small effects here. We find no difference in the sensitivity of four markers at subcritical temperatures, whereas the T_m's are different, as first reported by Roger and Hotchkiss[4] and by Marmur and Doty.[3] No striking effect has yet been found for dependence of subcritical sensitivity on molecular weight, but there is a dependence of the T_m's on molecular weight, which varies for different markers. This will be the subject of a future report.

Summary.—The sensitivity of the transforming activity of a DNA preparation to heating at temperatures below the critical melting temperature depends strongly on the method of preparing the DNA. DNA deproteinized by a few cycles of shaking with chloroform-isoamyl alcohol showed concave-downward, concentration-dependent, survival curves. The same DNA, treated further with a single phenol extraction, exhibited exponential survival curves, which were concentration-independent and more stable and were very similar to the previously observed curves for a phenol preparation from a different strain of pneumococcus. A fraction of the first preparation recovered from a CsCl gradient showed intermediate stability.

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MECHANISMS OF INACTIVATION OF DEOXYRIBONUCLEIC ACIDS BY HEAT

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There has been much interest lately in the structure of the deoxyribonucleic acid (DNA) molecule and the effects of various agents on that structure. In particular, the effect of heat on the properties,[1] structure,[2] and biological activity[3–6] has been the subject of several investigations. Heat is especially interesting in that it leads

to extensive physical changes in the state of the molecule in a critical narrow temperature range. It was recently shown[2, 5] that these changes are at least partly reversible. In addition, heat causes a slow loss of biological activity below the critical temperature[3]; in an accompanying paper[7] it is shown that this loss follows a simple first-order rate law when the DNA is properly prepared. The loss is probably the result of a chemical reaction. Heat further has mutagenic effects in living organisms[8]; these are very likely associated with physical and chemical changes in the chromosomal DNA.

The reported behavior of DNA on heating is in marked contrast to that of ribonucleic acid, which has been studied previously by one of the present authors.[9] In the case of the infectious ribonucleic acid from tobacco mosaic virus, the biological activity is lost by a simple first-order reaction whose rate increases exponentially with temperature.[9] The different behavior of the two kinds of nucleic acid is presumed to reflect the great difference in their configurations, DNA possessing in its native state a rigid, secondarily bonded, double-stranded helical structure,[10] while in RNA, the secondary structure, if any, is certainly less extensive.[9, 11]

The original aim of this investigation was to study in detail the loss of activity of the biologically active DNA, or transforming principle, from *Diplococcus pneumoniae* in order to see to what extent the reportedly complex behavior of this DNA could be related to the simpler behavior characteristic of the ribonucleic acid that one of us had previously studied.[9] To this end, data were taken consisting of the results of quantitative measurements of the transforming activity of the DNA after various heat treatments in aqueous solution. The resulting curves of activity versus time of heating showed so many interesting features that the following rather extensive analysis was undertaken.

Background.—Our general ideas about the effect of heat on DNA are derived from experimental studies such as those already cited and from the statistical mechanical theory that has recently been developed.[12–16] At low temperatures, the Watson-Crick double-stranded structure[10] is the stable form. Imperfections in the structure appear as the temperature is raised; these take the form of occasional sequences of unbonded base-pairs forming open loops. The ends of the chains are natural sites of weakness in the double-stranded structure and extensive opening occurs there also. The opening process leads finally to a catastrophic breakdown of the double-stranded structure at a critical temperature that is identified with the experimental denaturation temperature. At temperatures near but below the critical temperature, the equilibrium state of the molecule consists of a fluctuating alternation of sections of the double-stranded structure and of loop sections of unbonded base-pairs. The fraction of unbonded base-pairs increases rapidly with temperature just below the critical temperature. This change in configuration is accompanied by a change in the optical density of the ultraviolet absorption band at 260 mμ; the latter is frequently taken to be a measure of the fraction of base-pairs bonded, although it is not certain that this is completely justified.[17] Since the chain ends are sites of weakness, the critical temperature should become lower if the chain length becomes too short, although it is not yet known at what length the effect appears. The two strands separate into independent entities above the critical temperature. Since the number of independent particles increases when this occurs, it would be expected that concentration

would have an effect on the critical temperature, but in fact the concentration effect is probably very slight.[16]

A complicating factor is the differing strengths of the bonds in the two types of base-pairs. Marmur and Doty[18] have shown that the critical temperature of a double-stranded molecule containing only adenine-thymine pairs is about 60° lower than that of a guanine-cytosine molecule and that the critical temperatures of molecules with intermediate compositions vary linearly with the composition. Since we have no reason to expect that all the molecules or molecular fragments in a given DNA preparation should have the same composition, we must be prepared to encounter some diversity of critical temperatures within one sample.

