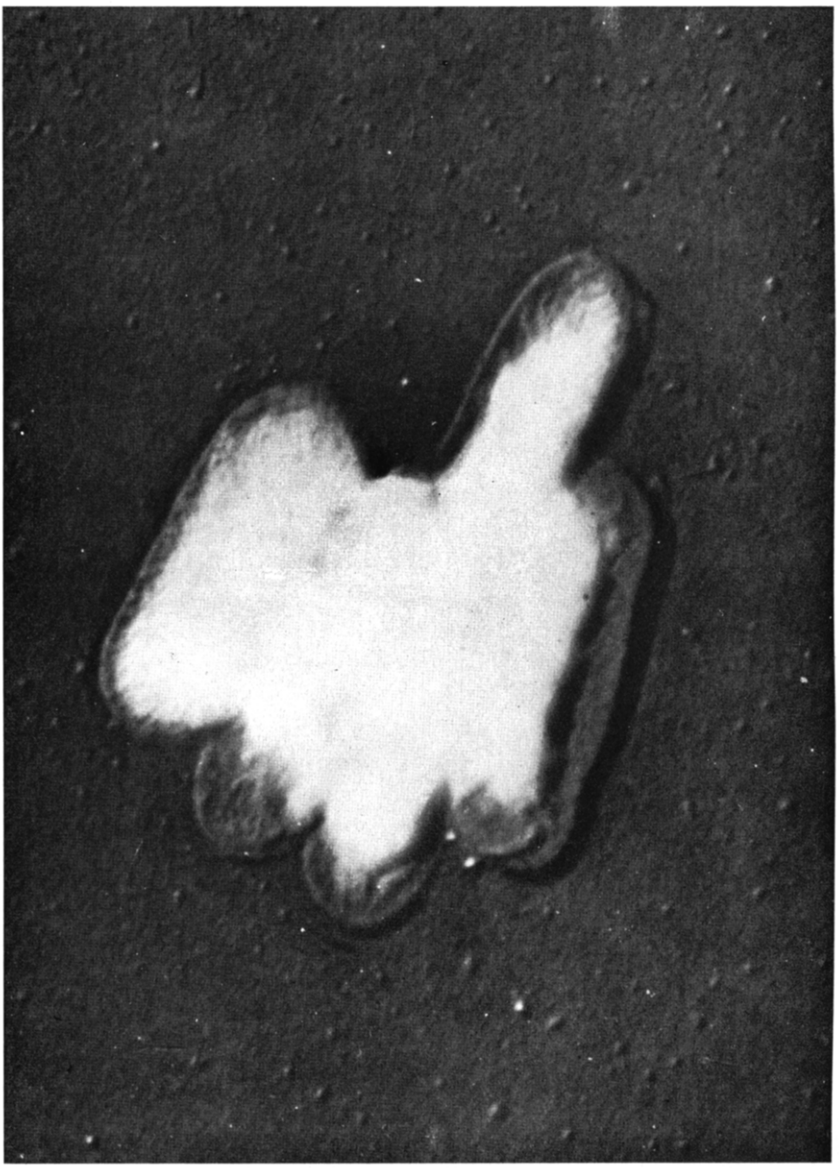

Fig. 10. *Salmonella pullorum*. Washed suspension of cells from 16 h culture. (26,500 ×)

