DEMONSTRATION, WITH THE ELECTRON MICROSCOPE, OF A NUCLEUS IN *BACILLUS MYCOIDES* GROWN IN A NITROGEN-FREE MEDIUM

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Although the bacterial cell has been extensively investigated with the electron microscope, only a few papers have dealt with the problem of the nucleus.

Piekarski (1939) stated that the nucleus like structures or nucleoids demonstrable with the Feulgen reaction and with the ultraviolet microscope in *Eberthella typhosa*, *Escherichia coli*, *Serratia marcescens*, *Proteus*, and *Pseudomonas aeruginosa* may be seen with the electron microscope in individual cells of most old cultures, but rarely in cells from young cultures. In old cultures, the cell contains a single, central nucleoid; in young cultures there is a nucleoid at each end of the cell. Piekarski and Ruska (1939) confirmed these conclusions, but found little structural differentiation in the cells of *Sarcina alba*, *Sarcina lutea*, and *Bacillus subtilis*.

Knaysi and Mudd (1943) studied a number of bacteria at accelerating potentials varying from 60 to 200 kv. At 60 kv, the young cells of *Staphylococcus flavo-cyaneus*, *Staphylococcus aureus*, and *Streptococcus pyogenes* appeared homogeneously opaque to the electrons; those of *Neisseria gonorrhoeae* were homogeneous and semitransparent; those of *Brucella abortus* and *Pasteurella pestis* showed a homogeneous, transparent protoplasm containing one to several bright vacuoles. Old cells of the latter two organisms showed a transparent protoplasm containing one or two opaque bodies of unknown nature. Mature cells of *Neisseria meningitidis* had a transparent protoplasm containing one opaque body per cell; this body was insoluble in water at 80 C, indicating that it was not volutin. Treatment of the opaque cells with dilute sodium bicarbonate obscurely revealed the presence of internal bodies in *Staphylococcus flavo-cyaneus*, but not in *Staphylococcus aureus*. With the high-voltage microscope, cells of *S. flavo-cyaneus* from a 2-day-old culture were transparent and contained beautiful, often paired, opaque bodies identified as the nuclei previously demonstrated by Knaysi (1942). Cells from young cultures were either less transparent with poorly defined nuclei or homogeneously opaque. The cells of *Streptococcus pyogenes* became transparent but showed no internal differentiation, although it was reported (Sevag, Smolens, and Lackman, 1940) that they contained relatively large quantities of thymonucleic acid. On the basis of these observations, Knaysi and Mudd concluded that bacteria contain nuclear material which, depending on conditions yet unknown but probably related to environment and development, may be diffuse in the protoplasm or may be partly or totally differentiated into a nucleus.

Baylor, Appleman, Sears, and Clark (1945) studied the internal structure of *Rhizobium leguminosarum* with and without previous treatment with various solvents and nuclear stains. Untreated cells and cells stained with Heidenhain's hematoxylin contained poorly visible opaque bodies. Hydrolysis with HCl, application of the Feulgen technique, or staining by several other procedures caused distortion and increase in the opacity of the protoplasm. Only in cells treated with dilute sodium bicarbonate were the internal bodies clearly visible. These bodies were soluble in acetone and in 2 per cent nitric acid. The authors concluded, however, that they may be nuclei.

The method used in this investigation is based on observations recently made by the senior author. It was shown (Knaysi, 1945a) that the endospore of *Bacillus mycoides* contains a relatively considerable quantity of a substance which is an excellent source of nitrogen but not a source of energy. More recently (Knaysi, 1946c), it was found that the volutin inclusions of the yeast *Hansenula anomala* are readily utilizable as a source of nitrogen but not for energy. Reinvestigation of germinating spores of *Bacillus mycoides* showed that the source of nitrogen they contain gives microchemical reactions of volutin, which is chiefly ribonucleic acid (Delaporte, 1939). When the spores are heavily planted in a nitrogen-free medium, many germinate; the nucleic acid is gradually used up and soon disappears from the vegetative cells. These cells have a low density, low refringence, and a low affinity for dyes. Fixed by heat and mounted in a film of an aqueous methylene blue solution of pH 3.5 to 4.0, they show deeply stained bodies in a faintly stained cytoplasm, a behavior similar to that of *Hansenula anomala*. When this yeast is grown in a glucose solution and the cells observed as described above, only the nucleus is deeply stained. At pH 3.0, the cells of both remain unstained. Since we have suspected for some time that homogeneous staining of young cells and their homogeneous opacity to the electrons may be due to the same cause, it seemed desirable to find out whether or not cells of usually opaque bacteria that show internal differentiation with the light microscope when grown in a nitrogen-free medium would also show differentiation with the electron microscope.

