mechanism of disaggregation of procarboxypeptidase A are now in progress.

Summary.—Evidence is presented to show that procarboxypeptidase A, although a single molecular species as judged by the criteria of protein chemistry, is actually a molecular complex, having two, and possibly three, distinctive enzymatic activities. The molecule consists of three polypeptide chains which can be separated from each other under certain conditions. One of these is carboxypeptidase A or an immediate precursor thereof; the second of these has been identified as a precursor of an endopeptidase which hydrolyzes acetyl-L-tyrosine ethyl ester and glucagon. The mechanism of this nonenzymatic disaggregation reaction and its relation to zymogen activation have been considered.

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TRANSFORMATION BY DENATURED DEOXYRIBONUCLEIC ACID

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On raising deoxyribonucleic acid (DNA) to temperatures above a critical "melting point," the helical secondary structure collapses[1] and the biological transforming activity declines to a few per cent of its initial value. It does not go to zero, however, and this "resistant" transforming activity, first reported by Lerman and Tolmach,[2] has been the subject of considerable investigation. Marmur and Lane[3] found that it resisted 120°C and therefore could not be due to an anomalous very high guanine-cytosine content creating a resistant species of molecule. In following up this problem, they discovered "renaturation" of dissociated single strands and suggested that this phenomenon, occurring to a slight extent even on fast cooling, would account for the resistant activity.

Ginoza and Zimm,[4] however, found that the level of the resistant fraction was constant over a 200-fold range of concentration during heating, whereas renatura-

tion of dissociated strands had been shown to be dependent on the square of the concentration.[3, 5] Their further studies provide very suggestive but not compelling evidence for the conclusion that the residual activity is a property of single-stranded denatured DNA itself. Roger and Hotchkiss[6] also suggest, on other grounds, that it is a property of the denatured DNA, but prefer to think of it as double-stranded rather than single.

One alternate possibility exists, however, namely, that there are some DNA molecules whose strands are held together by a small amount of a non-DNA material and that some of these will renature on fast cooling, independent of total DNA concentration. Since this appears to be what happens at temperatures just below the irreversible melting temperature, e.g. 88°C., where the reversible (ambient temperature) hyperchromic shift is over 80 per cent complete,[3] the rate should be adequate if only there is something holding occasional strands together. In this case then, the residual activity would be carried by essentially native renatured molecules, the alternative being that it resides in the denatured molecules which are merely less efficient at getting into the bacterial genome.

This question is readily settled by the use of CsCl density gradients, since denatured DNA has a density about 0.015 gm./ml greater than that of the corresponding native DNA, as first shown by Meselson and Stahl.[7] Pneumococcal DNAs containing streptomycin (S) and novobiocin (N) resistance markers respectively were prepared by a phenol method described elsewhere.[8] Portions of each were heated at a concentration of 23 μg/ml in 7.7 molal CsCl for seven minutes at 100°C and cooled in an ice bath. Mixtures of heated and unheated DNAs were made a follows: (1) Neither heated; (2) S heated, N unheated; (3) N heated, S unheated. 3.0 ml of each mixture were then placed in tubes of the SW-39 rotor, layered with 2.2 ml of mineral oil, and centrifuged for 77 hr at 35,000 rpm in the Spinco Model L Centrifuge.

The tubes were fractionated by puncturing with a needle and collecting each drop (mean volume 0.01 ± .001 ml) in a separate tube, which was then diluted for transformation assay. The final assay data were obtained using 1/250 of the DNA per drop, which put all points on the linear region of the concentration-response curve. The results are shown in Figure 1, where the values for the heated DNA have been magnified 10-fold for easy presentation, and we see that the residual transforming activity of the heated DNA (about 5 per cent of the initial) is associated with a well resolved peak 17–19 drops more dense than the unheated activity. The predicted value for denatured DNA, from calculation of the density gradient, drops per cm, etc., is 18–20 drops, which is excellent agreement considering the errors involved in drop size, mean radius, and so forth.