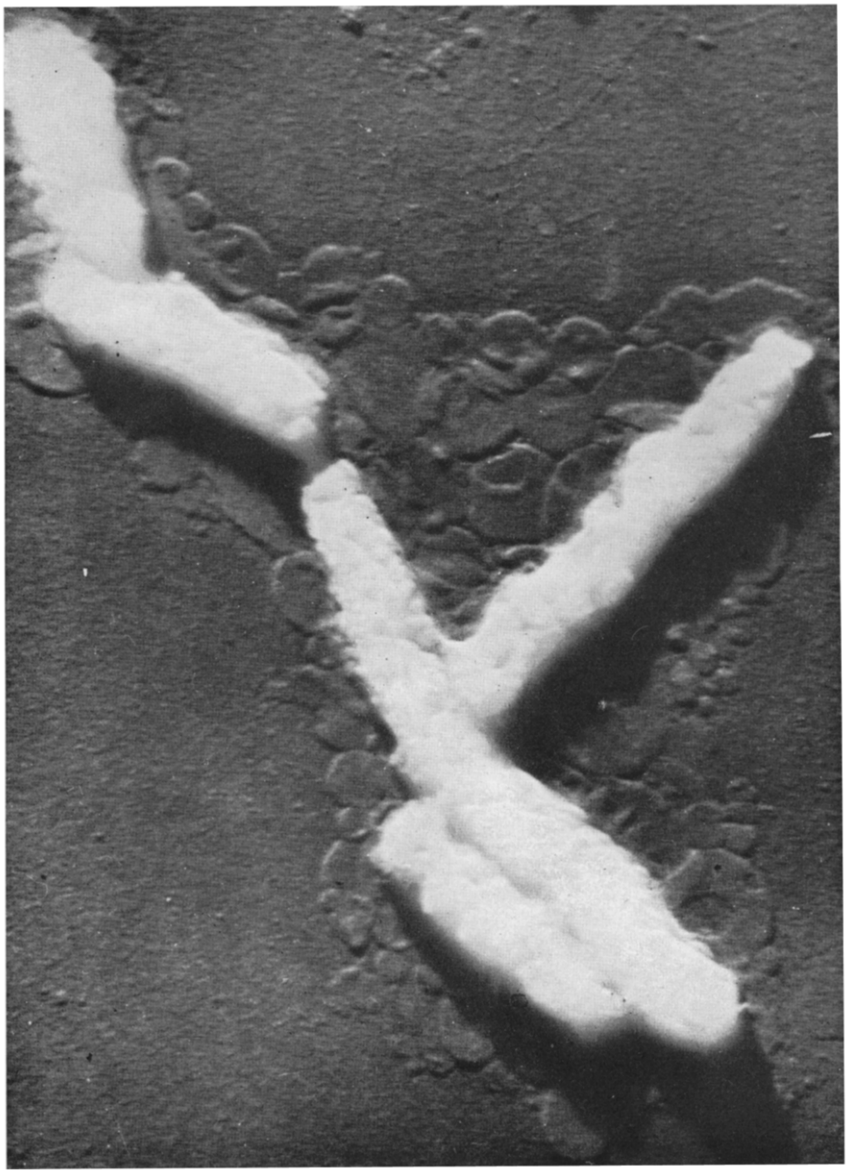

Fig. 11. *Salmonella pullorum* suspension heated for 5 minutes at 75° C. Ruptured cell walls adhering to cytoplasmic bodies. (22,500 ×)

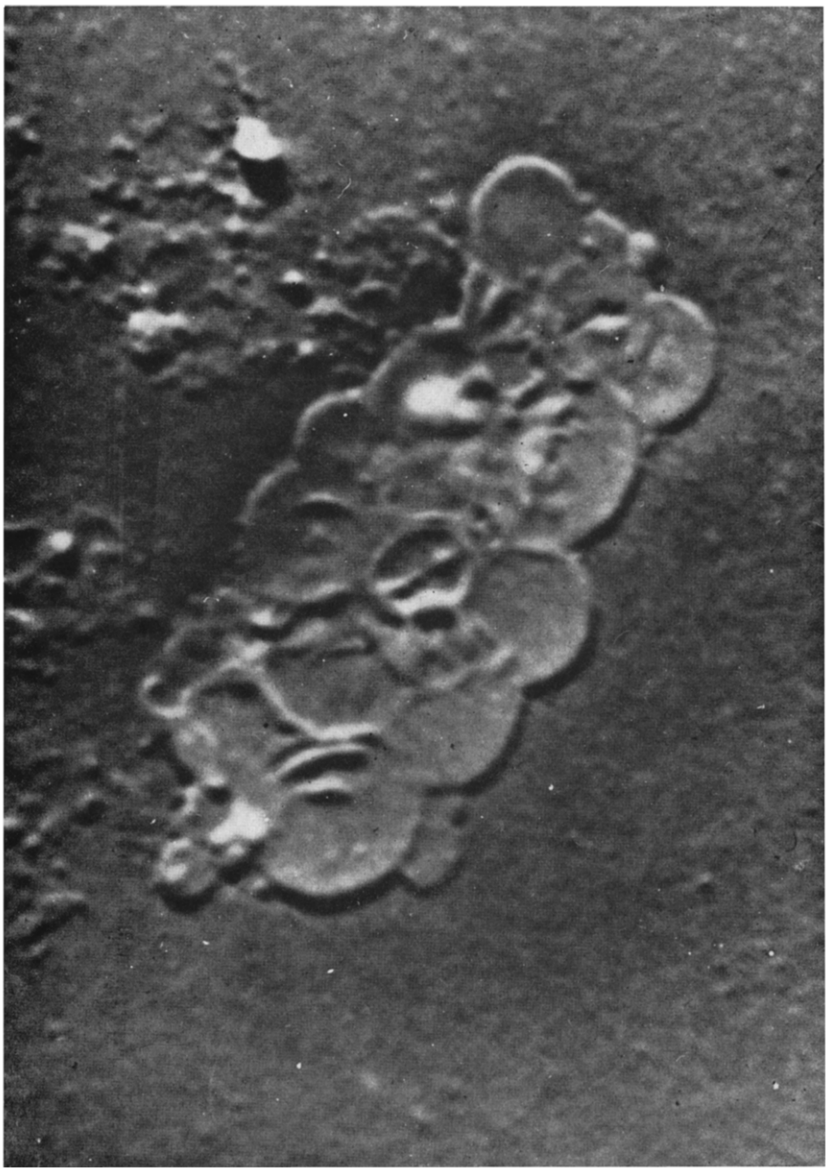

Fig. 12. *Salmonella pullorum*. Circular fragments of cell walls from suspension heated at 75° C. (25,000 ×)