ORGANISM AND METHOD

We selected, for this study, strain C₂ of *Bacillus mycoides*, on which the preliminary observations reported above were made. At 50 kv, the protoplasm of young cells of this organism grown on nutrient agar appears homogeneously opaque (figure 1); at 150 kv, it becomes homogeneously transparent (figure 2).

The nitrogen-free medium first used had the following composition: glucose, 0.2 g; equimolar mixture of KH₂PO₄ and K₂HPO₄, 0.23 g; distilled water, 100 ml. The spores were harvested from an agar slant culture, washed several times with sterile, distilled water, and resuspended in similar water. Enough of the heavy suspension was inoculated into the nitrogen-free medium to produce a definite haze. After various periods of incubation, the cells were centrifuged out, resuspended in distilled water, mounted on collodion, and examined in the usual manner with the high-voltage microscope. Cells thus obtained were highly vacuolated,

FIG. 1. Sister cells from a nutrient agar slant; culture $4\frac{1}{2}$ hours old at 35 C; 50 kv. $\times 23,500$.

FIG. 2. Cells grown on a nutrient agar slant; culture $51\frac{1}{2}$ hours old at 37 C; 150 kv. $\times 26,000$.

FIG. 3. Cells from a 17-hour-old culture in the glucose-phosphate medium, at room temperature. Note the many vacuoles and the several nuclei. In *a* the arrow points to a dividing nucleus. In *b*, note also the dense slime layer, the cell wall, and the cytoplasmic membrane. Both cells photographed at 150 kv. $\times 25,000$.

FIG. 4. Cells grown on a nutrient agar slant; culture 5 hours old at 37 C. Smear was heat-fixed and treated with a 0.5 per cent solution of sodium bicarbonate for 90 minutes at room temperature; 50 kv. $\times 15,000$.

Length of scales = 1μ .

FIG. 5. Two germ cells from a 16-hour-old culture, at room temperature, in the glucose-acetate medium. Note the transparent cytoplasm and the two types of bodies; 50 kv. $\times 23,500$.

FIG. 6. Vegetative cells showing evidence of growth. Culture, voltage, and magnification as for figure 5.

FIG. 7. Vegetative cells one of which appears to bear flagella. Note also two other flagella. Culture, voltage, and magnification as for figure 5. Note also nuclei and bodies of type B; the structure of the latter can be clearly seen in the two extreme cells.

Length of scales = $1\ \mu$.

encapsulated, and contained each 5 or 6 opaque bodies sometimes showing evidence of division (figure 3). On the other hand, the cells were not readily cen-

trifuged out and often became opaque again, resynthesizing nucleic acid from the glucose, the phosphate, and nitrogen probably liberated by autolysis. This was avoided by substituting sodium acetate for potassium phosphate in the medium. This medium has an initial pH of about 7.4, and the pH remains in the neighborhood of 6.8 even several days after inoculation. It is buffered dynamically; the acids produced, chiefly from the glucose, are continuously neutralized by the NaOH formed when the acetate radical is utilized. In this medium, the cells grow normally, free of vacuoles and slime, and many complete the life cycle; their protoplasm is so transparent that they have to be observed at 50 or 30 kv (figures 5 to 20).

OBSERVATIONS AND CONCLUSIONS

In the glucose-acetate medium many of the spores germinate (figure 5), and germination is followed by growth of the vegetative cells (figure 6); these may be motile (observation with the light microscope) and bear flagella (figure 7); they contain two types of internal bodies (figure 8) distinct from each other by the degree of their opacity, their size, apparent form, and their behavior. Both types are present at all stages of development (figures 5, 8, and 9).

Type A consists of large, highly opaque bodies, 0.22 by 0.29 μ to 0.43 by 0.50 μ . There may be 1 to 6 of these in a cell. A body of this type sometimes seems to be surrounded by a membrane which may be an artifact due to drying, but there is no sign of internal differentiation (figures 10 and 11). Evidence of division is frequent, but the manner in which division takes place is not yet clear; sometimes one observes two bodies in close proximity separated by a dark streak as broad as the bodies themselves and resembling nuclei in early telophase (figures 8, 11, and 12); at other times it appears as if a body, lying in the middle of a cell, has been split in two by division of the cell, although it may be possible that division of the body preceded that of the cell (figures 14 and 15). We never saw what may be considered as *clear* evidence of division by simple constriction. Further study is necessary before the mechanism of division can be definitely described.