There is a slight trail of a few per cent of the activity toward lower density. This is common with the drop-collecting procedure and presumably is due to mixing as the material comes down the tube. If any of the heated activity is due to renaturation, it is a very small fraction of the total. The three tubes behaved very similarly, the curves of Figures 1*a* and 1*b* being essentially superimposable. The native DNA peaks in all three have been sketched using the combined data. While there appear to be few points near the top of Figure 1*c* particularly, the near coincidence of the native peaks is amply confirmed by the low points when plotted on a semi-log scale.

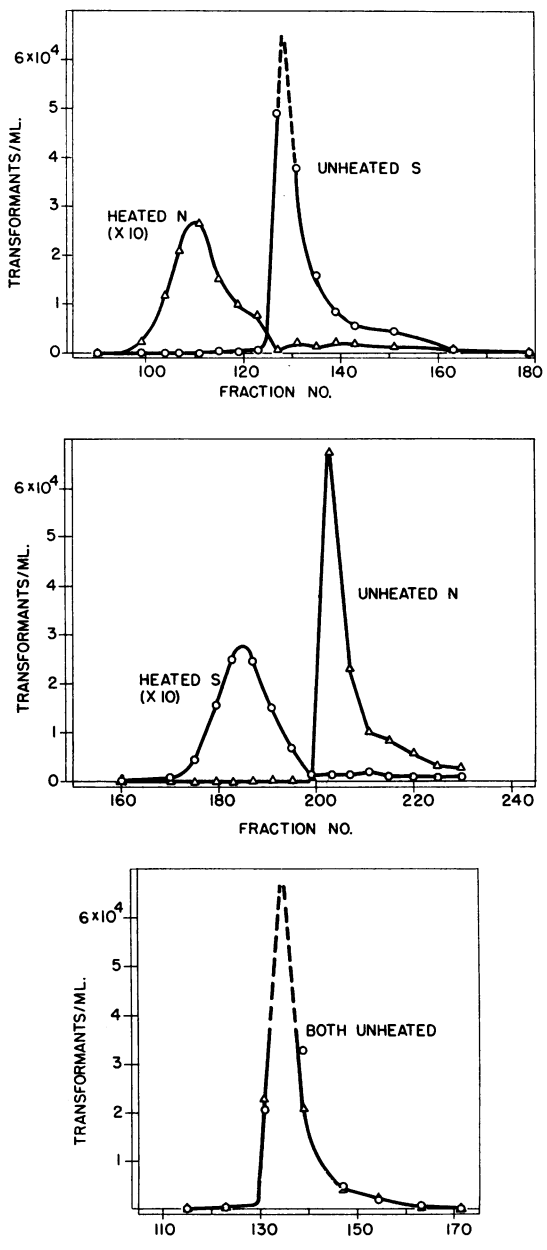

FIG. 1.—Transforming activity in fractions from mixtures of heated and unheated pneumococcus DNAs after separation in a CsCl gradient. N = novobiocin resistance marker, S = streptomycin resistance marker. Heated activities are multiplied by 10. Expected separation between native and denatured DNAs, 19±1 fractions. Other details in text.

From these data and the lack of concentration dependence mentioned earlier,[4] we conclude that the residual activity of heated DNA is in fact a biological property of single denatured molecules. We have not demonstrated here that these are

single strands, but we rely on the work of Marmur, *et al.*[3, 5] on this point. Whether or not this material is single-stranded, this result demonstrates once more that the Watson-Crick helical structure of native DNA is not required for biological activity, though transformation of bacteria is much more readily accomplished by such a molecule.