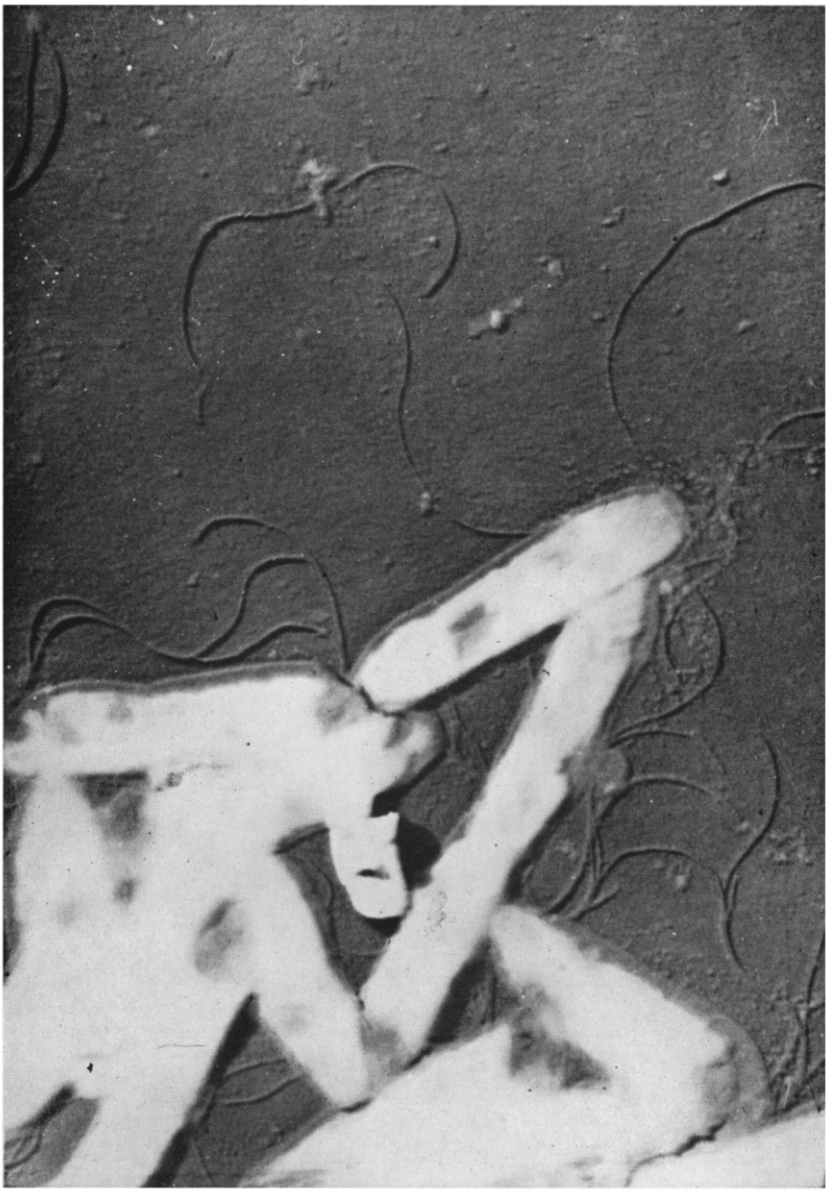

Fig. 13. *Pseudomonas fluorescens*. Washed suspension of cells from 16 h culture. (19,500 ×)

