BACTERIOLOGICAL REVIEWS
Vol. 28, No. 1, p. 14-29 March, 1964

EFFECTS OF HYDROSTATIC PRESSURE ON MICROBIAL SYSTEMS[1]

CARL-GÖRAN HEDÉN

Department of Bacteriology, Karolinska Institutet, Stockholm, Sweden

INTRODUCTION

Given the opportunity to choose the topic for this paper, I was torn between a wish to remain within my main area of interest, which in recent years has been the biotechnology of fermentation, and a desire to make a tangential excursion out of this field. I decided to take the second alternative, because it gives emphasis to an important theme: that biotechnology should function as a link between basic and applied research. This thesis is so far from being generally accepted that it is well worth special attention.

Usually I define biotechnology as the study of the *establishment and maintenance of artificial environments in which living cells or labile biological substances are produced, preserved, selectively destroyed, or modified as desired.* This definition clearly indicates that the subject is focused on application, but obviously it would become sterile if it had no room for basic studies. These may then involve "toolmaking," and this paper is concerned with the establishment of one, highly artificial environment: that attained under high hydrostatic pressure. Such techniques may provide useful tools both in the mapping of microbial control mechanisms and in the manipulation of those mechanisms with practical aims.

Much of the fundamental work on pressure effects on microbial systems has been performed in the United States by Johnson, Haight, Morita, ZoBell, and many others (for references, see 53), but here I will not consider enzyme kinetics, luminescence, or barophilic bacteria. Rather, I will draw from our own limited experience to illustrate the points I want to make.

ACTION OF PRESSURE ON FROZEN CELLS

My personal interest in subjecting microorganisms to high pressure was initiated by a practical problem of biotechnology: how to disrupt the bacteria in a frozen paste at very low temperatures. This was of importance for the removal of labile toxins from *Bordetella pertussis* (10), so the problem was studied in some detail, the initial point being some experiments aimed at improving existing methods, among them the classical freezing and thawing procedure. This, of course, has the drawback of involving many exposures to relatively high temperatures (9).

Considering the disruption caused by cycling across the phase-boundary between liquid and ice I (Fig. 1), it seemed reasonable to assume that passages across the boundary between ice I and ice III would have a similar effect, the crystal structure of ice III being almost 20%

[1] The Office of Naval Research Lecture by the same title, presented at the Annual Meeting of the American Society for Microbiology, at Cleveland, Ohio, in May 1963, formed the basis of this contribution. I should like to express my sincere thanks to the Office of Naval Research and to the Program Committee of the Society for making the lecture possible. This paper is not intended to be a comprehensive review, but rather aims at focusing attention on some interesting phenomena which we have recently studied in Stockholm under U.S. Public Health Service grant C 5799 from the National Cancer Institute. In preparing the paper, I have drawn freely from the data emerging from the lines of study followed by the different members of a team consisting of the following individuals besides myself: E. Hammarsten and T. Lindahl (biochemists), L. Rutberg (microbial geneticist), A. Rupprecht (physical chemist), and I. Toplin (chemical engineer).

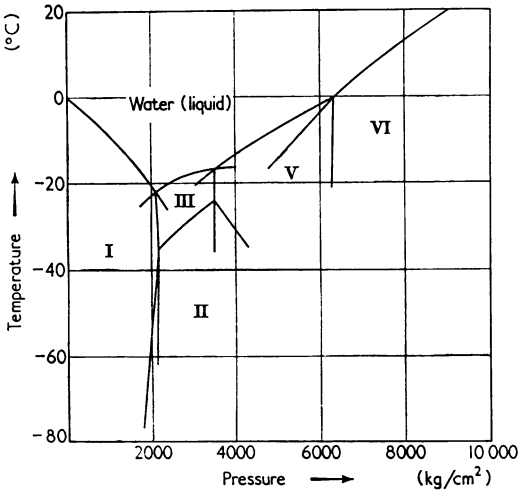

FIG. 1. *Different forms of ice.*

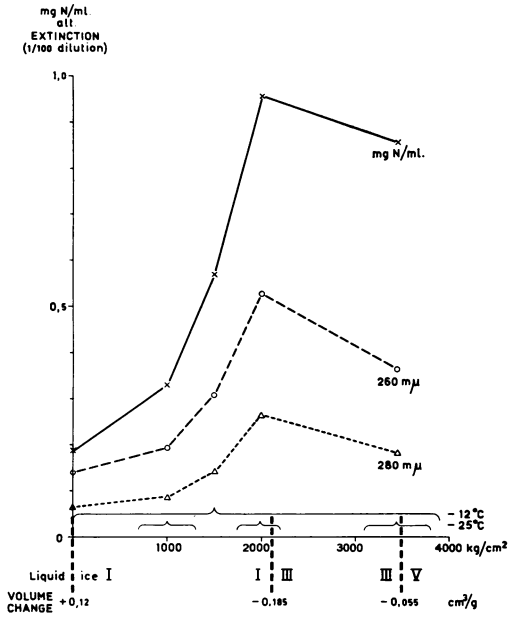

FIG. 2. *Disintegration effect of ice-phase transitions. The measurements were made on the supernatant from frozen and pressure-exposed cells of Escherichia coli B (10-ml suspension of 10^{11} cells per ml of buffer) centrifuged at 25,000 × g for 30 min, after extraction for 2 hr at 0 C with 0.1 M phosphate-citrate buffer. The brackets indicate the regions for ten pressure cycles.*

more compact than that of normal (I) ice (12, 13, 102). As indicated in. Fig. 1, ice III cannot, however, exist at normal pressures, so a special chamber was built for testing the hypothesis.

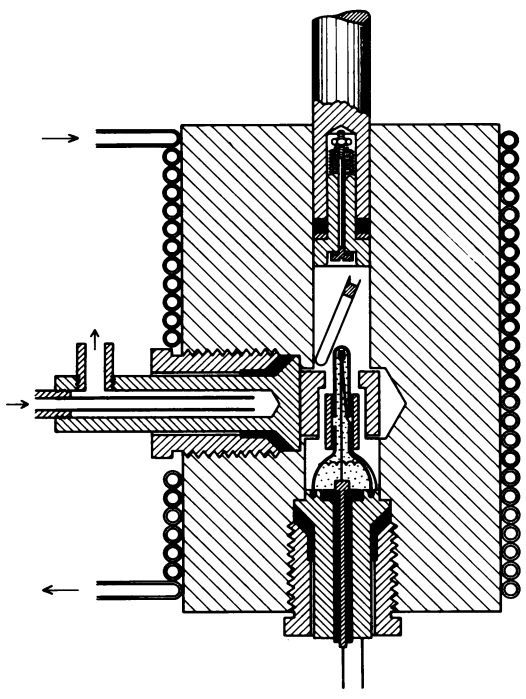

FIG. 3. *Cross section of cylindrical pressure chamber with slanting specimen tube in axial water-filled hole. The tube rests on the copper portion of a "cooling finger" inserted from the right-hand side. A small thermistor probe, housed in a glass capillary filled with paraffin oil, used as an electrical insulator, is located near the specimen. Nylon is used for all gaskets except for the auxiliary Neoprene rubber gasket below the nylon ring around the plunger. Nylon is also used for electrical insulation wherever needed.*

With this apparatus, it was found that when the pressure was pulsated up and down in the region of 2,000 atm at −25 C, disruption occurred as expected (Fig. 2), yielding cells which looked quite empty under an electron microscope in spite of the fact that the envelope was not too badly damaged (25). When Edebo added the shearing forces also used by Hughes (49), and forced the frozen material back and forth through a narrow cylindrical channel (24), the practical device needed for our work became available. It permitted subzero disintegration even down to a degree where cell-wall antigens become accessible to immunodiffusion analysis, and it has been used with success for the low-temperature preparation of cell walls from *B. pertussis* and staphylococci (10, 108, 109).