When the cell is ready to form a spore, one observes a faintly delimited forespore with these bodies grouped usually at each of its poles (figures 9, 13, and 16); occasionally, one or more bodies may be located elsewhere (figures 17, 18, and 19). In a sporangium that contained only two of these bodies, one is located at each pole of the forespore (figures 9 and 13). One rarely observes a body of this type, originally present in the mother cell, that does not become enclosed in the forespore. Almost always, the forespore seems to appear in its full size and characteristic form. Nevertheless, isolated observations (figures 13 and 20) seem to suggest the following possible sequence: Two bodies, or groups of bodies, move to a definite distance from each other; an area, slightly denser than the cytoplasm of the mother cell, appears to grow from each body, or group of bodies, toward the other; the forespore results from the merging of these two areas. We never recognized a forespore containing only one body. The length of the forespore is determined by the relative position of the two bodies, or groups of bodies, which seem to produce it, and this is not changed by subsequent development. However, the

FIG. 8. Vegetative cells from a culture in the glucose-acetate medium, 16 hours old at room temperature. At the lower end of cell *a*, there are two nuclei separated by a broad, dark zone, the whole resembling nuclei in early telophase. Note absence of constriction. All cells show numerous bodies of type *B*, the structure of which is best observed with a hand lens. A few of these appear free in the background and probably originated from autolyzed cells. Photographed at 50 kv. $\times 48,000$.
Length of scale = 1μ .

FIG. 9. Sister cells from a culture, two days old at room temperature, in the glucose-acetate medium. Note a young forespore in each cell, the position of the nuclei in the forespore, and the presence of bodies of type *B*. The bright spot in the upper cell is the image of the filament. Note the semicircular fringes of the split cross plate. Photographed at 50 kv. $\times 50,000$.

FIG. 10. Cell from a culture, 16 hours old at room temperature, in the glucose-acetate medium. Note possible evidence of a membrane around the nucleus. Photographed at 50 kv. $\times 48,000$.

FIG. 11. Culture as for figure 9. Upper end shows two neighboring nuclei joined by a broad, dark zone; the inner nucleus shows possible evidence of a membrane. Photographed at 50 kv. $\times 48,000$.

Length of scales = 1μ .

density of the forespore increases gradually. Mature spores were occasionally found in the medium used (figure 21), but the steps in the transition from a forespore to a spore were not observed.

FIG. 12. A cell from a culture, 2 days old at room temperature, in the glucose-acetate medium. Lower end contains what appears to be a dividing nucleus; 50 kv. $\times 48,000$.

FIG. 13. A flagellum-bearing cell. Culture 1 day + 16 hours old at room temperature in the glucose-acetate medium. Note the young forespore with a clue to its mode of formation; an earlier stage may be seen in figure 20. Note also position of the nuclei in the forespore. Photographed at 50 kv. $\times 47,000$.

Length of scales = 1μ .

In view of the fact that the function of a body is determined by its behavior, the observed behavior of these bodies of type A during the life cycle of *Bacillus mycoides* identifies them as the nuclei of the cell.

Type B (figures 7 and 8) consists of bodies, a few to about 40 per cell, smaller than the nuclei (0.08 by 0.1μ to 0.13 by 0.17μ) and much less opaque. Careful examination of many micrographs shows that they are extremely thin, each with a circular or elliptical outline consisting of a beaded thread, 40 to 60 A in thickness, and a central area which sometimes appears homogeneous but more

FIG. 14. Sister cells. Culture 1 day + 16 hours old at room temperature in the glucose-acetate medium. Note what appears to be division of the cell through the median nucleus. Photographed at 50 kv. $\times 23,500$.

FIG. 15. Sister cells. Culture 2 days old at room temperature in the glucose-acetate medium. Note what appears to be simultaneous division of cell and nucleus. Photographed at 50 kv. $\times 23,500$.

FIG. 16. A cell with a young forespore. Culture 2 days old at room temperature in the glucose-acetate medium. Note groups of nuclei and their polar positions in the forespore. Photographed at 50 kv. $\times 23,500$.