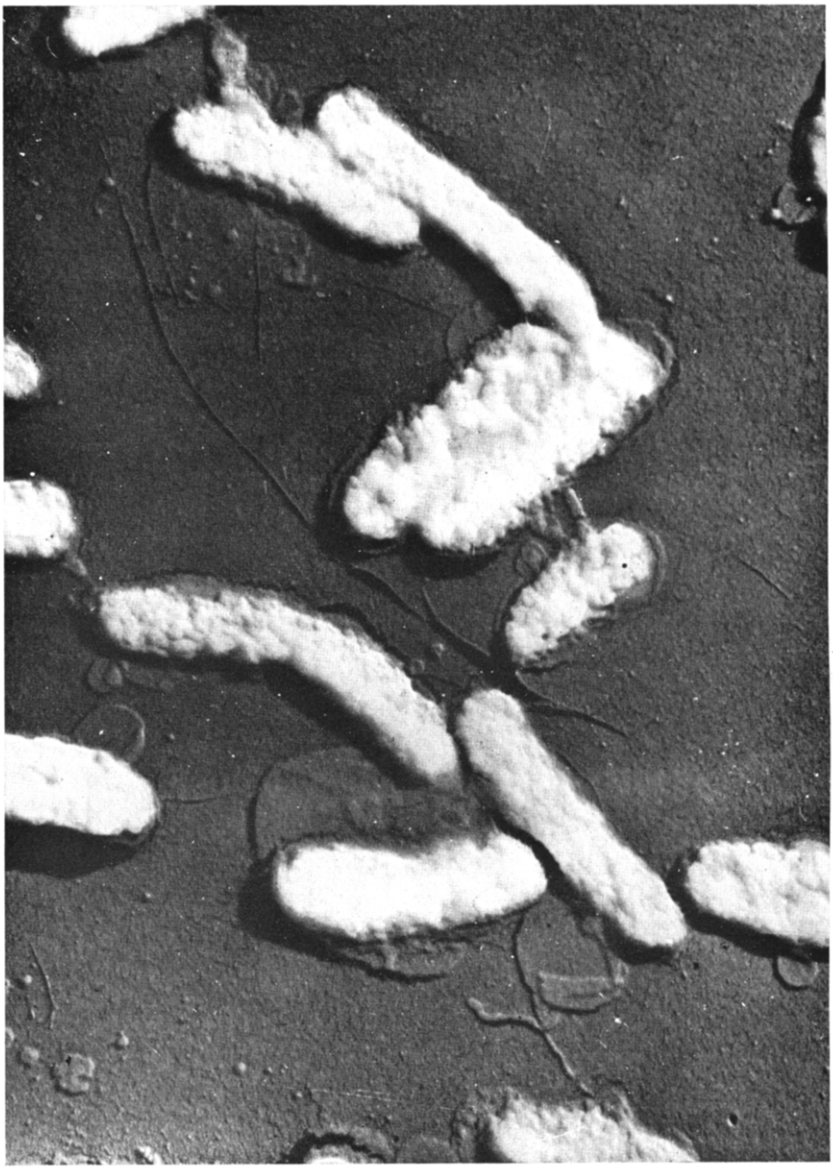

Fig. 14. *Pseudomonas fluorescens* suspension heated for 5 minutes at 75° C. (20,000 ×)

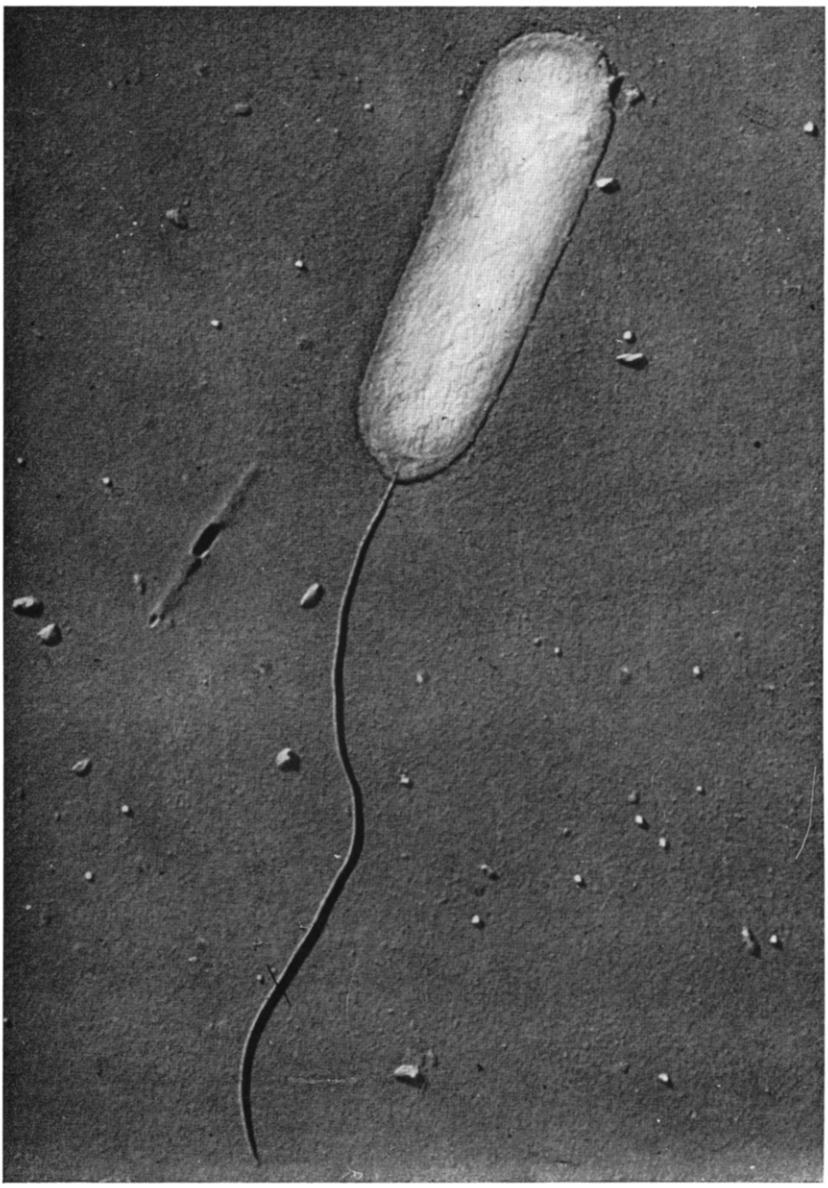

Fig. 15. *Pseudomonas pyocyanea.* A typical cell from washed suspension of 16 h culture. (25,500 ×)