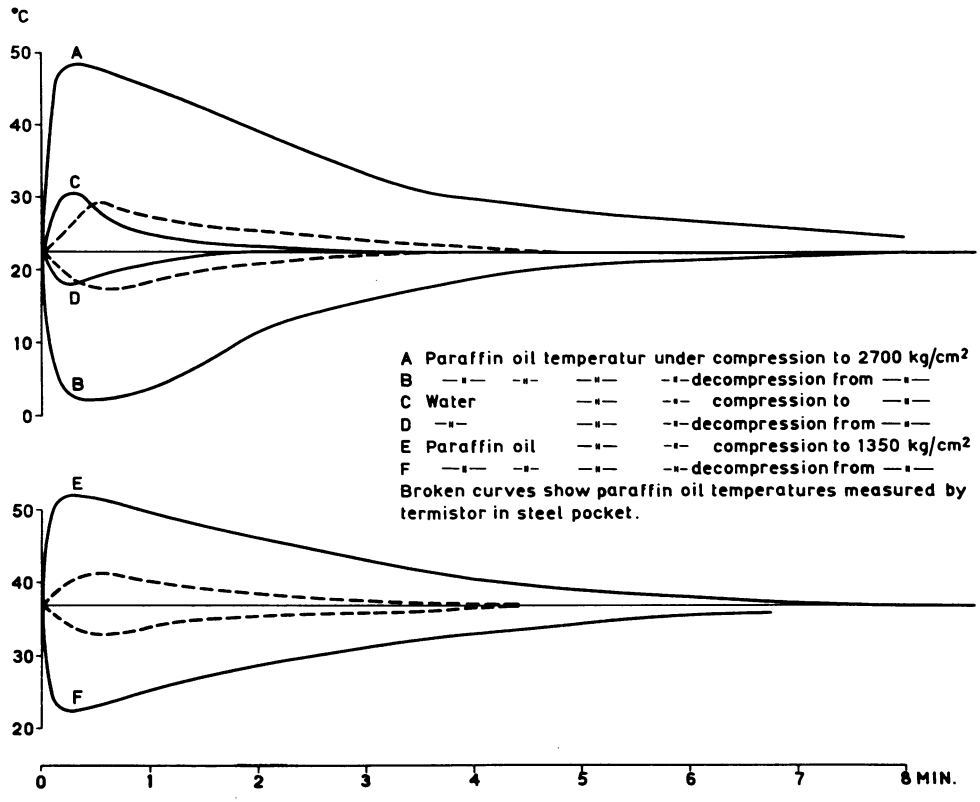

FIG. 4. *Temperature pulses associated with compression-decompression of paraffin oil and water. The measurements were made both with a free thermistor and with the same probe "delayed" by housing in a 1-mm thick pocket of tempered steel (Uddeholm, Sweden; quality Arne).*

General Effects of Pressure at Physiological Temperatures

Nucleic acid metabolism has been a department interest for many years (18, 42, 43, 70, 85), and with the high-pressure equipment available it was tempting to investigate such phases of the cellular control mechanisms, where large volume changes could be expected to occur. The basis is, of course, that pressure will accelerate processes leading to a volume decrease, whereas any synthesis involving a volume increase will be inhibited. The particular interest of pressure data in regard to biological processes derives from the fact that, whereas reactions involving only small molecules are likely to have small volume changes, of the order of a few milliliters per mole, the reactions involving large molecules, such as protein, may exhibit very large volume changes, of the order of 100 ml per mole (54).

Affecting volume changes is, however, not the only way in which pressure may act on the control systems of the cell. These systems might also be affected by increased solation of the cytoplasm (72), which could cause acceleration of particle interactions, or by denaturation of critical enzymes, or changes in pH (53, 76). Obviously, we could anticipate difficult problems of interpretation, but we also ran into some difficult technical problems.

The pressure equipment originally used for disrupting frozen cells proved inadequate, because it became necessary to measure pressure and temperature directly in the chamber. Figure 3 shows a cross section of one of the devices now used, and Fig. 4 illustrates the value of being able to measure the temperature pulses associated with compression and decompression (78). It is shown as a warning to those who might tend to forget this effect. We did so ourselves in the early work on phage and bacteria (91, 92), where the expos-

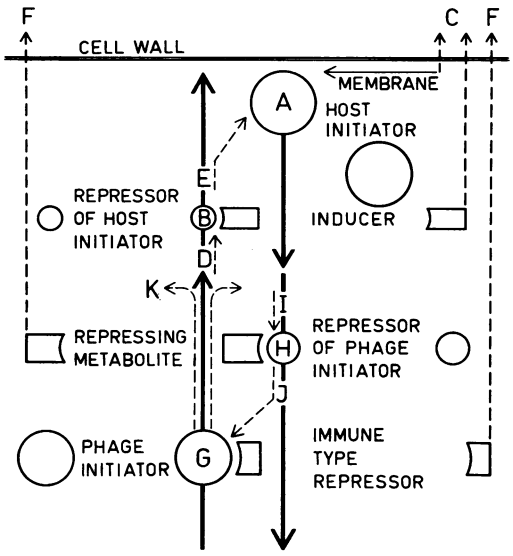

FIG. 5. *Model of cellular control mechanisms with hypothetical pressure "targets" indicated by letters: A, B, G, H (= desensitization); C, F (= leakage) and D, E, I, J, K (= direct effects on DNA). Considering the evidence for linear processes both in replication (86) and protein synthesis (57) and also the theoretical (106) and experimental data (56, 77), which indicate opposed orientation of the phosphodiesterbonds in the two coils making up the double helix, the two strands are shown as heavy vertical arrows. Initiators and repressors (51) are shown as spheres not only free in the cytoplasm but also attached to the chromosome. When located at specific sites (replicator and operator) on the chromosome, they could be influenced by small molecule effectors (inducers or repressing metabolites). Those have been drawn between the strands to illustrate an intimate relation to the transcription process and a function of the nontranscribed strand (41) as part of an anchoring or "switch" mechanism, which could be either reinforced or weakened. Synthesis of repressors and initiators is shown as fine arrows which indicate a control by specific loci (D, E, I, J). Replication and protein synthesis in general are supposed to follow similar linear patterns, except for the fact that the synthesis of labile messenger RNA would require both strands (15, 27, 80, 100, 107). DNA synthesis, on the other hand, would proceed according to the "semiconservative" scheme, i.e., the chains would separate without breakage and each would serve as a template for the formation of a complementary chain (22, 33, 62, 74). The initiator can, for instance, be visualized as a specific depolymerase (60). It, or an electrical event which it triggers, would expose two primer strands (23, 71, 93), and they would both (101) attract complementary*

ures were done in paraffin oil. However, for instance in the studies on transforming deoxyribonucleic acid (DNA), which will be described below, water was used for transmitting the pressure, and the samples were carefully precooled to compensate for the unavoidable small temperature increase (45).

SPECULATIVE MODEL FOR DISCUSSING SOME POSSIBLE PRESSURE TARGETS

As mentioned above, high hydrostatic pressure may act on many different levels in the cell, so it might be useful to consider some possible targets in terms of some concepts of biochemical genetics. It must be realized, however, that this necessarily involves a lot of speculation; therefore, the ideas condensed in Fig. 5 should only be regarded as a hypothesis formulated to link up the experiments reported later.

I use the terminology of Jacob and Monod (51), who suggest that two types of receivers for signals from specific regulator genes (regulators) are located on the chromosome, one triggering replication (the replicator) and the other protein synthesis (the operator). In Fig. 5, both a host and a phage genome are included. The heavy arrows are DNA strands, and the fine ones

bases and participate in a polymerization event, which might proceed over a four-stranded structure (16, 17) held together by unreplicating chromosome segments by the DNA attached proteins, which may take over the interbase hydrogen bonds still keeping the helix in a "quasi-one-strand-helix" grip (98), or by some other mechanism. The finishing of one replication would not necessarily require protein synthesis (40, 68), but a new cycle would require at least so much RNA or protein synthesis, or both, that the initiator level could be maintained. An element of competition between protein and DNA synthesis (83, 96) would seem natural, at least in the small portion of the chromosome, perhaps 10% (59), which is supposed to be single stranded at any one time during the DNA synthesis proceeding during most of the cell cycle (1, 67, 94, 110). The diagram, of course, does not exclude that the balance between replication and synthesis of messenger RNA may depend on the rate of deoxyribonucleotide formation from ribonucleotides (20, 84), the synthesis of thymidylic acid (36), or the phosphorylation of the deoxyribonucleotides (61). The phage genome has been drawn in line with the host genome, but this does, of course, not exclude the more probable types of attachment.

indicate signals to the receivers. The latter can be considered as specific points on the strands. They are covered by spheres corresponding to cytoplasmic switch molecules, which activate replication (the initiator) or block the operator (the repressor).

In the cell, both the initiators and the repressors obviously occur attached to specific codes on the chromosome, as well as in a diffusible form. Let us now assume that the DNA-attached molecules are of special significance, and that they themselves—or the electrical events which they trigger (46)—travel along one particular nucleotide chain in a direction determined by the polarity of that strand.

The prerequisite necessary for an electrical event to run as indicated seems to be that the strands are unlinked by hydrogen bonds. Such a situation, unlikely as it might seem, was actually considered by Spitkovsky (98) as an explanation for certain spectrophotometrical data and the great mechanical elasticity of nucleoprotein. He suggested that protein may take over the interbase bonds and hold the DNA in a "quasi-one-strand-helix" configuration. Such a structure might actually have been very useful for the development of the molecule, because it would tend to preserve the genetic code against "proton tunneling" (63), making tautomeric bases in both strands at the same time. If proteins, or ribonucleic acid (RNA) for that matter, took over the hydrogen bonds, except during the brief periods of replication, this would greatly stabilize the genetic message.