The above paragraphs describe the situation at equilibrium, but nonequilibrium effects are also important. The dissociation process is expected to take several seconds or more[19, 20] because of the extensive unwinding that is necessary before the two strands can separate. The rate may be even slower immediately above the critical temperature because the amount of free energy available for driving the unwinding is very small. The reverse process, association, is even more strikingly slow; until recently it was not thought to go at all. However Marmur, Doty *et al.*[2, 5] have recently shown that reassociation will occur if the solutions are held for a sufficiently long time, a period of hours with *D. pneumoniae* DNA, at a temperature somewhat below the critical temperature. This sluggishness is apparently the result of the partial association of various parts of the two strands into improperly matched configurations, which must then be "annealed out" before proper rematching can occur. To distinguish reassociation with reformation of the native double-stranded structure from reassociation into improperly matched configurations, Marmur, Doty *et al.* have coined the term "renaturation" for the former process.

Experimental.—The property that was examined in this study was the transforming activity of the DNA. Cells from a drug-resistant strain of *D. pneumoniae* were lysed and the DNA separated. After having been subjected to various treatments, the ability of the DNA to transform a drug-sensitive strain into a drug-resistant one was assayed quantitatively. The assay was carried out in the linear region of DNA concentrations, where the number of cells transformed is presumed to be proportional to the number of active DNA molecules. Experimental details are given in the previous paper.[7] The results are usually expressed as percentages of the activity of the untreated samples.

Two preparations of DNA were used. The first came from a strain of cells obtained from Dr. J. Marmur, bearing resistance to (among others) the drugs streptomycin, bryamycin, novobiocin, and erythromycin. In the preparation used in this work, the protein was removed from the DNA by extraction with phenol; for this reason, the preparation is called "Phenoled TP." The second preparation came from another strain of cells carrying a streptomycin-resistance marker only; although it too was extracted with phenol, the accidents of laboratory bookkeeping led to its being designated "III-DNA-A." Further details are in the previous paper.[7]

The Collapse Process.—The decay of transforming activity as a function of time at a series of temperatures is shown in Figures 1 and 2 for the various markers tested in the two preparations. One feature is immediately obvious: most of the

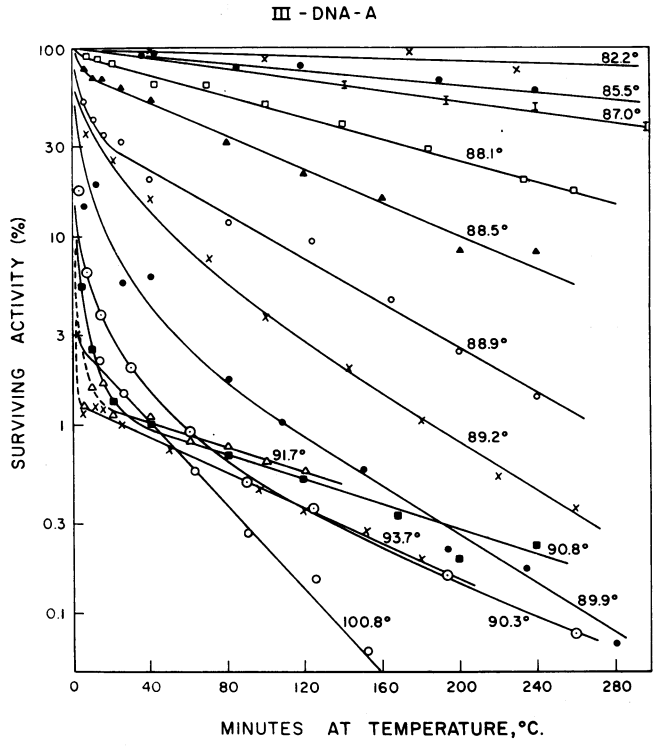

FIG. 1.—Activity for transformation to streptomycin resistance of preparation III-DNA-A after heating for various times at various temperatures. 0.1 *M* sodium phosphate buffer, pH 7.25 at room temperature, DNA concentration between 0.05 and 2.0 μg/ml.

activity curves have two regions; an initial rapid loss of activity followed by a slower decay that is linear on the semilogarithmic scale. Presumably, each of these regions corresponds to its own molecular process with its own characteristic dependence on temperature. For convenience, we name the first process the "collapse process," while the linearity on the semilog scale suggests the name "single-hit process" for the second.

Let us examine the collapse process first. A measure of its extent at any temperature can be obtained by extrapolating the linear part of the curve to zero time and noting the intercept. The latter is presumably the level to which the activity would fall as a result of the collapse process alone if the single-hit process did not occur, and hence, it is a measure of the former.

In Figure 3, the extent of collapse, as measured by the intercepts, is plotted against temperature for all five sets of data. Included also is the optical density curve taken by Marmur and Doty[18] on a similar preparation under similar conditions. The similarity of the six curves is striking, suggesting that the "collapse" in question is indeed the collapse of the native base-paired structure, as has been generally believed.[1, 2, 4, 6]

The data shown in Figure 4 shed further light on the nature of the collapse. These experiments, where the material was returned to a temperature below the