FIG. 17. Cell showing a forespore with a lateral nucleus. Culture, voltage, and magnification as for figure 16.

FIG. 18. Cell with a forespore containing 5 nuclei, 2 of which are polar. Culture 1 day old at room temperature in the glucose-acetate medium. Photographed at 50 kv. $\times 24,000$.

FIG. 19. Two cells each containing a forespore. In *a* the forespore almost fills the cells. Culture 2 days old at room temperature in the glucose-acetate medium. Photographed at 50 kv. $\times 24,000$.

Length of scales = 1μ .

often consists of minute granules and threads in a wide variety of patterns. The central area is often separated from the boundary line by a transparent ring, and may be surrounded by a second boundary line similar to the outer one and concentric with it. These bodies appear to be part of a background also consisting of a network of granules and beaded threads similar in dimensions but of much lower opacity to the electrons. Like the spots of a leopard, they are groups of dark elements in an otherwise similar background. Because of the large depth

FIG. 20. Cell showing two groups of nuclei and the beginning of forespore formation. Note the semiellipsoidal, dense region to the right of the pair of nuclei; a more advanced stage can be seen in figure 13. Culture 16 hours old at room temperature. Photographed at 50 kv. $\times 23,500$.

FIG. 21. A sporangium containing a mature endospore. Culture 4 days old at room temperature in the glucose-acetate medium. Photographed at 50 kv. $\times 13,000$.

FIG. 22. Culture, voltage, and magnification as for figure 21. Note ghost cells still showing bodies of type B. Photographed at 50 kv. $\times 13,000$.

Length of scales = 1μ .

of focus of the electron microscope, it is not possible to determine directly the location of these bodies in the cell. However, since they are present in autolyzed cells (figures 22 and 23c), and are semielliptical along the margin of the cytoplasm (figure 23), it may be concluded that they are in the cytoplasmic membrane. They do not visibly participate in the formation of the forespore as do the nuclei, nor do they move away from the forespore area (figure 9) as do the lipoprotein inclusions usually liberated into the cytoplasm before sporulation (Knaysi, 1945b, 1946b). Further discussion of these bodies will be found in the following section.

FIG. 23. Two normal vegetative cells (*a* and *b*), and one ghost cell (*c*), all showing marginal bodies of type *B* with semielliptical outlines, indicating that they are thin and present in the cytoplasmic membrane. Culture 16 hours old in the glucose-acetate medium. Photographed at 50 kv. $\times 35,500$. Length of scale = 1μ .

DISCUSSION

The observations reported in the previous section prove, we believe, the existence of one to several nuclei in the cell of *Bacillus mycoides*. The evidence does not rest solely on similarity in staining reactions, of doubtful specificity, between the bodies observed and the nuclei of more highly developed organisms, but on the behavior of these bodies during the life cycle of the organism, which is the only sure criterion of nuclear function: constancy of presence, evidence of division, and a leading role in the formation of the endospore. Since the only treatment the cells received was quick drying, it cannot be said that the observed bodies may have been coagulated nuclear material or other constituents of the protoplasm. Since they are readily demonstrable with the light microscope, it cannot be said that they were formed by the action of the electrons. We are aware, however, that the novelty of the procedure may raise certain questions regarding the viability of the cells investigated, the genuineness of the forespores, the possibility that the bodies we observed are mere inclusions or, granting that they are nuclei, that they would also be present in cells grown on ordinary culture media.

The first question can be answered by stating that cells similar to those observed were able to grow in microcultures and, therefore, were viable. It can also be answered by pointing out that dead cells do not form endospores, or by *reductio ad absurdum*, since our inoculum consisted entirely of free spores which germinated (figure 5) and grew (figure 6) and formed endospores in the medium used (figure 21). The only vegetative cells found in the cultures at various stages of their development were transparent and contained the bodies we recognized as nuclei: some of the cells bore flagella (figure 7), and motility was observed with the light microscope. Therefore, it would be absurd to assume that all the cells we observed were dead and partially autolyzed.

We believe that we observed genuine forespores because, in a medium like the one used, vacuoles would be more transparent than the cytoplasm; their number per cell and their size and form would vary considerably. Furthermore, in a medium which induces vacuolation, the presence of vacuoles would have been more general, as in the glucose-phosphate medium (figure 3), and would not have been restricted to a relatively few cells. The forespores are denser than the cytoplasm of the mother cell; only one per cell was observed, which is the case in non-filamentous growth of *Bacillus mycoides*; their size and shape also correspond to what would be expected in this organism. In one case we observed a forespore which almost filled the mother cell (figure 19), a condition we often observed in this and several other members of the genus *Bacillus*.

