subcellular fractions as well as previously in the global RNA of green *Euglena* as compared with the colorless variety (Brawerman and Chargaff, 1959).

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Studies on Polynucleotides. XV.* Enzymic Degradation. The Mode of Action of Pancreatic Deoxyribonuclease on Thymidine, Deoxycytidine, and Deoxyadenosine Polynucleotides†

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The degradation by crystalline pancreatic deoxyribonuclease of synthetic homologous thymidine, deoxycytidine, and deoxyadenosine polynucleotides and of thymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-deoxycytidine (de-TpApC) has been studied. Divalent ion activation, which was necessary, was provided by manganous ions, the manganous ions being about twice as effective as magnesium ions, in agreement with previous results. Under the standard conditions used (a large amount of enzyme was employed), all of the tetranucleotides and higher homologues bearing 5′-phosphomonoester end-groups were attacked. The di- and trinucleotides bearing 5′-phosphomonoester groups and de-TpApC were resistant under the conditions used. The mode of action was identical in all the series bearing 5′-phosphomonoester end-groups. Cleavage occurred mostly at internal bonds; thus, the tetranucleotides gave mainly dinucleotides; pentanucleotides gave mainly di- and trinucleotides; hexanucleotides gave initially di-, tri-, and tetranucleotides. With the oligonucleotide de-TpTpTpTpTp, which bears a 3′-phosphomonoester end-group, cleavage occurred, in addition, at the terminal linkage so as to release de-pTp and de-TpTpTpT, indicating that the 3′-phosphomonoester group simulates a phosphodiester bond for the enzymic action. That attack occurs equally well at multiple points was shown by studying the substrates de-pTpTpTpTpC and de-pT[pT]$_3$pT. The former gave de-pTpT and de-pTpTpC as well as de-pTpTpT and de-pTpC, while the latter after partial degradation gave all the possible lower homologues. In each series of homologous compounds, the rate of degradation increased with increase in chain length. Of the three analogous pentanucleotides, de-pTpTpTpTpT, de-pCpCpCpCpC, and de-pApApApApA, the last was attacked faster than the pyrimidine analogues. The mode of action of the enzyme on polynucleotides and on DNA is discussed in the light of the present findings.

The action of crystalline pancreatic deoxyribonuclease on deoxyribonucleates (DNA) has been studied by a large number of investigators. Both the kinetics of its action and the composition of the ultimate digestion products have received attention (for a recent review see Laskowski, 1961). Detailed analyses of the products have shown them to consist of about 1% mononucleotides (nucleoside-5′-phosphates), 13–18% dinucleotides, and the remainder a complex mixture of higher oligonucleotides (Sinsheimer, 1954, 1955). The main clearly established feature of the action of the enzyme is that all the products bear 5′-phosphomonoester end-groups. Any further questions regarding the mode of action of the enzyme have remained unanswered, however, although certain interpretations concerning preferential specificity toward purine or pyrimidine bonds have been advanced (Laskowski, 1961; Vanecko and Laskowski, 1961). These conclusions have been derived from studies of (a) the composition of the dinucleotide mixture

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resulting from the DNA degradation and (b) the degradation of mixtures of oligonucleotides bearing 3′-phosphomonoester groups (Vanecko and Laskowski, 1961).

The recent availability of synthetic, well-characterized homologous series of short-chain polynucleotides of thymidine (Khorana and Vizsolyi, 1961; Tener *et al.*, 1958; Turner and Khorana, 1959), deoxycytidine (Khorana *et al.*, 1961), and deoxyadenosine (Ralph and Khorana, 1961), as well as a mixed trinucleotide (Gilham and Khorana, 1959), offered an opportunity to ascertain if more could be learned about the mode of action of the pancreatic enzyme. The results which are reported in the present communication show that under the conditions used all the tetra- and higher polynucleotides are attacked at "internal" bonds to give smaller fragments and that, where possible, the attack occurs at multiple points. The di- and trinucleotides bearing 5′-phosphomonoester groups are resistant under the conditions used in the present work. Further, while the mode of action on the adenine and pyrimidine oligonucleotides is identical, the rate of attack on the adenine compounds is distinctly higher than on the pyrimidine analogues. Brief reports of parts of this work have already appeared (Khorana, 1959; Ralph *et al.*, 1961).