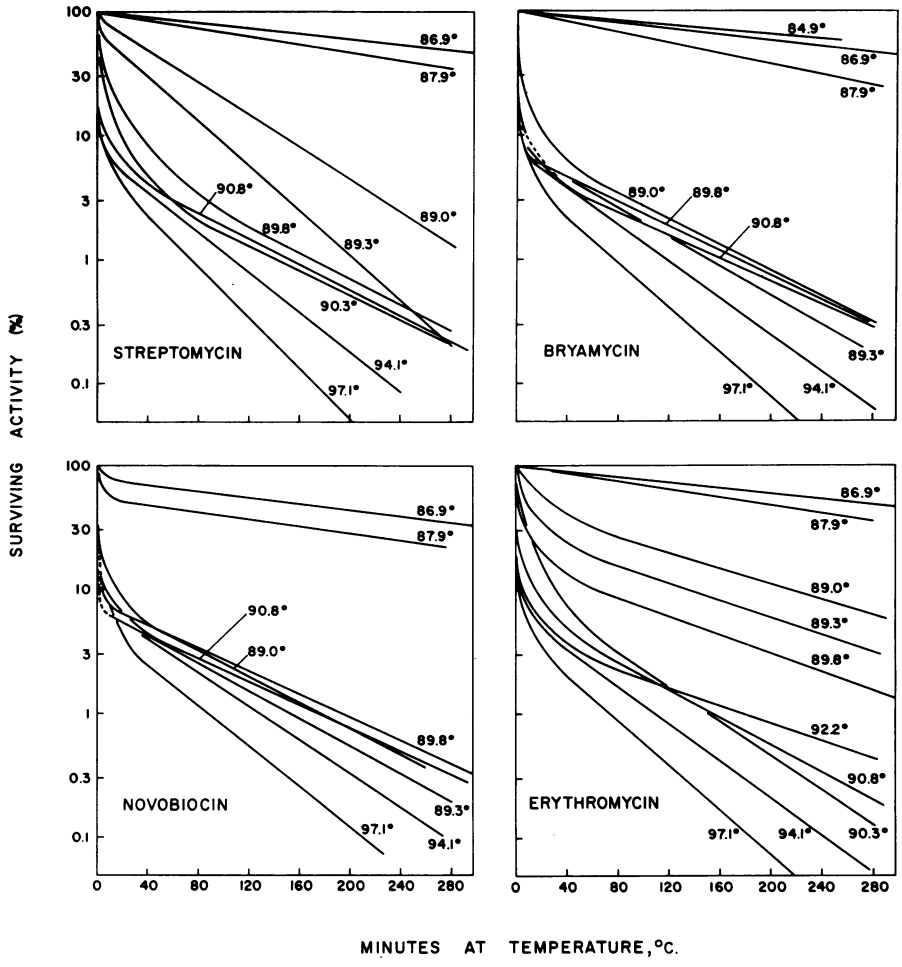

FIG. 2.—Transforming activity of DNA preparation "Phenoled TP" as a function of time and temperature. Four different drug-resistance markers are shown. Same solvent as in Figure 1, DNA concentration between 0.05 and 0.5 μg/ml.

collapse region after a short time above it, show that the process is largely irreversible at the times and temperatures used. This irreversibility suggests, following the considerations outlined in the introduction, that the two strands of the Watson-Crick structure have separated completely.[21]

As was remarked above, the physical separation of the two strands must involve the relative rotation of the ends by as many turns as there are initially in the helix, a rather slow process.[19, 20] The speed of the untwisting should increase with temperature as the free energy available to drive the process increases. This increase is in fact easily seen in the data of Figure 1. Another way the process can be speeded up is by reducing the necessary number of rotations per chain end, for example, by breaking up the strands into smaller pieces before untwisting. In this connection, the data of Figure 5 are interesting. Here, the material was exposed briefly to the degradative enzyme, DNase, before heating. This short

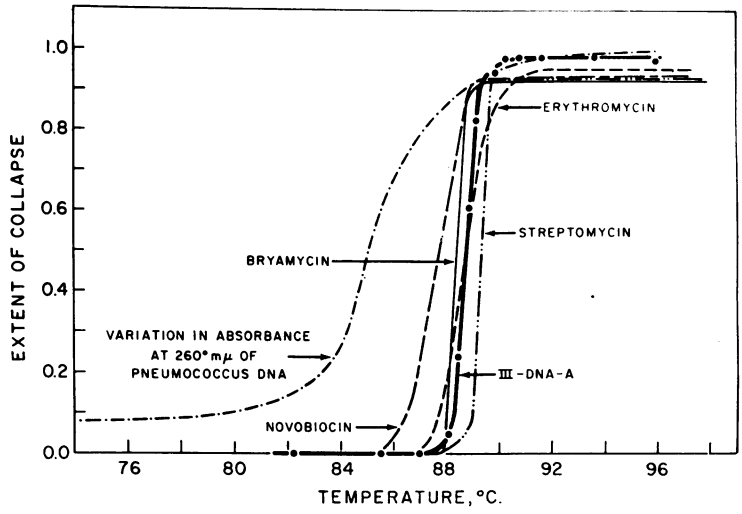

FIG. 3.—"Extent of collapse" plotted against temperature. The straight lines of Figures 1 and 2 were extrapolated to the ordinate; the *loss* of activity at these extrapolated points is taken as the extent of collapse. Also included is the optical density curve of Marmur and Doty.[18]