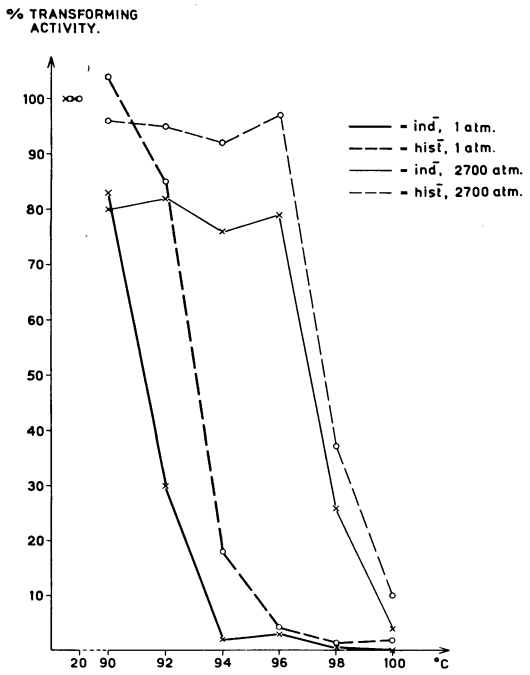

FIG. 6. *Residual transforming activity of Bacillus subtilis DNA in 0.15 M NaCl and 0.015 M sodium citrate (pH 7.9) heated, at a concentration of about 2 μg/ml, to different temperatures for 30 min (see 45).*

Let us suppose that the molecules or charges traveling along a DNA strand are uninterrupted by interpositioned switches or blocking mechanisms (inducers and repressors?). They would then eventually reach a "linker" or a special code, perhaps one complementary to that characteristic for their point of origin, and would there find the conditions for switching over, either to the other strand of the original double helix, or to a newly synthesized strand. Löwdin (63) pointed out that, in the case of transcription, the possibility for a "return journey" along the complementary strand suggests a simple explanation for the base ratios found in RNA and for the unwinding of messenger RNA. Another interesting consequence of the strand-switching model is that an accumulation of molecules or charges might occur at both ends of the double helix, but on different strands. This could be significant for the formation of ring structures (32, 52, 99) and the type of tertiary organization which requires alternating polarities of the replicons making up some chromosomes (35, 58, 81, 103).

When considering high pressure, which certainly may affect orbital overlaps and π-electron pathways, it becomes tempting to think in terms of electrical events, but in Fig. 5 the targets have been illustrated on the physiological level. They fall into three categories: (i) desensitization of trigger molecules attached to DNA (A, B, G, H), (ii) leakage (C, F), and (iii) direct effects on DNA (D, E, I, J, K).

We may take them in the reverse order and start by asking: what effect does pressure have on isolated, transforming DNA?

EFFECTS ON DNA

To determine the effect of pressure on transforming DNA (45), we studied two unlinked markers (indole and histidine) in the DNA from *Bacillus subtilis* (2). This DNA melts between 86 and 92 C, and above that temperature the biological activity rapidly disappears. A pressure

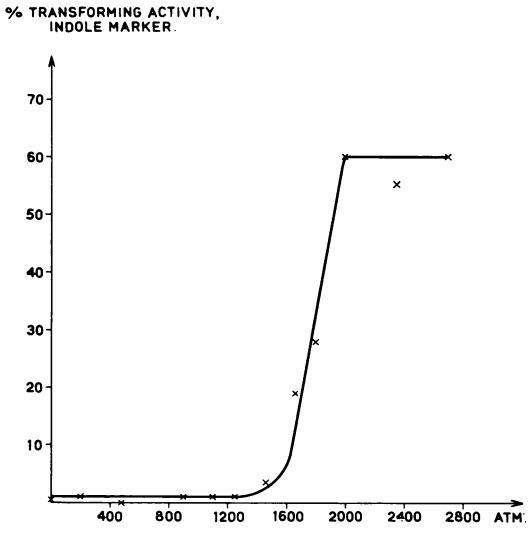

FIG. 7. *Residual transforming activity of Bacillus subtilis DNA in 2 M NaCl and 0.001 M sodium citrate after heating to 100 C for 30 min at various pressures (see 45).*

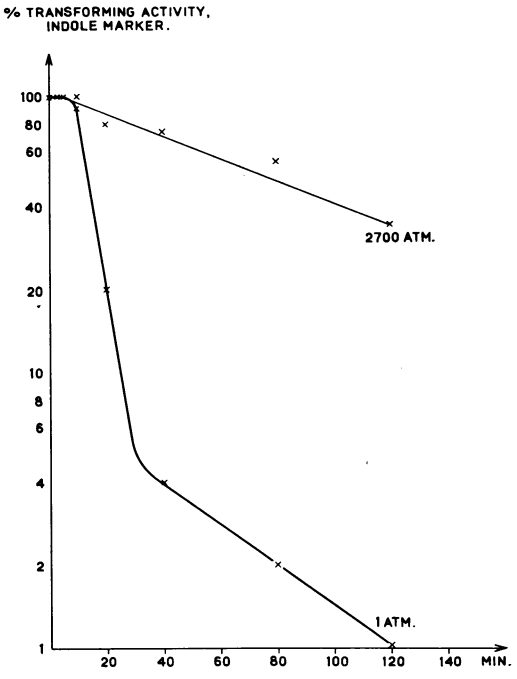

FIG. 8. *Residual transforming activity of Bacillus subtilis DNA in 2 M NaCl and 0.001 M sodium citrate after heating to 100 C for various times (see 45).*

of 2,700 atm, however, increases the melting temperature and protects the biological activity against thermal inactivation (Fig. 6). Similar effects were observed earlier with enzymes (6, 39, 76). Even pressures lower than 2,700 atm have an effect on DNA, provided that a certain threshold is passed. Figure 7 shows that at 100 C this is close to 2,000 atm.

If the loss of transforming activity with time is studied, the protective function of pressure becomes conspicuous (Fig. 8); the slow first-order decrease at 100 C and 2,700 atm probably illustrates the hydrolytic splitting off of purine bases, a phenomenon known to occur at high temperatures (38).

If we assume that the double helix is preserved under pressure, it is also reasonable to assume that in such a structure the bases are partly protected from contact with the solvent. In the single strand, they would not be similarly protected, which would explain the higher rate of breakdown.

Pressure obviously opposes single-stranding, but will it also affect DNA more directly, for instance, by causing permanent changes in the bases at more physiological temperatures than around 100 C? Considering the induction effects, which will be described later, we rather expected that the pressure levels used in our experiments would be mutagenic; however, when Holme and Edebo (47) tested this by the streptomycin technique described by Bertani (8), they could detect no effects at 2,000 or 3,000 atm applied for 30 sec or at pressures ranging from 150 to 700 atm with longer exposure times. Actually, an antimutational effect of pressure had earlier been reported by McElroy (66), who regarded it as an indication of a volume increase in the chromosome caused by certain mutagens.

EXPERIMENTS ON BACTERIA AND EXTRACELLULAR PHAGE

The brief pressure period employed in the experiments just described does not have much effect on viability. On the other hand, longer exposures kill many bacteria. The literature concerning this phenomenon goes back almost a century and will not be reviewed here, but a few experiments on *Escherichia coli* will be mentioned, because this is the host for the viruses which will be discussed later.

In the case of *E. coli*, Johnson and Lewin (54, 55) showed that the temperature inactivation

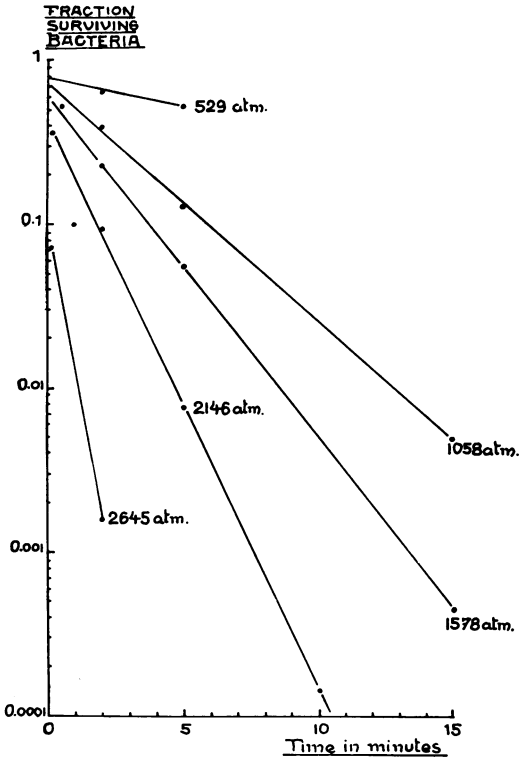

FIG. 9. *Inactivation rates of Escherichia coli B at different pressures (see 88).*