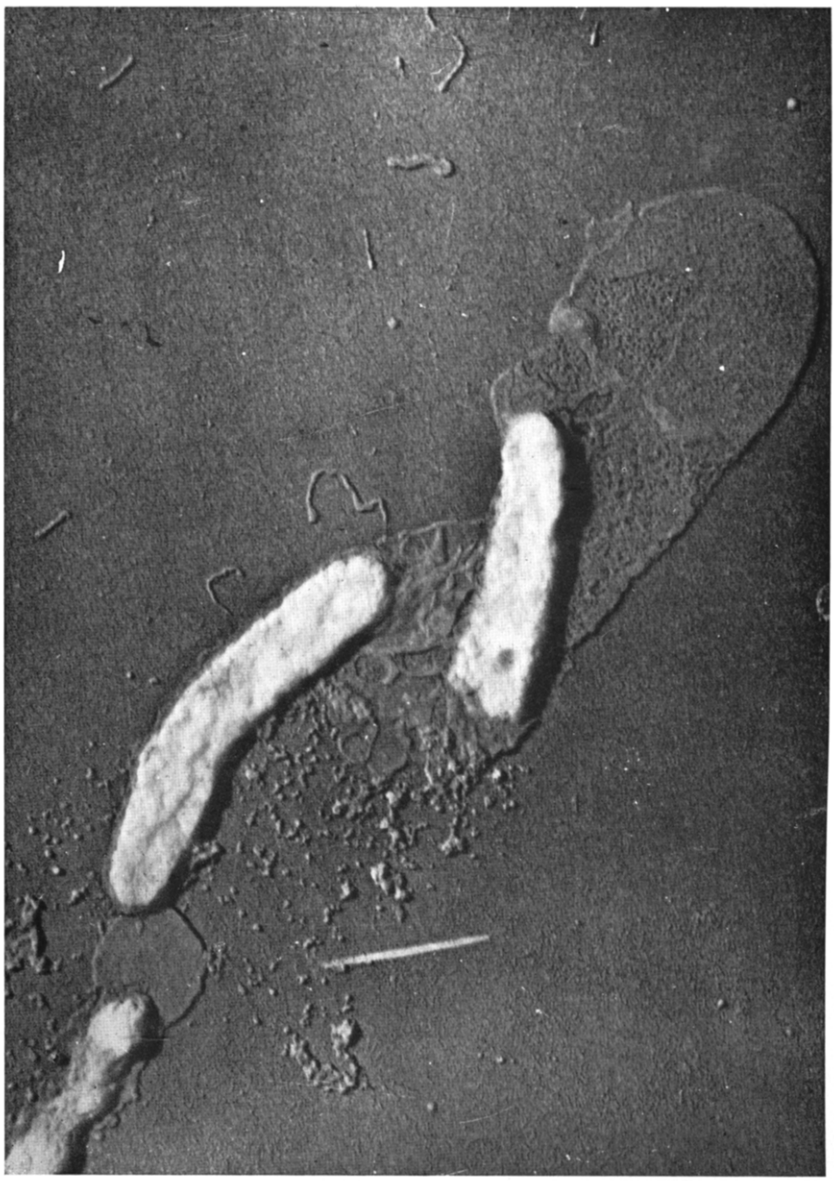

Fig. 16. *Pseudomonas pyocyanea* suspension heated for 5 minutes at 75° C. Note cell wall rupture and detached flagella. (19,000 ×)