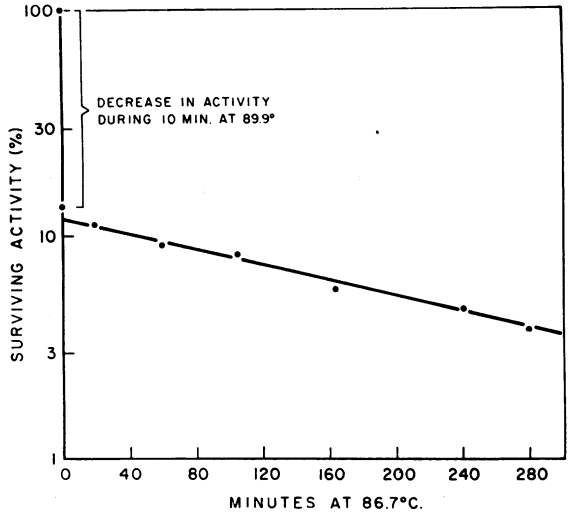

FIG. 4.—Transformation activity of III-DNA-A after initial heating at high temperature and immediate transfer to and holding at a lower temperature. Same solvent as in Figure 1, DNA concentration 2.0 μg/ml at 89.9°, 0.05 μg/ml at 86.7°.

DNase pretreatment has the effect of speeding up the collapse of the surviving activity, in good accord with the expectations.

A remarkable thing about the collapse process is the fact that it never leads to complete loss of activity; a few per cent of the initial activity seem to remain even when the temperature is raised well above that necessary to start the collapse.[21] Several possible explanations present themselves: the activity may be the result of the "renaturation" discovered by Marmur, Doty and others;[2, 5] the sample may contain a small fraction unusually resistant to heat; or the activity may be a true property of denatured DNA.

The possibility of renaturation was tested by heating and cooling at diverse concentrations of DNA. Doty and Marmur [2, 5] found that renaturation was highly concentration-dependent, as would be expected in a reaction involving two

particles. In contrast, we found that changing the concentration of III-DNA-A from 0.05 μg/ml to 10 μg/ml led invariably to between 1 per cent and 1.5 per cent residual activity as determined by the intercept at zero time. Only if the solution were held at 65°C for hours could a different result be obtained, and then only at the higher concentration.[22] This independence of concentration shows that the residual activity does not depend on interactions between the particles and hence rules out renaturation of the kind observed by Doty, Marmur *et al.*

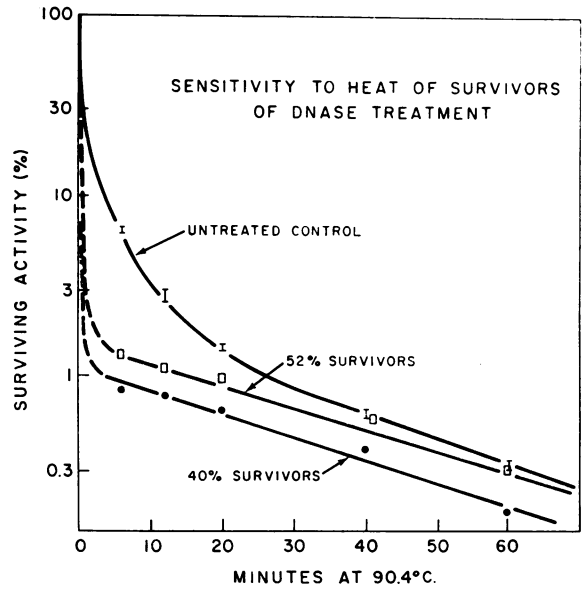

FIG. 5.—Effect of pretreatment with DNase on transformation activity after heating. The ordinate is the per cent of the survivors of the enzyme treatment that also survived the heating. Details: 6.3 μg of III-DNA-A in 0.1 ml of buffer was added to 3.05 ml of the following enzyme solution: 0.01 μg Worthington DNase, 0.4 per cent bovine serum albumin, 0.001 Mg^{++}, physiological saline, and 0.01 *M* phosphate buffer at neutral pH. Samples were incubated at 37° for 7 and for 10 minutes and quickly frozen until the heating experiment. The surviving activity of these two samples before heating was 52% and 40% respectively. After thawing, 0.1 ml of above samples was pipetted into 3.9 ml of 0.1 *M* phosphate in a bath at 90.4°. The control was treated in the same way, except that the incubation at 37° was omitted. The control gave results identical to those of another experiment at 90.4° without enzyme, showing that the DNase was immediately inactivated by the heat.

Decision between the remaining two possibilities is difficult, although good reasons for preferring the third are developed later. If the third is correct, it means that denatured DNA, composed principally of unmatched strands, is effective in transferring information to the receptor cells, though with relatively low efficiency. This idea, though somewhat unorthodox, does not seem to be contrary to any known fact, the details of the transformation process being at present quite incompletely understood. We return to the discussion of this point later.