The nuclei are not inclusions. They take nuclear stains in the conventional manner. They are distinct from volutin by their relatively high isoelectric point and their insolubility in 0.04 per cent sodium bicarbonate in 90 minutes at room temperature; they are distinct from lipid inclusions by their relatively low refringence, their presence in the germ cells and subsequent early generations, and their unstainability with Sudan black B. Furthermore, they are not to be con-

sidered a new type of inclusions, since inclusions would not occupy a definite position in a vacuole or in a forespore and, when several inclusions are present, some would remain free in the cytoplasm of the mother cell, a situation never clearly recognized during the present investigation. It must be added that, with the light microscope, the forespore appears homogeneous in the living cell (Knaysi, 1946b), as well as in cells stained to show inclusions. There are certainly no lipoid granules, and the ribonucleic acid the forespore contains is diffuse throughout the cytoplasm.

The question as to whether or not nuclei are present in cells grown on ordinary media is more difficult to answer, since cells grown on ordinary media show no internal differentiation. It may be remembered, however, that cells treated with 0.5 per cent NaHCO_3 suggest the existence of bodies which resemble the nuclei observed in transparent cells (figure 4). More definite information is obtained when a culture in the glucose-acetate medium is examined with the light microscope. In such a culture one observes vegetative cells, numerous refractile spores, and many nonrefractile spores which used up their reserve material but failed to germinate. When these are mounted in a drop of methylene blue solution of pH 3.5 to 4.0, they show a faintly stained cytoplasm and two deeply staining bodies, one at each pole of a spore, which have a considerable resemblance in size and form to the nuclei observed with the electron microscope. Since these spores were formed on ordinary media, it seems logical to conclude that nuclei similar to those observed in the forespores of transparent cells are also present when the spores are formed on ordinary media.

Other questions may also be asked. For instance, does the nucleus contain more than one chromosome? Are the results now obtained with *Bacillus mycoides*, and those previously obtained by Knaysi and Mudd (1943) with *Staphylococcus flavo-cyanus*, applicable to other bacteria? What is the biological nature of the endospore?

The correct answers to these questions will have to await further research. It is safe to predict, however, that answers will soon be forthcoming, since we now have a procedure which, directly applied or modified, makes investigation of the internal structure of bacteria with the electron microscope possible, thus eliminating much of the uncertainty which, in the past, has characterized such investigations.

The nature and function of the bodies of type *B* are not definitively established. They are part of the cytoplasmic membrane and, except when they appear homogeneous, they cannot be structurally differentiated from the rest of that membrane. Both seem to consist chiefly of a network of beaded threads and granules, probably representing complex molecules. Their only distinguishing feature is the greater opacity of their threads and granules, probably acquired through synthetic activity. Their general appearance is that of dark spots in an otherwise similar background. They bear close resemblance in form and dimensions to the dark disks observed during the prespore stage of the same organism by Knaysi, Baker, and Hillier (1947). It is not unlikely that the bodies of type *B*, which are present throughout the life cycle, become gradually more opaque by

continuous synthesis, and that they become visible in the prespore stage even in cells grown on ordinary media. It may be recalled that the formation of lipoprotein granules in the cytoplasmic membrane of *Bacillus mycoides* and *Bacillus cereus* and elimination of these granules into the cytoplasm were observed by Knaysi (1945b, 1946a). Thus the bodies of type B may be the primordia of the prespore inclusions. Their chief role, however, may be the formation of cross plates at the time of cell division, a function which is suggested by the observations of Knaysi, by a frequently linear arrangement of these bodies across the cell (figure 7), and by the occasional presence of cross plates the borders of which seem to consist of consecutive semielliptical fringes (figure 9).

A relation between ribonucleic acid and the gram reaction was found by Dubos and MacLeod (1938) and by Henry and Stacey (1943). However, the function of ribonucleic acid in the bacterial cell, its correct location, and its causal relation to uniform staining and uniform opacity to electrons were recognized, for the first time, in the present investigation. This led to a procedure by which ribonucleic acid is removed, and its reformation is prevented, without injury to the cell or interference with its normal development. Work now in progress indicates that application of this procedure transcends the limits of the gram reaction.