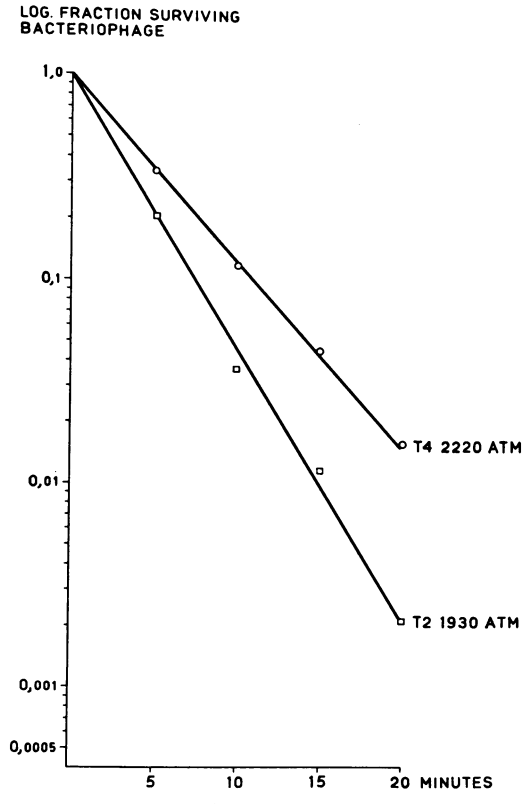

FIG. 10. *Inactivation rates of phages T_2 and T_4 (see 89).*

is opposed by pressure; later, Foster and Johnson (34) found that it also reduces the ability of infected cells to support phage growth as measured by burst sizes and latent periods.

In our group, Rutberg (88, 92) studied the effects on *E. coli* B in some detail, and found an exponential increase in the killing effect with time, the rate being related to the pressure level (Fig. 9).

We found similar curves for the phages T_2 and T_4 (Fig. 10), the former being the most sensitive (89, 92). This is actually the same relation as found with ultraviolet exposure, which might perhaps be regarded as an argument for a DNA target at very high pressure levels and long exposure times.

It has been shown that a hybrid from a cross $T_2 \times T_4$—having the ultraviolet sensitivity of T_4 and backcrossed nine times with T_2, selecting for the ultraviolet sensitivity of T_4—has the pressure sensitivity of T_4 (89). Marker rescue can also be demonstrated with T-even pressure-inactivated phage. These experiments strongly support the idea that the target for very high pressures is the same as that for ultraviolet irradiation.

If we want to think of the DNA as the "target" in the low-pressure range also, it becomes necessary to think of the sensitive element as being exposed in its labile, single-stranded form.

Let us then consider two types of replication, that associated with cell division and that required for phage multiplication.

With regard to cell division of normal terrestrial organisms, this was shown by ZoBell and his co-workers to be blocked at very moderate pressures, around 50 to 200 atm, and to be resumed when the pressure is released (111, 112, 113). All these authors worked with unaerated cultures, but we also obtained the effect in actively aerated cultures (44). In this case, one must of course consider the toxic effects of the diradical oxygen, but under purely hydrostatic pressure such an effect does not seem very likely. However, such pressures were found by Britten

and McClure (14) to cause a conspicuous leakage of the amino acid pool, and Berger (7) found an accumulation in the medium of building blocks for the cell walls of *E. coli* grown under 50 to 400 atm.

In the model referred to earlier (Fig. 5), leakage is indicated by the letter C. It will be noted that the chromosome is illustrated as being intimately related to the cell wall, a situation suggested by Jacob and Monod as an explanation for the physiological synchronization of replication and cell division. Obviously, a leakage of material required for finishing the cell membrane might prevent the initiator release and, consequently, also the replication required for cell division. However, the cell metabolism would continue, and swollen forms and "snakes" would appear, just as was actually found by several investigators (7, 113).

Other alternatives for the blocking of chromosome replication would be a more or less direct DNA effect influencing the initiator-repressor synthesis (D, E), or an interference with the physiological single-stranding. The low pressures required, and the fact that phage and bacteria are not killed by such pressures, however, seem to make the former alternative unlikely, and the fact that other types of replication, such as phage multiplication, are unaffected at the pressure levels in question argues against the second possibility. This would leave us with essentially one alternative apart from leakage: desensitization, i.e., partial denaturation of the cytoplasmic switch molecules of Jacob and Monod (51).

These critical control molecules (initiators and repressors) are supposed to be subjected to delicate allosteric influences by inducers or repressing metabolites (75). The latter modify the signals which reach the chromosome triggers. The allosteric influence is pictured as a desensitization similar to the loss of sensitivity toward the inhibitor, without the loss of activity toward substrate which is known to exist in enzymes subjected to partial inactivation or denaturation (19, 37, 73). Such phenomena are well-known effects of the application of hydraulic pressure (53, 69).

EFFECTS ON THE MULTIPLICATION OF VIRULENT AND TEMPERATE PHAGE

Much information is available concerning a very active and highly specialized nucleic acid protein synthesis, namely phage multiplication. What can such a system tell us about the intracellular target of high pressure? How does phage multiplication, for instance, proceed in bacteria that are first pressure-treated and then infected?

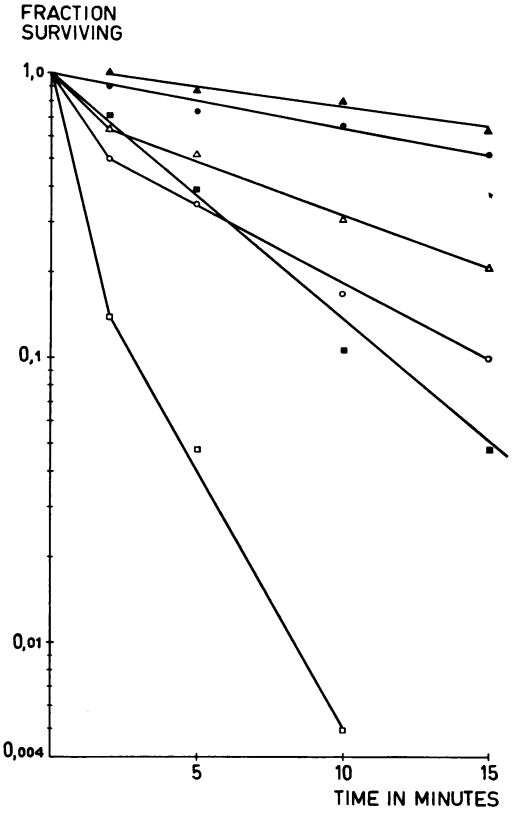

FIG. 11. *Inactivation rates of Escherichia coli B at different pressures measured by capacity to form colonies (△ at 740 atm, ○ at 1,040 atm, and □ at 1,480 atm) and to support T_2 multiplication (▲ at 740 atm, ○ at 1,040 atm, and ■ at 1,480 atm). (See 88.)*

Figure 11 shows that the capacity to support T_2 multiplication falls off with increasing pressure at a rate which is not too different from that showing survival of uninfected cells (88, 92). This is actually one of the many facts which indicate that pressure and ultraviolet irradiation have different targets, ultraviolet treatment being much more potent in killing the cells than in destroying the above capacity (3).

A second problem concerns the effect on phage multiplication, if the pressure is applied *after* infection. In this case, Rutberg was able to demonstrate a sensitivity to 2-min exposures of

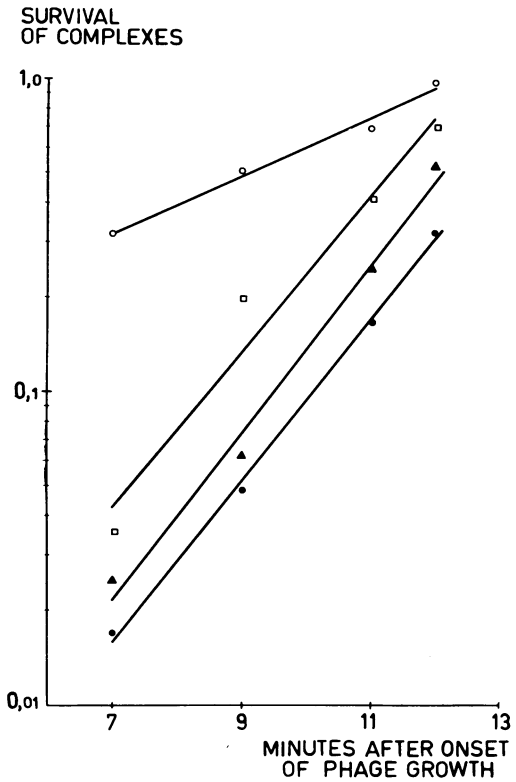

FIG. 12. *Effect of 1,260 atm, applied for different lengths of time, on intracellular multiplication of T_2 phage. Symbols:* ○ = *1 min,* □ = *2 min,* ▲ = *4 min,* ● = *6 min. (See 88.)*