The Single-Hit Process.—The linearity on a semilog scale of part or all of the inactivation curves is characteristic of a process in which a single randomly occurring event inactivates each genetic marker. We leave aside for the moment the question of the precise chemical nature of this event, referring to it for convenience simply as a "hit."

The remarkable temperature dependence of the rate of this single-hit process, as given by the slope of the inactivation curves, is shown in Figure 6. The process proceeds below the collapse region but slows down very rapidly as the temperature drops. There is a striking similarity between these curves and the dependence of optical density on temperature (Fig. 3). We are led to believe that the site of the "hit" must be at those occasional base pairs that, as the result of thermal fluctuations, are not hydrogen-bonded and to which reference was made in the introduc-

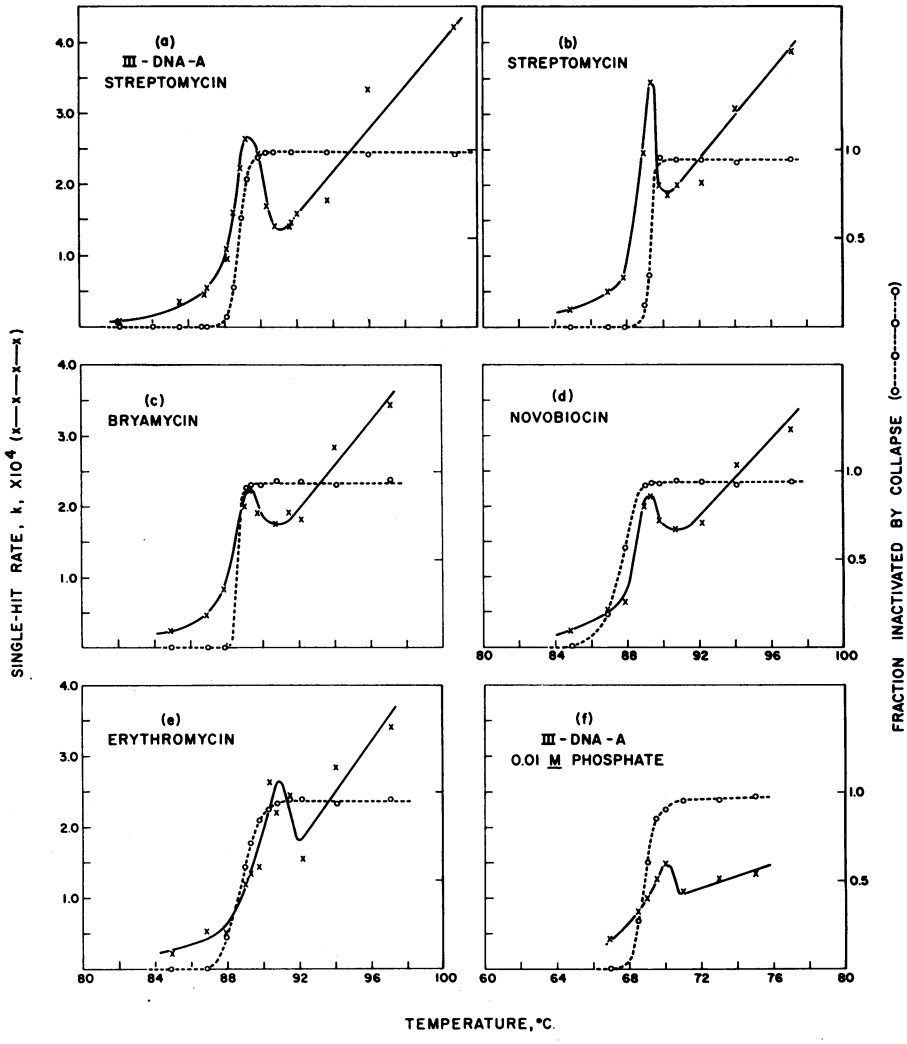

FIG. 6.—Rate of single-hit process, in reciprocal seconds (solid curves) and extent of collapse (dashed curves). Graphs (a) and (f), III-DNA-A in 0.1 *M* (pH 7.25) and 0.01 *M* phosphate (pH 6.6) respectively; graphs (b)–(e), Phenoled TP in 0.1 *M* phosphate.

tion. The rate of the single-hit process thus measures the degree to which the native helical structure is broken down, as does the optical density, but with the difference that the hit is in the narrow region of the genetic marker, whereas the optical density is averaged over the whole sample.

At temperatures immediately above the collapse region, the curve of the single-hit process takes a new course, first dropping precipitously as the collapse becomes complete and then rising smoothly with temperature at a rate quite usual for simple chemical reactions (Q_{10} = 3.4, activation energy of about 34 kcal). In order to understand the meaning of this curve, we must discover what the material is that retains its activity after having been heated above the collapse temperature. We still have two hypotheses: there may be a small fraction that is unusually resistant

to heat, or the activity may be a true property of denatured DNA.