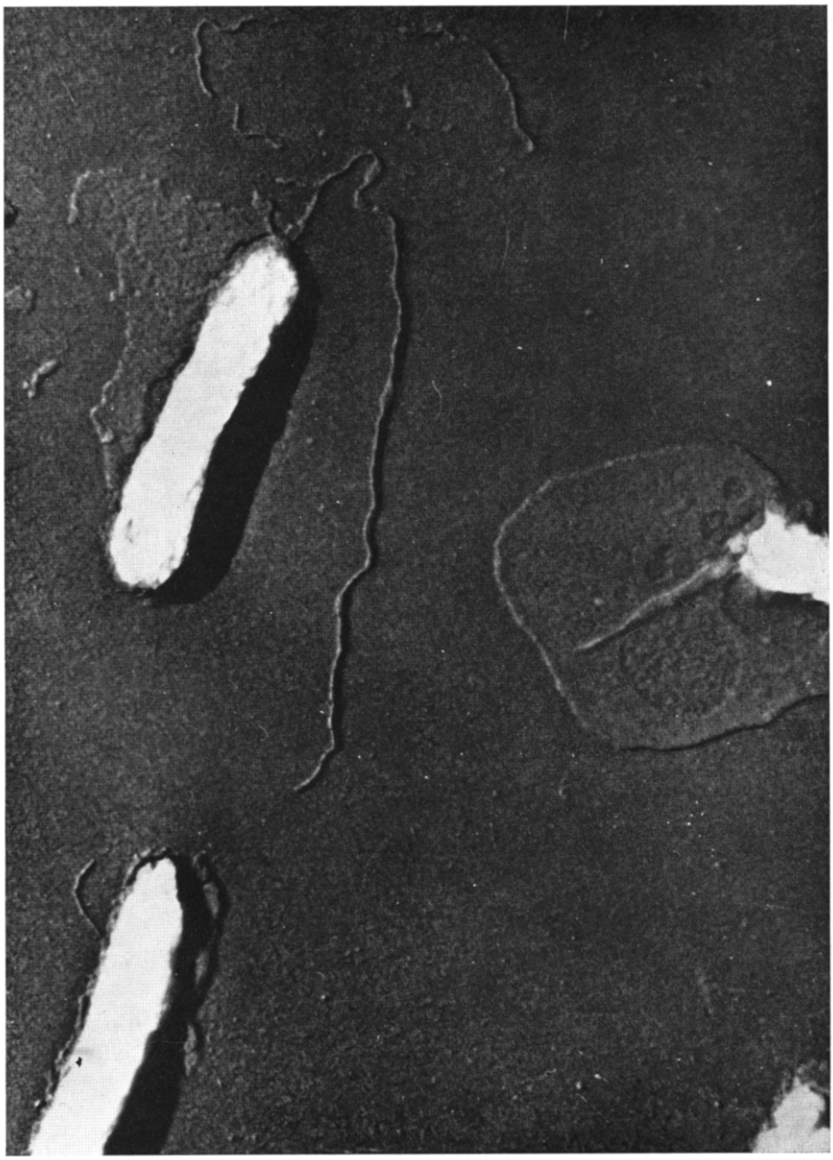

Fig. 17. *Pseudomonas pyocyanea* suspension heated for 5 minutes at 75° C. Note terminal flagellum which appears to be attached to the cytoplasmic body. (19,000 ×)

Fig. 18. *Streptococcus faecalis*. Washed suspension of cells from 16 h culture. (27,000 ×)