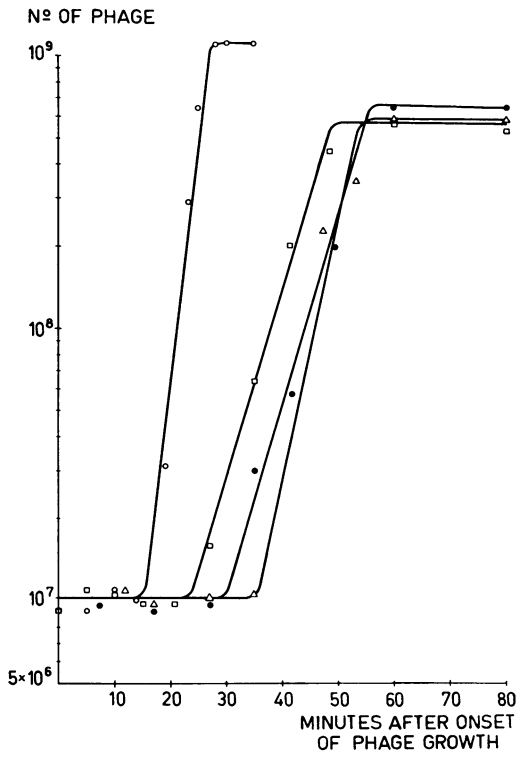

FIG. 13. *Effect of 2-min exposure to 740 atm on the latency period and burst size measured on Escherichia coli B infected with T_2 phage. Pressure-treated at time 0,* □; *at 7 min,* ●; *and at 12 min,* △ *(control,* ○*). (See 88.)*

the intracellular phage, which was much greater than that exhibited by mature particles (88). As shown in Fig. 12, the sensitivity is particularly high during the first 7 min. The one-hit type curves for the different pressure levels then tend to converge. After 10 to 12 min, mature particles could be detected by lysis from without, and bursts then started to appear, confusing the phage counting. It seems likely that the extrapolated curves give the time required for the particles to become resistant to the pressure level used. The fact that the sensitivity of the developing complexes shows a pattern different from that observed for ultraviolet treatment (5, 64) is an additional argument for the assumption that ultraviolet treatment and low pressure have different targets.

Figure 13 shows that 2-min pressure exposures (740 atm) during the period of intracellular phage development reduce the burst sizes somewhat (88). It also demonstrates that the latency period is largely constant after the pressure exposure, irrespective of whether this takes place immediately after infection or 7 or 12 min later. This fact, together with the fact that the late exposures do not yield bigger bursts than the early ones, makes it reasonable to assume that pressure stops phage multiplication, which has to begin from the start when the pressure is released. In terms of the model described earlier, this can hardly be explained as simple leakage of building blocks of low molecular weight, and more profound effects, such as destruction of initiator, a reversal of single-stranding, or both, seem more likely.

The existence of such profound changes is also indicated by another pressure phenomenon, phage induction, which was observed in connection with some early T_2 burst-size studies (91). In this case, we noticed an irregular appearance of an unknown phage after treatment of *E. coli* B (26). The system was, however, very difficult

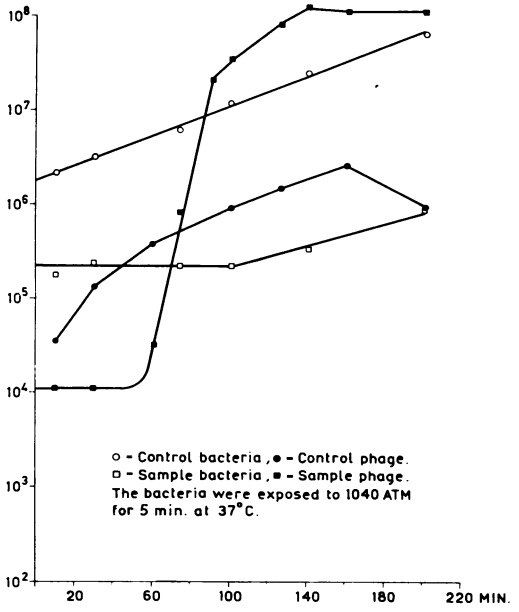

FIG. 14. *Induction of phage lambda in Escherichia coli K-12 by means of pressure. (See 90.)*

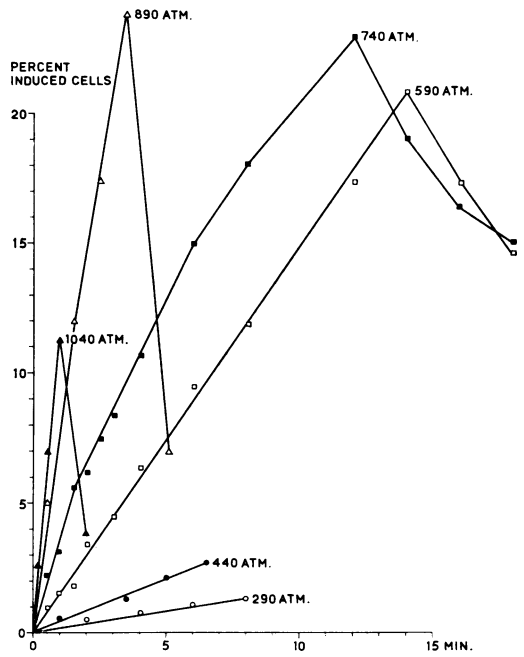

FIG. 15. *Induction of phage lambda by different pressure levels. (See 73.)*

to work with, so induction will be illustrated here with *E. coli* K-12, where Rutberg had studied the lambda phage (90). This was a fortunate choice, because the system has proved to be unusually apt for this type of induction.

As shown in Fig. 14, *E. coli* K-12 lyses after the same latency period as observed in the case of ultraviolet induction, i.e., about 60 min. By applying the spraying technique of Six (97), which depends on blocking the noninduced cells by spraying the plates with streptomycin (using, of course, a streptomycin-resistant strain for plating), Rutberg then determined the actual number of induced cells. The response followed a pattern quite different from that seen with ultraviolet treatment (Fig. 15). It is proportional to the time of exposure at any one pressure level. These levels can be quite low, and, by a special technique, it was actually possible to show induction of 2-min exposures down to a pressure of 150 atm. This should be a memento in ultracentrifugation, where such pressures are easily attained (Fig. 16).

In the high-pressure range, optimal induction obviously requires short exposure times; otherwise one may get into the sharp-decline range. On the one hand, too short an exposure might return the released phage to an environment of immune-type repression, and, on the other, an extended pressure treatment might interfere with the escape mechanisms. In both cases, there would be a reduced induction.

The model used in this paper as a basis for discussing pressure effects (Fig. 5) indicates that leakage of a repressing metabolite or an immune-type repressor could be an explanation (F), but it seems unlikely that leakage would increase and decrease with pressure in a way which could explain the results just described. An inactivation of repressing metabolites or immune-type repressor might, however, be an alternative explanation. Earlier, a series of arguments against direct DNA effects of relatively low pressures were indicated, and in this particular case one might add that cell suspensions which were aerated in buffer for 2 hr lost their aptitude for ultraviolet induction but could still be induced with pressure.

Pressure actually seems to belong to a different category than the mutagens and carcinogens normally used for induction purposes (50, 65). Unfortunately, at the present time, we can only guess at the mechanism involved. However, considerable volume changes take place. Estimates of about 220 ml per mole can actually be

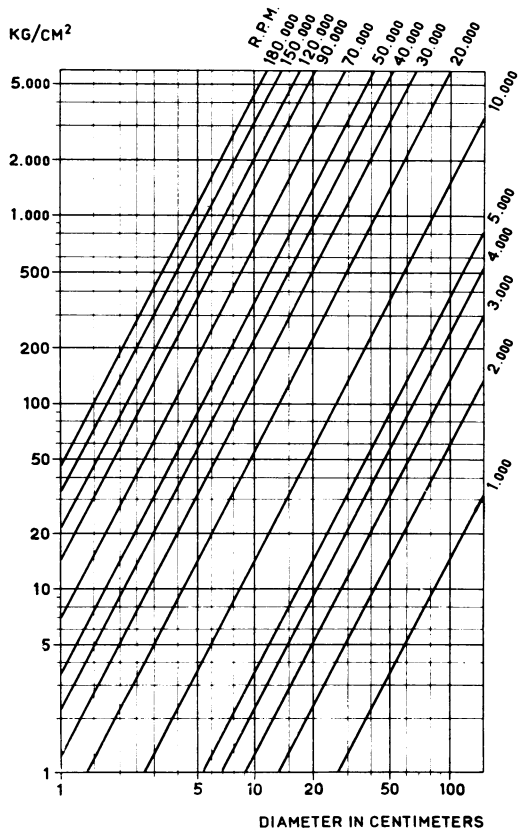

FIG. 16. *Nomogram illustrating the centrifugally produced liquid pressures in water.*

made on the basis of induction data obtained at 30 and 37 C. Such volume changes might perhaps be expressions of a desensitization of the type mentioned earlier or of steric alterations in the replication process (K).