Let us begin by examining the first of these alternatives. The data of Figure 6 show that the resistant fraction, while resisting collapse, is nearly as sensitive to the single-hit process as the main fraction. To examine this point further, the experiments shown in Figure 7 were carried out. Here, two samples of DNA were kept for a while at 87°, just below the collapse region, until they retained only 37 per cent and 20 per cent, respectively, of their initial activity. They were then raised to a temperature above the collapse region and the activity followed as a function of time by removing aliquots for assay. This activity, when extrapolated back to the time at which the samples were raised to the higher temperature, gives a measure of the sensitivity of the resistant fraction to heating at 87°, below the collapse temperature of the main fraction. From the straight line, B, in Figure 7, we can obtain the rate constant of the single-hit process operating on the resistant fraction at 87°; it is 2.7×10^{-5} sec^{-1}. This may be compared with the rate constants of 12×10^{-5} at 91.7° and 37×10^{-5} at 100.8° from which the value of 3.4 for Q_{10} was derived; using the same value of Q_{10}, we would expect that the rate constant would be 8.9×10^{-5} at 87°, much greater than observed. We are therefore forced to believe that the single-hit curve for the resistant fraction has a sharp bend in the collapse region of the main fraction.

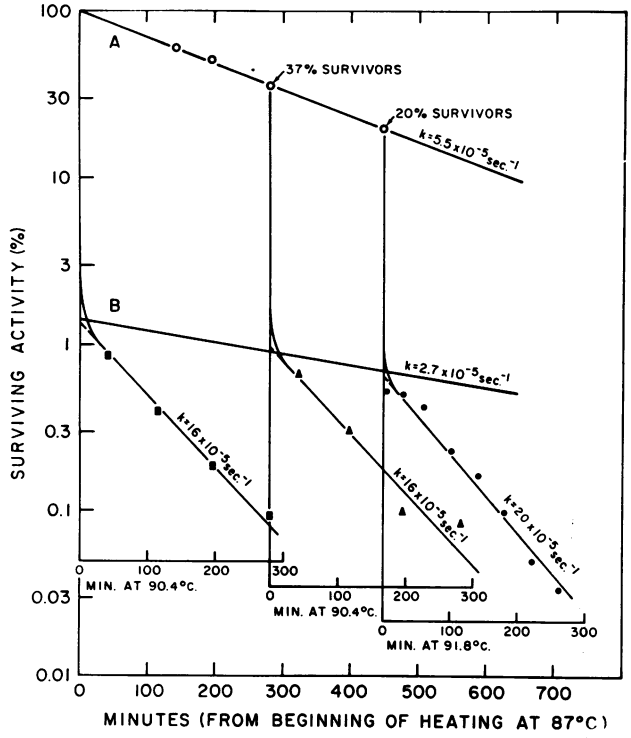

FIG. 7.—Experiments on samples of III-DNA-A heated in 0.1 *M* phosphate for various times at 87° (below collapse temperature) and subsequently at temperatures above the collapse temperature. Samples heated at 87° were assayed for activity at various times (curve A). Two of these and a control were then immediately heated at the higher temperature, as shown. Curve B is the straight line drawn through the extrapolated intercepts of the high-temperature curves. DNA concentration 2.0 μg/ml during 87° heating, 0.05 μg/ml during second heating.

A further test of the idea is given by the inverse experiment to that of Figure 7. The results have already been shown in Figure 4. Here the material was first heated above the collapse region and then its sensitivity to the single-hit process studied by maintaining it at a temperature below the collapse region. We would expect that the inactivation rate would be the same as that of curve B in Figure 7, but we find that it is now considerably larger, 7.0×10^{-5} sec^{-1}, forcing us to con-

clude that a preliminary exposure to high temperature sensitizes the "resistant" fraction to further heating at a lower temperature.

Thus we see that each new experiment demands a new hypothesis, an undesirable state of affairs and one that argues against the correctness of the idea of a resistant fraction in the DNA.

One alternative remains, the idea that the transforming activity that survives heating above the collapse temperature is a true property of denatured DNA. Let us examine from this point of view the facts that have been presented. Since we have already seen that concentration and time of cooling have no effect in the range that we are using, we must believe that the activity does not depend on the interaction of the single DNA strands, or reformation of double strands, that is known to occur to some extent on cooling. It must then be a property of the single strands themselves.

When we look at the data with this idea in mind, one result immediately assumes new significance. In Figure 6, it appears that the drop in the single-hit rate that occurs at the collapse temperature is, within the accuracy of the data, nearly a factor of two.[23] This would correspond nicely to the reduction of the "target" for the single-hit process from two strands just below the collapse temperature to one strand above. Below the collapse temperature, the two strands are still attached and function as a unit on cooling, although many individual base pairs may be unbonded while hot. We must assume, then, that a hit on either strand blocks the activity of both as long as they are attached. Above the collapse temperature, the two strands are separate and remain so on cooling; a hit on one strand blocks this strand alone.