It has been pointed out in a previous section that many spores fail to germinate in the medium we used; this is in no way contrary to the expected behavior of endospores. However, since a high percentage of germination would be desirable, we are now investigating the problem and the results will be the subject of a future communication. It suffices now to state that when the spore suspension is obtained from a week-old slant culture on a medium made up of meat infusion (half strength), 100 ml + tryptone, 0.5 g + glucose, 0.5 g + agar, 1.5 g, and when the inoculum is such that it amounts to 5 or 6 million spores per ml of the glucose-acetate medium, a culture one day old at 35 C may consist of 30 to 50 per cent of vegetative cells. To obtain such results, however, it is necessary that the spore suspension be concentrated and, preferably, less than 2 weeks old.

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SUMMARY

When the endospores of *Bacillus mycoides*, C₂, are washed with sterile, distilled water and heavily inoculated into a nitrogen-free medium, many germinate and gradually use up their supply of ribonucleic acid. The germ cells and subsequent generations of vegetative cells are transparent to electrons even at 50 or 30 kv and show two types of opaque bodies.

Bodies of the first type are relatively large and highly opaque; they show evidence of division and are totally enclosed in the forespore; they do not seem to divide by simple constriction. These are the nuclei of the cell.

The bodies of the second type are smaller and very thin; they consist of beaded threads and granules located in the cytoplasmic membrane; they do not, visibly, take part in the formation of the endospore. There is evidence that they are endowed with synthetic power and that they are involved in the formation of cross plates at the time of cell division.

REFERENCES

- BAYLOR, M. B., APPLEMAN, M. D., SEARS, O. H., AND CLARK, G. L. 1945 Some morphological characteristics of nodule bacteria as shown by the electron microscope. II. *J. Bact.*, **50**, 249-256.
- DELAPORTE, B. 1939 Sur les acides nucléiques des levures et leur localisation. *Rev. gén. botan.*, **51**, 449-482.
- DUBOS, R. J., AND MACLEOD, C. M. 1938 The effect of a tissue enzyme upon pneumococci. *J. Exptl. Med.*, **67**, 791-797.
- HENRY, H., AND STACEY, M. 1943 Histochemistry of the gram-staining reaction for micro-organisms. *Nature*, **151**, 671.
- KNAYSI, G. 1942 The demonstration of a nucleus in the cell of a *Staphylococcus*. *J. Bact.*, **43**, 365-386.
- KNAYSI, G. 1945a Investigation of the existence and nature of reserve material in the endospore of a strain of *Bacillus mycoides* by an indirect method. *J. Bact.*, **49**, 617-622.
- KNAYSI, G. 1945b On the origin and fate of the fatty inclusions in a strain of *Bacillus cereus*. *Science*, **102**, 424.
- KNAYSI, G. 1946a On the existence, morphology, nature, and functions of the cytoplasmic membrane in the bacterial cell. *J. Bact.*, **51**, 113-121.
- KNAYSI, G. 1946b On the process of sporulation in a strain of *Bacillus cereus*. *J. Bact.*, **51**, 187-197.
- KNAYSI, G. 1946c On the inclusions of *Hansenula anomala*. *J. Bact.*, **52**, 487-488.
- KNAYSI, G., BAKER, R. F., AND HILLIER, J. 1947 A study, with the high-voltage electron microscope, of the life cycle and structure of the endospore in *Bacillus mycoides*. *J. Bact.*, **53**, 525-537.
- KNAYSI, G., AND MUDD, S. 1943 The internal structure of certain bacteria as revealed by the electron microscope—a contribution to the study of the bacterial nucleus. *J. Bact.*, **45**, 349-359.
- PIEKARSKI, G. 1939 Lichtoptische und übermikroskopische Untersuchungen zum Problem des Bakterienzellkerns. *Zentr. Bakt. Parasitenk.*, I., Orig., **144**, 140-147.
- PIEKARSKI, G., AND RUSKA, H. 1939 Übermikroskopische Untersuchungen an Bakterien unter besonderer Berücksichtigung der sogenannten Nucleoide. *Arch. Mikrobiol.*, **10**, 302-321.
- SEVAG, M. G., SMOLENS, J., AND LACKMAN, D. B. 1940 The nucleic acid content and distribution in *Streptococcus pyogenes*. *J. Biol. Chem.*, **134**, 523-529.