The latter possibility would include alterations in newly formed single strands of DNA or RNA. Such strands are probably rather vulnerable before being stabilized by double-helix formation, and they might tend to fold to gain a smaller volume. It is interesting to note that such a folding or looping process might give us structures similar to transfer RNA, and that segments with secondary structure in messenger RNA have been assumed to be of importance for their biological function (79).

CONCLUDING REMARKS

As mentioned earlier, the DNA-attached molecules might be important for ring formation and, consequently, also for the formation of the type of secondary structure just mentioned. Stedman and his group proposed that histones act as gene inhibitors (21; *see also* 4), and later such proteins were actually shown to repress DNA-dependent RNA synthesis (48). However, much remains to be learned about the DNA-attached proteins before one can start to consider the pressure sensitivity of the DNA-protein complex. A start in this direction is being made in our laboratory by Hammersten and his co-workers, who developed counterflow electrophoresis in the presence of ethylene glycol as a means of preparing "functional" nucleoprotein without a denaturation, which might irreversibly attach extraneous proteins to the molecule complex (28, 29, 30, 31). Nucleoprotein isolated in this manner contains less than 0.1% protein, but this has a strong nuclease activity. The finding of Macheboeuf and his group (104, 105), that both polymerization and depolymerization of RNA varies with the pressure and with the size of the macromolecules exposed, adds interest to studies aimed at determining the effect of pressure on the type of nucleoprotein mentioned.

DNA in the cell exists in the form of nucleoprotein and, as pointed out before, we must keep this high degree of organization in mind when discussing pressure effects. The electron-transport pathways in nucleoproteins may, of course, be pressure-sensitive, but information must be obtained about the electrical properties of the simple deproteinized DNA molecule before one can consider the nucleoproteins from this point of view. In this area, our own efforts have so far been limited to the development of a technique for bulk preparation of oriented DNA with retained transforming activity (87).

Hoffman and Ladik (46) regard DNA as a semiconductor, which has completely filled valence bands and completely empty conduction bands; i.e., it behaves as an insulator. These authors think that electron donors, thermal excitation, or irradiation may turn the molecule into a conductor by moving electrons into the conduction bands. Under the influence of intracellular electrostatic fields, these electrons might then migrate to the ends of the double helix and initiate single-stranding by a process of electrostatic repulsion. This hypothesis, which in fact can easily be fitted into the model presented earlier, would indicate that pressure, which is known to increase the electrical conductivity of

organic semiconducting polymers (82), might have a very direct effect on the molecule. It is of interest that electrical fields recently were shown to stimulate cell division (11).

Apart from the approaches just mentioned, there are certainly many other roads which may lead to a better understanding of the mechanisms of pressure effects. At present, the interpretations are, of necessity, as loosely knit as indicated by this paper. Actually, the only general conclusion which can be drawn at the present time is that the effects of very low pressures are compatible with the hypothesis of leakage, that higher pressures add involvements of cellular control mechanisms, and that the highest pressure levels may cause irreversible DNA changes. However, the effects on the cellular control mechanisms are obscure, and when I have focused on replication and DNA transcription into RNA it is little more than a guess, which must be proved or disproved by future experiments. In any event, such experiments will certainly further emphasize one fact, clearly shown by the investigations of Johnson et al., namely, that pressure may be a very useful tool in molecular biology. It might some day also be of value in the applied areas, where it might be put to use in blocking cell division, without necessarily stopping the metabolism, or in releasing defective viruses, for example.

However, not only the microbiologist, but also other scientists, might find it rewarding to remember to include pressure among their parameters. Schramm and his co-workers (95) synthesized polynucleotides at atmospheric pressures in a nonenzymatic environment, and it is, of course, well known that pressure accelerates many polymerizations. Temperature denaturation and protection against this effect by pressure may also have been two important links in a development process of such macromolecules as nucleic acid in the abysmal depths of the oceans on our primitive earth.

ACKNOWLEDGMENTS

I am indebted to my colleagues at the Bacteriology Department, Karolinska Institutet, for their help and criticism. I also want to thank G. Bertani and T. A. Hoffman for the guidance and advice they have given me.

LITERATURE CITED

1. ABBO, F. E., AND A. B. PARDEE. 1960. Synthesis of macromolecules in synchronously dividing bacteria. Biochim. Biophys. Acta **39**:478–485.
2. ANAGNOSTOPOULOS, C., AND J. SPIZIZEN. 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
3. ANDERSON, T. F. 1948. The growth of T2 virus on ultraviolet-killed host cells. J. Bacteriol. **56**:403–410.
4. BARTALOS, M. 1963. A possible chemical term for the operator gene. Nature **198**:109.
5. BENZER, S. 1952. Resistance to ultraviolet light as an index to the reproduction of bacteriophage. J. Bacteriol. **63**:59–72.
6. BERGER, L. R. 1958. Some effects of pressure on a phenylglycosidase. Biochim. Biophys. Acta **30**:522–528.
7. BERGER, L. R. 1959. The effect of hydrostatic pressure on cell wall formation. Bacteriol. Proc., p. 129.
8. BERTANI, G. 1951. A method for detection of mutations, using streptomycin dependence in Escherichia coli. Genetics **36**:598–611.
9. BILLAUDELLE, H., AND P. O. BRUNÉR. 1959. An automatic apparatus for extraction of cell suspensions by repeated freezing and thawing. J. Biochem. Microbiol. Technol. Eng. **1**:229–238.
10. BILLAUDELLE, H., L. EDEBO, E. HAMMARSTEN, C.-G. HEDÉN, B. MALMGREN, AND H. PALMSTIERNA. 1960. Studies on the chemical and immunological structure of Bordetella pertussis. Acta Pathol. Microbiol. Scand. **50**:208–224.
11. BOZOKY, L., G. KISZELY, T. A. HOFFMAN, AND J. LADIK. 1963. Effects of electrostatic fields on cell mitosis. Nature **199**:1306.
12. BRIDGMAN, P. W. 1912. Water in the liquid and five solid forms under pressure. Proc. Am. Acad. Arts Sci. **47**:439–559.
13. BRIDGMAN, P. W. 1937. The phase diagram of water to 45000 kg/cm^2. J. Chem. Phys. **5**:964–966.
14. BRITTEN, R. J., AND F. T. MCCLURE. 1962. The amino acid pool in *Escherichia coli*. Bacteriol. Rev. **26**:292–335.
15. BURMA, D., H. KROGER, S. OCHOA, R. WARNER, AND J. WEILL. 1961. Further studies on deoxyribonucleic acid-dependent enzymatic synthesis of ribonucleic acid. Proc. Natl. Acad. Sci. U.S. **47**:749–752.
16. CAVALIERI, L. F., AND B. H. ROSENBERG. 1961. The replication of DNA. I. Two molecular classes of DNA. II. The number of polynucleotide strands in the conserved unit of DNA. Biophys. J. **1**:317–336.
17. CAVALIERI, L. F., AND B. H. ROSENBERG. 1961. The replication of DNA. III. Changes in the number of strands in E. Coli DNA

during its replication cycle. Biophys. J. **1**:337–351.