The data of Figure 7 are likewise easily interpreted on this basis. The slope of line B, 2.7×10^{-5}, gives the rate of inactivation of single strands in double molecules which were broken up by heating above the collapse temperature after the inactivation hit occurred. The slope of line A in Figure 7 is the rate of inactivation of the double molecules themselves, and it is just twice, 5.5×10^{-5}, the rate of inactivation of the single constituent strands as given by line B. The results of Figure 7 are thus naturally explained without recourse to additional hypotheses. The same may be said for Figure 4, which is now viewed as giving the rate of inactivation of single-stranded material at 86.7°; it is not expected to have any close relation to the experiment of Figure 7, where the material during the low temperature heating is in the form of double strands with a fraction of the bases unbonded.

Another minor but perplexing observation can also now be explained. In some of the cases shown in Figures 1 and 2, there is a tendency at long times for the activity curves of a temperature in the collapse region to cross those of higher temperatures. We can now say that the lower temperature curves express the inactivation of the double-stranded molecules which inactivate faster than the same material in single-stranded form at a higher temperature. These crossovers are inexplicable on the basis of the resistant fraction hypothesis and furnish the only definite, as opposed to suggestive, evidence against that hypothesis.

To insure that the apparent close relation between the onset of collapse and the rapid rise in the single-hit rate was not the result of an unfortunate coincidence, the series of experiments was repeated with III-DNA-A in 0.01 *M* phosphate buffer. Doty, Marmur *et al.*[2] have reported that the denaturation of DNA occurs between

60° and 70° in this buffer, and we were curious to see if the interrelations found near 90° with 0.1 *M* phosphate would be repeated. The results are summarized in Figure 6(f), and it can be seen that indeed the picture has the same appearance except for the downward shift in the temperatures. Our confidence in the general correctness of interpretation is thus reinforced.

The Nature of the Single Hit.—The question naturally arises: what is the nature of the "hit" that inactivates the transforming activity? Two chemical reactions are known that are likely candidates for the honor, chain scission and depurination. The rate of chain scission of single-stranded DNA was recently determined[2] by measurement of the rate of molecular weight decrease; it amounts to 10^{-6} events per base-pair per minute in 0.01 *M* phosphate at 79°. Zamenhof and Greer[24] have measured the rate of separation of purines from DNA in this buffer; it is about 0.11 per cent per hour at 81° or 1.8×10^{-5} events per base-pair per minute. Clearly, the depurination is much more likely to be the significant event because of its much greater frequency.

We may also estimate the size of the sensitive region. For III-DNA-A at 75° in 0.01 *M* phosphate, we find that the rate of the single-hit process is 8.22×10^{-3} per minute. When this is divided by the frequency of depurination plus chain scission, a total of 1.9×10^{-5} events per base pair per minute, we obtain a quotient of 430 base pairs, which is our estimate of the size of the sensitive region for streptomycin resistance in this material. This is in good agreement with the target molecular weight of 3×10^5, or about 500 nucleotide pairs, which was calculated by Guild and Defilippes for the same marker from data obtained by irradiation with cyclotron particles in the Mev range.[25]

Attempts have also been made by Rosenberg, Sirotnak, and Cavalieri to determine the marker size by measuring the rate of loss of transforming activity as the molecule is broken by sonication or shear.[26] They concluded that the marker was "small" in comparison to the molecules used in that study, which were 1400 base-pairs or more in length. Examination of their published data leads us to believe that there is no essential contradiction between their results and ours, in that a marker size of 430 base-pairs could not be distinguished from one much smaller within the precision of their data on the streptomycin marker.

Heterogeneity.—It is apparent in Figure 3 that there are differences among the markers with respect to the precise temperatures, and the temperature ranges, associated with the collapse process. Similar differences have been observed by other workers,[1-6] and those observed by Marmur and Lane,[5] who worked with the same strain of organisms, are consistent in detail with ours. Hence, it seems unlikely that the differences are the result of random experimental errors. It is tempting to attribute the differences to the presence of different amounts of guanine-cytosine base-pairs in the various markers or possibly to differences in the chain length of the DNA strands bearing the markers. The latter hypothesis could also be invoked to explain the variable sharpness of the collapse transitions as seen in Figure 3. However, these explanations are confronted by a difficulty as soon as the rate curves of the single-hit process are considered, since similar effects would be expected to appear in the rates, while in fact the rates behave otherwise.

We have already presented arguments supporting the idea that the rapid rise in the single-hit rate that occurs just below the collapse temperature is a reflection of

the increasing number of base-pairs that are opened up as a result of thermal excitation. We would expect that those DNA molecules that were especially susceptible to collapse for any of the reasons given in the preceding paragraph would also have an especially large fraction of opened base-pairs, and hence would be especially susceptible to being "hit." However, the experiments show that at any given temperature below the collapse region the inactivation rates of all the markers in both samples of DNA are precisely the same, in apparent contradiction to our explanation of the collapse differences.

We can get around the difficulty by postulating that the separation of the strands that causes the collapse is affected more by the properties of that small most stable region that is the last to come apart than by the average composition of the DNA molecule as a whole. If, for example, the molecule bearing the streptomycin marker in Phenoled TP had a sequence of a few score base-pairs that was particularly rich in the strong guanine-cytosine combination, this sequence would hold the two strands together even when the rest of the molecule was ready to separate. If the molecules bearing the bryamycin marker lacked such an unusually stable sequence, they would come apart at a lower temperature, as in fact is the case. At the same time the gross composition of the two markers themselves could be nearly the same, the fraction of base-pairs opened up at a given temperature likewise the same, and the rate of the single-hit process the same, as is observed.