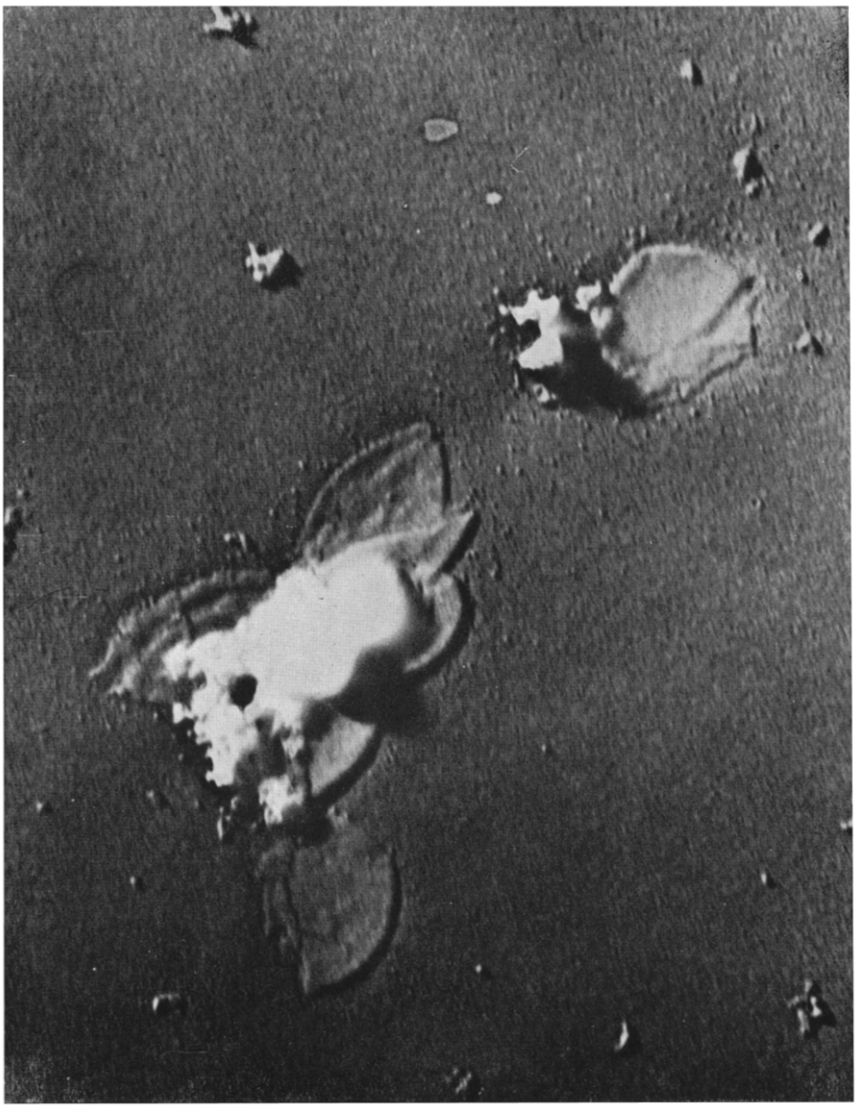

Fig. 19. *Streptococcus faecalis* suspension heated for 5 minutes at 100° C showing appearance of ruptured cell walls. (23,000 ×)

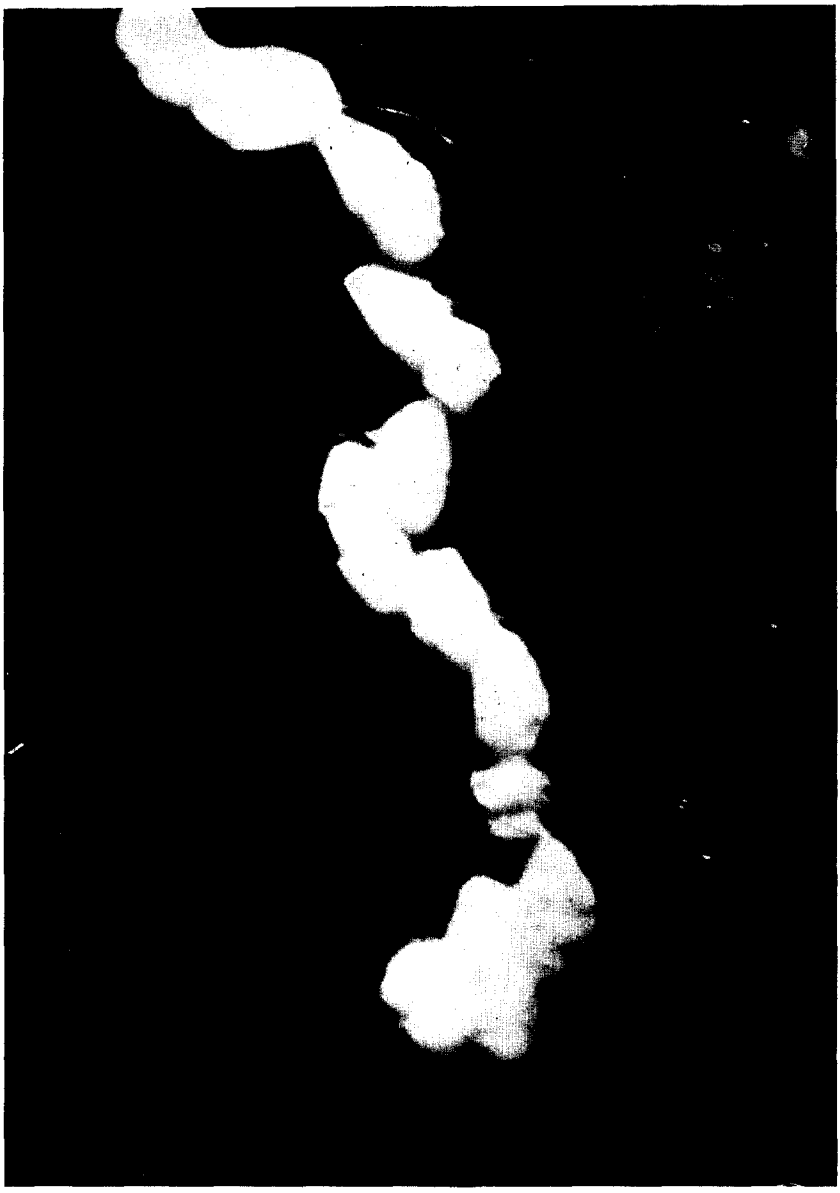

Fig. 20. *Streptococcus faecalis* suspension heated for 5 minutes at 100° C showing mostly intact cell walls. (28,000 ×)

bacterial species to temperatures ranging between 70–75° C for 5 min results in the rupture of their cell walls and marked granulation of the cytoplasm. The effectiveness with which heat causes rupture of the cell wall would appear to vary from one organism to another, since rupture had occurred in only a small proportion of cells of *Strep. faecalis* heated at 100° C. These observations would appear to offer a reasonable explanation for the findings of KANTOROWICZ[11], who found that undamaged cells of a number of organisms were resistant to pepsin and trypsin, but that the Gram-negative species became susceptible to digestion if they were first heated to 70° C.