We can now interpret more fully the observations of Lerman and Tolmach.[2] They compared the effect of various damaging agents on transforming activity and on the ability of the DNA to be permanently incorporated into receptor cells, as measured by P^{32} uptake. For most agents (UV, X ray, DNase, etc.), P^{32} incorporation fell one-fifth to one-fourth as rapidly as did transformation, showing that most of the damage was to processes occurring after incorporation of the DNA. In the case of heat at 95°C, however, the P^{32} incorporation fell as rapidly as did transformation to a value between 5 and 10% per cent of the initial activity. At this point, both P^{32} and transforming activity leveled off, and in the subsequent slow decline (Ginoza and Zimm's "single hit" process[4]), P^{32} incorporation again fell more slowly than the transforming activity. The intercepts of the slow component suggested, in this one experiment, that the transformation per unit P^{32} incorporation was if anything higher than that of the control.

It is therefore clear that the loss of transforming activity on denaturation is due entirely to a decreased, but nonzero, efficiency of incorporation per molecule and that material which is incorporated is as good as or better than the starting material at carrying out the remaining biological steps of the process.

There is a further point of interest. Ginoza and Zimm[4] found that the survival curves of activity versus time of heating actually crossed one another for small temperature increments near 90°C; i.e., the rate constant of the slow component decreased nearly a factor of two in a narrow temperature interval at the point where complete irreversible collapse had occurred. After four hours' heating, e.g., the surviving activity at 90.8°C was three times that at 90.3°C. This was the chief result leading to the conclusion that the slow component represents chemical damage to single strands and that above the melting temperature the single strands act independently in transforming bacteria. Since the latter conclusion is now confirmed, we can agree with their argument that at temperatures just below the complete dissociation point, damage to either strand inactivates both for transformation purposes. This result is consistent with the observations of single-hit inactivation kinetics seen for damage to transforming activity by other agents, such as ionizing and ultraviolet radiation, deoxyribonuclease, mustards, etc.[2, 9]

Note added: Since this work was done we have learned that Rownd, Lanyi, and Doty[10] also have found by density-gradient studies that the residual activity is in the denatured band.

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*THE SELECTIVE SYNTHESIS OF INFORMATIONAL RNA IN BACTERIA**

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Introduction.—For the past several years, this laboratory has employed virus-infected cells to examine the nature of the mechanism involved in transcribing information from DNA for the protein-synthesizing apparatus. The data obtained are consistent with the simplest of transcription devices, namely, that RNA strands complementary to homologous DNA are the informed intermediaries. The experimental steps leading to this conclusion may be briefly noted, since they served as an operational guide in the present attempts to extend this mechanism to uninfected cells.

Existence proof of RNA homologous to DNA: The existence of a "T2-specific RNA" inferred from the P^{32} experiments of Volkin and Astrachan[1] was established by Nomura, Hall, and Spiegelman.[2] The proof was attained by separating the newly synthesized RNA from the bulk of the cellular RNA using both zone electrophoresis in starch columns and centrifugation in sucrose gradients. The T2-specific RNA was found to have a higher electrophoretic mobility and a greater heterogeneity in size than the three principal normal RNA components (23*S*, 16*S*, and 4*S*). It was further shown that T2-specific RNA was ribosome-bound but with a linkage very sensitive to disruption by low magnesium levels.

Sequence complementarity: Having established T2-specific RNA as a physical entity and provided methods for its selective enrichment, it was possible to proceed to an inquiry into the significance of the homology in base ratios between it and T2-DNA. To examine this question, Hall and Spiegelman[3] employed the device described by Marmur and Lane[4] and Doty *et al.*[5] for the reconstitution of double-stranded structures. It was possible to show that RNA-DNA complexes were indeed formed in mixtures of single-stranded T2-DNA and purified T2-RNA subjected to a slow cooling process. The success of the hybridizing experiment suggested immediately that the original observation[1] of a similarity in base composition between T2-RNA and DNA was indeed a reflection of a more profound homology. The fact that hybrid formation was found to be unique to the homologous pair led to the conclusion that the nucleotide sequences of T2-RNA and DNA are complementary.

Existence of natural RNA-DNA complexes: If continued formation of complementary RNA is a necessary concomitant, it should be possible to find RNA-DNA hybrids in any cell actively engaged in protein synthesis. Again, the T2–*E. coli*