The different steepnesses of the collapse curves, with some actually crossing others, can now be explained. We have only to postulate that a given unusually stable sequence is not invariably associated with a given marker. This might result from random breakage of the DNA during preparation, the breaks sometimes leaving a given marker attached to one stable sequence, sometimes to another.

Some Unanswered Questions.—A curious difference occurs in the rates of inactivation above the collapse temperature. While the four markers of the Phenoled TP inactivate at about the same rate, the rate of the streptomycin marker in III-DNA-A is only about half as fast. The simplest explanation would be that the size of the marker region in the latter sample is only about half as large as in the former, an idea that would lend interest to a genetic investigation of the relationships between the two strains of cells concerned. But how does one reconcile this explanation with the virtual identity of the inactivation rates of the same two samples below the collapse temperature? More probably, something resulting from the different modes of preparation, perhaps the presence of a large amount of RNA in III-DNA-A, is involved.

The transforming activity of the DNA after having been heated above the collapse temperature, which we have interpreted as activity of single-stranded DNA, is different in the two samples, being about one and a half per cent of the activity of the native form in III-DNA-A and about 8 per cent in the Phenoled TP. This difference also is probably the result of some otherwise inconsequential difference between the two samples, such as molecular weight. It has been shown that transforming activity is highly dependent on the molecular weight, largely as a result of a change in the absorption of the DNA.[26]

Finally, we wish to direct attention again to one part of the interpretation of the sudden drop in the single-hit rate at the collapse temperature, namely, the necessity for assuming that a hit in one strand of the duplex molecule inactivates also the

complementary strand for as long as the two strands are attached but that the complementary strand recovers its activity as soon as the two strands are separated by heat. In other words, a hit in one strand "blocks" the activity of the other only as long as the two strands are attached. We might hypothesize that the cell has two independent mechanisms for using DNA, one for double-stranded DNA and another for single. This seems unlikely. We might alternatively suppose that a hit in one strand produces a lethal mutation, that is, a cell receiving such DNA not only fails to transform but is actually killed. There are many difficulties with this interpretation; to mention one, it is hard to see why the drop in the single-hit rate at the collapse temperature, here considered as the rate of the lethal mutation, should depend on which marker is being assayed, as it obviously does in the experiments of Figure 6. Hence, this hypothesis also seems unlikely. We await the devising of a better explanation.

Summary.—The inactivation by heat of two preparations of the transforming principle of *D. pneumoniae* has been studied quantitatively with the object of elucidating the mechanisms of heat inactivation. These preparations were both deproteinated by the phenol method, which gives preparations showing simple first-order kinetics. Two processes are found, in accordance with previous workers. The first, or "collapse," process is apparently the actual separation of the complementary strands of the DNA and leads to a rapid and kinetically complex loss of from 93 to 98 per cent of the activity. The remaining few per cent of activity is apparently inherent in the denatured DNA and does not depend on "renaturation." The other process, the "single-hit" process, is a first-order reaction that apparently proceeds only at those base-pairs that are open as a result of thermal excitation, and hence its rate increases anomalously fast with temperature in the temperature region just below that at which the collapse occurs. It seems likely that the first-order reaction is the loss of a purine from one of the strands. With this assumption and the known rate of depurination, we estimate the size of the streptomycin resistance marker to be about 430 base-pairs, although this estimate must be considered as very tentative in view of differences between the two samples. The collapse temperature is different with different markers in the same sample, while the dependence of the single-hit process on temperature is nearly the same for all markers as long as the collapse process does not intrude. Following other workers, we attribute the differences in the collapse temperatures to heterogeneities in the guanine-cytosine content of the different markers, but in order to reconcile this idea with the rate data, we must assume that the heterogeneities are very local and that the over-all compositions of all the markers are effectively the same.

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Note added in proof: We would like to call attention to a paper by M. Roger and R. D. Hotchkiss elsewhere in this issue, in which is discussed the works by these investigators in this field.

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[21] This residual activity was previously noted by other workers. In particular, we wish to acknowledge private communications from M. Roger and from J. Marmur and P. Doty in October 1959.

[22] In other experiments, some recovery of activity was noted when the solutions were held at 65° for two hours. The exact activity figures were 16% after either 4 or 8 minutes at 92° and 2 hours at 65° at a concentration of 4 μg/ml; 3.5% and 2.5% respectively after 4 or after 8 minutes at 92° and 2 hours at 65° at a concentration of 0.05 μg/ml, in reasonable agreement with the observations of Marmur, Doty *et al.*[2,5] on "renaturation."

[23] We should not forget that it is especially difficult to determine accurately the rate of the single-hit process in this region, since the collapse process itself is prolonged under these conditions and interferes with a clear-cut delineation of the single-hit curve. In fact, it is conceivable that the two processes are not completely independent in this critical temperature range.

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