It is of interest to note that the cytoplasmic bodies of the rod-shaped organisms, *Esch. coli*, *Salmonella pullorum* and the two *Pseudomonas* species, have retained the original shape of the organism. This could be explained by the existence of another "membrane" enclosing the cytoplasm, or by coagulation of the cytoplasm by the heat-treatment, or by a combination of both. With the two flagellated species there were instances when flagella appeared to be attached to the cytoplasmic bodies (*e.g.* Fig. 17). Although the heat-treatment is a drastic procedure, it is perhaps significant that in no instances did the flagella appear to be attached to the ruptured cell walls. Rupture of the cell wall by heat-treatment may provide some evidence as to the place of origin of the flagella, a question discussed by HOUWINK AND VAN ITERSON[8].

Many of the ruptured cell walls observed in these studies appear to have fragmented into roughly circular discs. This was especially noticeable with the cell walls from *Salmonella pullorum* (see Fig. 12). JOHNSON, ZWORYKIN AND WARREN[12] reported that ghosts of cytolysed luminous bacteria "reveal a mosaic density, with relatively transparent, roughly circular, or slit-like areas". HILLIER, MUDD AND SMITH[13] have also observed elliptical and circular segments of cell wall in phage-lysed preparations of bacteria. As pointed out by HILLIER *et al.*[13], it seems probable that the intact cell wall may be composed of a mosaic of these circular segments, which become apparent under stress of phage-lysis or stress due to heat-treatment of bacterial cells.

The cell walls observed on heat-treatment of bacteria have features in common with those observed in studies of phage-lysis, sonic disintegration and disintegration of bacteria with minute glass beads. In all instances the cell walls appear to be very thin transparent structures possessing properties of elasticity and rigidity.

ACKNOWLEDGEMENTS

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SUMMARY

Observations of the effects of heating bacterial suspensions have been carried out with the electron microscope.

1. Suspensions of *Esch. coli* have been subjected to temperatures ranging from 55–100° C;the electron micrographs show that marked granulation and shrinkage of the cytoplasm has occurred at temperatures below 70° C, but at 70–100° C these effects are accompanied by rupture of the cell wall.

2. Similar results were obtained with *Salmonella pullorum*, cell wall rupture being apparent at 70–75° C.

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3. Suspensions of *Ps. fluorescens* and *Ps. pyocyanea* heated at 75° C both showed rupture of the cell walls. There was no evidence of attachment of flagella to the cell walls.

4. Only a small proportion of the cell walls of *Strep. faecalis* had ruptured as a result of being heated at 100° C.

RÉSUMÉ

Nous avons observé au microscope électronique les effets du chauffage sur des suspensions de bactéries.

1. Des suspensions de *Esch. coli* ont été soumises à des températures allant de 55–100°; les micrographies électroniques montrent qu'une granulation et un rétrécissement marqués ont eu lieu à des températures au-dessous de 70°, mais que, à 70–100°, ces effets sont accompagnés de la rupture de la paroi cellulaire.

2. Nous avons obtenu des résultats semblables avec *Salmonella pullorum*, la rupture de la paroi cellulaire apparaissant à 70–75°.

3. Des suspensions de *Ps. fluorescens* et de *Ps. pyocyanea* chauffées à 75° montraient toutes deux la rupture des parois cellulaires. Il n'y avait aucun signe d'attachement de flagelles aux parois cellulaires.

4. Seule une faible proportion des parois cellulaires de *Strep. faecalis* étaient rompue saprés chauffage à 100°.

ZUSAMMENFASSUNG

Bakteriensuspensionen wurden erhitzt und die Folgen mit Hilfe des Elektronenmikroskopes beobachtet.

1. Suspensionen von *Esch. coli* wurden Temperaturen von 55–100° unterworfen; die Elektronenmikrographien zeigten, dass eine bedeutende Granulation und Schrumpfung des Cytoplasmas bei Temperaturen unter 70° stattgefunden hatte, aber diese Effekte waren bei 70–100° vom Zerreissen der Zellwand begleitet.

2. Ähnliche Ergebnisse wurden mit *Salmonella pullorum* erhalten; die Zellwand war bei 70–75° sichtbar zerrissen.

3. Beim Erhitzen auf 75° zeigten Suspensionen von *Ps. fluorescens* und *Ps. pyocyanea* beide zerrissene Zellwände. Es war kein Anzeichen eines Anhaftens von Geisseln an der Zellwand vorhanden.

4. Nur ein kleiner Teil der Zellwände von *Strep. faecalis* war nach Erhitzen auf 100° zerrissen.

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