18. CAVANNA, R., E. B. CHAIN, C.-G. HEDÉN, B. MALMGREN, N. A. ELIASSON, E. HAMMARSTEN, AND S. ÅQVIST. 1955. Interrelation of protein and polynucleotide synthesis in Escherichia coli. Rend. Ist. Super. Sanita (English Ed.) **17**:168–186.
19. CHANGEUX, J. P. 1961. The feedback control mechanism of biosynthetic L-threonine deaminase by L-isoleucine. Cold Spring Harbor Symp. Quant. Biol. **26**:313–318.
20. COHEN, S. S., H. D. BARNER, AND J. LICHTENSTEIN. 1961. The conversion of a phage-induced ribonucleic acid to deoxyribonucleotides in vitro. J. Biol. Chem. **236**: 1448–1457.
21. CRUFT, H. J., C. M. MAURITZEN, AND E. STEDMAN. 1954. Abnormal properties of histones from malignant cells. Nature **174**:580–585.
22. DELBRÜCK, M., AND G. S. STENT. 1957. On the mechanism of DNA replication, p. 699–736. *In* W. D. McElroy and B. Glass [ed.], The chemical basis of heredity. Johns Hopkins Press, Baltimore.
23. DOUNCE, A. L., N. K. SARKAR, AND E. R. KAY. 1961. The possible role of DNA-ase I in DNA replication. J. Cellular Comp. Physiol. **57**:47–54.
24. EDEBO, L. 1960. A new press for the disruption of microorganisms and other cells. J. Biochem. Microbiol. Technol. Eng. **2**:453–479.
25. EDEBO, L., AND C.-G. HEDÉN. 1960. Disruption of frozen bacteria as a consequence of changes in the crystal structure of ice. J. Biochem. Microbiol. Technol. Eng. **2**: 113–120.
26. EDEBO, L., AND L. RUTBERG. 1960. Electron-microscopical observations on a pressure-induced phage. J. Ultrastruct. Res. **4**: 88–91.
27. EISENSTADT, J. M., T. KAMEYAMA, AND G. D. NOVELLI. 1962. A requirement for gene-specific deoxyribonucleic acid for the cell-free synthesis of β-galactosidase. Proc. Natl. Acad. Sci. U.S. **48**:652–659.
28. ELIASSON, R., E. HAMMARSTEN, AND T. LINDAHL. 1962. Separation of polynucleotides and proteins from cell extracts at low temperatures by counter flow electrophoresis. Arch. Biochem. Biophys. Suppl. 1, p. 139–146.
29. ELIASSON, R., E. HAMMARSTEN, AND T. LINDAHL. 1962. Use of ethylene glycol in counter flow electrophoresis and in cold concentration of DNA and protein solutions. Biotechnol. Bioeng. **4**:53–56.
30. ELIASSON, R., E. HAMMARSTEN, T. LINDAHL, I. BJÖRK, AND T. C. LAURENT. 1963. The stability of deoxyribonucleic acid in glycol solution. Biochim. Biophys. Acta **63**:234–239.
31. ELIASSON, R., E. HAMMARSTEN, T. LINDAHL, AND H. PALMSTIERNA. 1961. Preliminary notes on the separation of nucleic acids and proteins by counter flow electrophoresis. Acta Chem. Scand. **15**:570–574.
32. FIERS, W., AND R. L. SINSHEIMER. 1962. The structure of the DNA of bacteriophage ϕ X174. J. Mol. Biol. **5**:408–434.
33. FORRO, F., AND S. A. WERTHEIMER. 1960. The organization and replication of deoxyribonucleic acid in thymine-deficient strains of Escherichia coli. Biochim. Biophys. Acta **40**:9–21.
34. FOSTER, R. A. C., AND F. H. JOHNSON. 1950. Influence of urethane and of hydrostatic pressure on growth of bacteriophages T2, T5, T6 and T7. J. Gen. Physiol. **34**:529–550.
35. FREESE, E. 1958. The arrangement of DNA in the chromosome. Cold Spring Harbor Symp. Quant. Biol. **23**:13–18.
36. FRIEDKIN, M. 1959. Speculations on the significance of thymidylic acid synthesis in the regulation of cellular proliferation, p. 97–103. *In* F. Stohlman [ed.], The kinetics of cellular proliferation. Grune and Stratton, New York.
37. GERHARDT, J. C., AND A. B. PARDEE. 1962. The enzymology of control by feedback inhibition. J. Biol. Chem. **237**:891–896.
38. GINOZA, W., AND B. ZIMM. 1961. Mechanisms of inactivation of deoxyribonucleic acids by heat. Proc. Natl. Acad. Sci. U.S. **47**: 639–652.
39. HAIGHT, R. D., AND R. Y. MORITA. 1962. Interaction between the parameters of hydrostatic pressure and temperature on aspartase of *Escherichia coli*. J. Bacteriol. **83**:112–120.
40. HANAWALT, P. C., O. MAALØE, D. I. CUMMINGS, AND M. SCHAECHTER. 1961. The normal DNA replication cycle. II. J. Mol. Biol. **31**:156–165.
41. HAYASHI, M., S. SPIEGELMAN, R. E. FRANKLIN, AND S. E. LURIA. 1963. Separation of the RNA message transcribed in response to a specific inducer. Proc. Natl. Acad. Sci. U.S. **49**:729–736.
42. HEDÉN, C.-G. 1951. Studies of the infection of E. coli B with the bacteriophage T2.

Acta Pathol. Microbiol. Scand. Suppl. **88**:1–126.

43. HEDÉN, C.-G., T. HOLME, AND B. MALMGREN. 1955. Application of the continuous cultivation technique to problems connected with growth rate and nucleic acid synthesis. Acta Pathol. Microbiol. Scand. **37**:50–57.
44. HÉDEN, C.-G., AND A. S. MALMBORG. 1961. Aeration under pressure and the question of free radicals. Sci. Rept. Ist. Super. Sanita **1**:213–221.
45. HÉDEN, C.-G., T. LINDAHL, AND I. TOPLIN. 1964. The stability of DNA solutions under high pressure. Acta Chem. Scand. *in press*.
46. HOFFMAN, T. A., AND J. LADIK. 1964. Quantum mechanical properties of DNA. *In* J. J. Duchesne [ed.], Advances in chemical physics, vol. 6, Structure and properties of biomolecules and biological systems. Interscience Publishers, Inc., New York.
47. HOLME, T., AND L. EDEBO. 1960. Undersökningar över mutagen effekt av vissa fysikaliska agentia. Rept. R-318/1960, Swedish Med. Res. Council.
48. HUANG, R. C., AND J. BONNÉR. 1962. Histone, a suppressor of chromosomal RNA synthesis. Proc. Natl. Acad. Sci. U.S. **48**:1216–1222.
49. HUGHES, D. E. 1951. A press for disrupting bacteria and other microorganisms. Brit. J. Exptl. Pathol. **32**:97–109.
50. JACOB, F. 1954. Les bactéries lysogène et la notion de provirus, p. 1–176. Monographies de l'Institut Pasteur, Masson et Cie, Paris.
51. JACOB, F., AND J. MONOD. 1963. Elements of regulatory circuits in bacteria, p. 1–24. UNESCO Symposium on biological organization. Biological organization at the cellular and supercellular level.
52. JACOB, F., AND E. WOLLMAN. 1957. Analyse des groupes de liaison génétique de différentes souches donatrices d'Escherichia coli K 12. Compt. Rend. **245**:1840–1843.
53. JOHNSON, F. H., et al. 1954. Hydrostatic pressure and molecular volume changes, p. 286–368. *In* F. H. Johnson, H. Eyring, and M. J. Polissar [ed.], The kinetic basis of molecular biology. John Wiley & Sons, Inc., New York.
54. JOHNSON, F. H., AND I. LEWIN. 1946. The disinfection of E. coli in relation to temperature, hydrostatic pressure and quinine. J. Cellular Comp. Physiol. **28**:23–45.
55. JOHNSON, F. H., AND I. LEWIN. 1946. The influence of pressure, temperature, and quinine on the rates of growth and disinfection of E. coli in the logarithmic growth phase. J. Cellular Comp. Physiol. **28**: 77–97.
56. JOSSE, J., A. D. KAISER, AND A. KORNBERG. 1961. Enzymatic synthesis of deoxyribonucleic acid. VIII. Frequencies of nearest neighbour base sequences in deoxyribonucleic acid. J. Biol. Chem. **236**:864–875.
57. KANO, S., AND S. SPIEGELMAN. 1962. Evidence for a nonrandom reading of the genome. Proc. Natl. Acad. Sci. U.S. **48**:1942–1949.
58. KELLENBERGER, E. 1960. The physical state of the bacterial nucleus. Symp. Soc. Gen. Microbiol. **10**:39–66.
59. LARK, K. G., L. F. CAVALIERI, AND B. H. ROSENBERG. 1963. Cellular control of DNA biosynthesis. Molecular biology, part I, ref. p. 184. Academic Press, Inc., New York.
60. LASKOWSKI, M., SR. 1961. Deoxyribonucleases, p. 123–147. *In* P. D. Boyer, H. Lardy, and K. Myrbäck [ed.], The enzymes, vol. 5. Academic Press, Inc., New York.
61. LEHMAN, I. R., M. J. BESSMAN, E. S. SIMMS, AND A. KORNBERG. 1958. Enzymatic synthesis of deoxyribonucleic acid. J. Biol. Chem. **233**:163–170.
62. LEVINTHAL, C. 1956. The mechanism of DNA replication and genetic recombination in phage. Proc. Natl. Acad. Sci. U.S. **42**: 394–404.
63. LÖWDIN, P.-O. 1962. Quantum genetics and the aperiodic solid. Some aspects on the biological problems of heredity, mutations, ageing and tumors in view of the quantum theory of the DNA molecule. Preprint No. 85, Quantum Chemistry Group, Uppsala, Sweden.
64. LURIA, S. E., AND R. LATARJET. 1947. Ultraviolet irradiation of bacteriophage during intracellular growth. J. Bacteriol. **53**:149–163.
65. LWOFF, A. 1953. Lysogeny. Bacteriol. Rev. **17**:269–337.
66. MCELROY, W. D. 1952. Evidence for the occurrence of intermediates during mutation. Science **115**:623–626.
67. MCFALL, E., AND G. STENT. 1959. Continuous synthesis of deoxyribonucleic acid in *Escherichia coli*. Biochim. Biophys. Acta **34**:580.
68. MAALØE, O., AND P. C. HANAWALT. 1961. Thymine deficiency and the normal DNA replication cycle. I. J. Mol. Biol. **3**:144–155.
69. MACHEBOEUF, M., J. BASSET, E. BARBU, M.

LE SAGET, AND G. NUNEZ. 1950. Influence de la pression sur l'hydrolyse des protéines par les acides ou les alcalis. Compt. Rend. Soc. Biol. **144**:962–964.

70. MALMGREN, B., AND C.-G. HEDÉN. 1947. Studies on the nucleotide metabolism of bacteria. Acta Pathol. Microbiol. Scand. **24**:417–504.
71. MANTSAVINOS, R., AND E. S. CANELLAKIS. 1959. Studies on the biosynthesis of DNA by cell-free extracts of mouse leukemic cells. Cancer Res. **19**:1239–1243.
72. MARSLAND, D. A., AND D. E. BROWN. 1942. The effects of pressure on sol-gel equilibria, with special reference to myosin and other protoplasmic gels. J. Cellular Comp. Physiol. **20**:295–305.
73. MARTIN, R. G. 1963. The first enzyme in histidine biosynthesis: the nature of feedback inhibition by histidine. J. Biol. Chem. **238**:257–268.
74. MESELSON, M., AND F. W. STENT. 1958. The replication of DNA in Escherichia coli. Proc. Natl. Acad. Sci. U.S. **44**:671–682.
75. MONOD, J., AND F. JACOB. 1961. General conclusions. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. Cold Spring Harbor Symp. Quant. Biol. **26**:389–401.
76. MORITA, R. Y., AND R. D. HAIGHT. 1962. Malic dehydrogenase activity at 101 C under hydrostatic pressure. J. Bacteriol. **83**:1341–1346.
77. NAGATA, T. 1962. Polarity and synchrony in the replication of DNA molecules of bacteria. Biochem. Biophys. Res. Commun. **8**:348–351.
78. NATHAN, M. I., AND W. PAUL. 1962. Effect of pressure on the energy levels of impurities in semiconductors. II. Gold in silicon. Phys. Rev. **128**:38–42.
79. NIERENBERG, M. W. 1963. The genetic code. Sci. Am. **208**:80–94.
80. OCHOA, S., D. BURMA, H. KROGER, AND J. WEILL. 1961. Deoxyribonucleic acid-dependent incorporation of nucleotides from nucleoside triphosphates into ribonucleic acid. Proc. Natl. Acad. Sci. U.S. **47**:670–679.
81. PAPPAS, G. D., AND P. W. BRANDT. 1960. Helical structure in the nuclei of free-living amebas. Intern. Kongr. Elektronenmikroscopie, 2, Berlin, 1958, p. 244–246.
82. POHL, H. A., A. REMBAUM, AND A. HENRY. 1962. Effects of high pressure on some organic semiconducting polymers. J. Am. Chem. Soc. **84**:2699.
83. PRESCOTT, D. M., AND R. F. KIMBALL. 1961. Relation between RNA, DNA, and protein synthesis in the replication nucleus of Euplotes. Proc. Natl. Acad. Sci. U.S. **47**: 686–693.
84. REICHARD, P., Z. N. CANELLAKIS, AND E. S. CANELLAKIS. 1961. Studies on a possible regulatory mechanism for the biosynthesis of deoxyribonucleic acid. J. Biol. Chem. **236**:2514–2519.
85. REICHARD, P., AND L. RUTBERG. 1960. Formation of deoxycytidine 5′-phosphate from cytidine 5′-phosphate with enzymes from Escherichia coli. Biochim. Biophys. Acta **37**:554–555.
86. RUDNER, R. 1960. Mutation as an error in base pairing. Biochem. Biophys. Res. Commun. **3**:275–280.
87. RUPPRECHT, A. 1963. Preparation of oriented DNA in large amounts. Biochem. Biophys. Res. Commun. **12**:163.
88. RUTBERG, L. 1964. On the effects of high hydrostatic pressure on bacteria and bacteriophage. I. Action on the reproduction of bacteria and their ability to support growth of bacteriophage T2. Acta Pathol. Microbiol. Scand. *In press.*
89. RUTBERG, L. 1964. On the effects of high hydrostatic pressure on bacteria and bacteriophage. II. Inactivation of bacteriophage. Acta Pathol. Microbiol. Scand. *In press.*
90. RUTBERG, L. 1964. On the effects of high hydrostatic pressure on bacteria and bacteriophage. III. Induction with high hydrostatic pressure of E. coli K lysogenic for phage lambda. Acta Pathol. Microbiol. Scand. *In press.*
91. RUTBERG, L., AND C.-G. HEDÉN. 1960. The activation of prophage in E. coli B by high pressure. Biochem. Biophys. Res. Commun. **2**:114–116.
92. RUTBERG, L., AND C.-G. HEDÉN. 1963. Effecten av Höga Tryck på Backterier och Bakterievirus. Rept. 1962, Swedish Cancer Society, p. 290–293.
93. SARKAR, N. K., AND A. L. DOUNCE. 1961. A spectroscopic study of the reaction of formaldehyde with deoxyribonucleic and ribonucleic acids. Biochim. Biophys. Acta **49**:160–169.
94. SCHAECHTER, M., M. W. BENTZON, AND O. MAALØE. 1959. Synthesis of deoxyribonucleic acid during the division cycle of bacteria. Nature **183**:1207–1208.
95. SCHRAMM, G., H. GRÖTSCH, AND W. POLLMAN. 1962. Nicht-enzymatische Synthese von Polysacchariden, Nucleosiden und Nu-

cleinsäuren und die Entstehung selbstvermehrungsfähiger Systeme. Angew. Chem. **74**:53–92.

96. SISKEN, J. E. 1959. The synthesis of nucleic acids and proteins in the nuclei of tradescantia root tips. Exptl. Cell Res. **16**: 602–614.
97. SIX, E. 1959. The rate of spontaneous lysis of lysogenic bacteria. Virology **7**:328–346.
98. SPITKOVSKY, D. M. 1963. To the problem of quasisinglecoil condition of DNA in DNP (to the mechanism of dNA reduplication). Biofizika **8**:140–141.
99. STREISINGER, G., R. S. EDGAR, AND G. HARRAR. 1961. The organization of DNA in bacteriophage and bacteria by C. A. Thomas, Jr., ref. p. 132. *In* J. H. Taylor [ed.], Molecular biology, part I. Academic Press, Inc., New York.
100. STRELZOFF, E., AND F. J. RYAN. 1962. The necessary involvement of both complementary strands of DNA in the specification of messenger RNA. Biochem. Biophys. Res. Commun. **7**:471–476.
101. SWARTZ, M. N., T. A. TRAUTNER, AND A. KORNBERG. 1962. Enzymatic synthesis of deoxyribonucleic acid. XI. Further studies on nearest neighbour base sequences in deoxyribonucleic acids. J. Biol. Chem. **237**:1961–1967.
102. TAMANN, N. 1903. Kristallisieren und Schmelzen, p. 315–344. Bart, Leipzig.
103. TAYLOR, J. H. 1962. Chromosome reproduction. Intern. Rev. Cytol. **13**:39–73.
104. VIGNAIS, P., E. BARBU, J. BASSET, AND M. MACHEBOEUF. 1951. Modifications de l'état des acides ribonucléiques sous hautes pressions en présence ou en absence de nucléodépolymérase. Compt. Rend. **232**:2364–2366.
105. VIGNAIS, P., AND M. MACHEBOEUF. 1954. Influence des hautes pressions hydrostatiques sur l'action enzymatique de la ribonucléase. Ann. Inst. Pasteur **86**:180–188.
106. WATSON, J. D., AND F. H. C. CRICK. 1953. Molecular structure of nucleic acids. A structure for deoxyribose nucleic acid. Nature **171**:737–738.
107. WOOD, W. B., AND P. BERG. 1962. The effect of enzymatically synthesized ribonucleic acid on amino acid incorporation by a soluble protein-ribosome system from Escherichia coli. Proc. Natl. Acad. Sci. U.S. **48**:94–104.
108. YOSHIDA, A., AND C.-G. HEDÉN. 1962. Some serological properties of staphylococcal cells and cell walls. J. Immunol. **88**:389–393.
109. YOSHIDA, A., C.-G. HEDÉN, B. CEDERGREN, AND L. EDEBO. 1961. A method for the preparation of undigested bacterial cell walls. J. Biochem. Microbiol. Technol. Eng. **3**:151–159.
110. YOUNG, I. E., AND P. C. FITZ-JAMES. 1959. Pattern of synthesis of deoxyribonucleic acid in Bacillus cereus growing synchronously out of spores. Nature **183**:371–373.
111. ZOBELL, C. E., AND F. H. JOHNSON. 1949. The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. J. Bacteriol. **57**:179–189.
112. ZOBELL, C. E., AND R. Y. MORITA. 1957. Barophilic bacteria in some deep sea sediments. J. Bacteriol. **73**:563–568.
113. ZOBELL, C. E., AND C. H. OPPENHEIMER. 1950. Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. J. Bacteriol. **60**:771–781.