Experimental

Substrates.—All substrates used were synthetic compounds which have already been reported from this laboratory. These were de-pTpT, de-pTpTpT, de-pTpTpTpT,[1] and higher homologues up to the decanucleotide (Khorana and Vizsolyi, 1961); the isomeric pentanucleotide bearing the 3′-phosphomonoester group (Turner and Khorana, 1959), de-TpTpTpTpTp; de-pTpTpTpTpC (Khorana and Vizsolyi, 1961); de-pCpCpCpC and homologues (Khorana *et al.*, 1961); de-pApApApA and homologues (Ralph and Khorana, 1961); and de-TpApC (Gilham and Khorana, 1959).

Enzymes and Methods.—Pancreatic deoxyribonuclease was a once-crystallized sample purchased from Worthington Biochemical Corporation. Solutions of the enzyme were made fresh every week in water at a concentration of 5 mg/ml and stored at 2°. In experiments in which the activation by different metal ions was tested, standard solutions of the enzyme were made by dissolving 10 mg of the enzyme in 1.5 ml of cold 0.05 M ethylenediaminetetraacetate (EDTA) (previously adjusted to pH 7.5). After 1 hour, the solution was dialyzed at 4° against two changes of 0.05 M ammonium acetate (pH 5.3).

Prostatic phosphomonoesterase used for the removal of the terminal phosphomonoester group was prepared by the method of Boman (1958). The enzyme was dialyzed at 4° against two changes (100 volumes) of 0.05 M ammonium acetate buffer (pH 5.3) made 0.001 M with EDTA. Standard assays were performed at 37° with *p*-nitrophenyl phosphate as the substrate. The enzyme solution was diluted so that 0.002 ml cleaved 0.10 μmole of *p*-nitrophenyl phosphate in 5 minutes at 37°. The enzyme assay contained the following components in a total volume of 0.5 ml: ammonium acetate buffer (pH 5.3), 100 μmoles; *p*-nitrophenyl phosphate, 2 μmoles; and enzyme. The reaction was stopped by the addition of 1 ml of 0.1 M sodium hydroxide and the absorbance read at 400 mμ. Dephosphorylations of phosphomonoester groups in oligonucleotides were performed with 0.05 ml of the standard phosphomonoesterase solution. The determination of the phosphate released by the phosphomonoesterase was performed by the method of Chen *et al.* (1956) as modified by Ames and Dubin (1960).

Experiments performed over a prolonged period on degradation of oligo- and polynucleotides by pancreatic deoxyribonuclease were carried out with one of the following three incubation mixtures:

(a) From 0.4–0.6 μmole (in oligonucleotide concentration) of a lyophilized powder of the substrate, 0.1 ml of a 0.5 M solution of tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.3), 0.02 ml of a 0.1 M solution of magnesium acetate, and 0.1 ml of a 5 mg/ml solution of the enzyme. Incubation was carried out for a total of 21 hours at 37°, a drop of toluene being added after 7 hours. The procedure is described for individual oligonucleotides under Results.

(b) Incubation mixture was similar to above, except that it contained 0.03 ml of 1 M manganous acetate or magnesium acetate in a total volume of 0.5 ml. Incubation was for 1–18 hours at 37°. After incubation one of the following two procedures was used: (1) The total incubation mixture was boiled for 2 minutes and subsequently incubated at pH 5.3 with 0.1 ml of standard prostatic phosphomonoesterase solution for 2–3 hours at 37°. Subsequently, the amount of phosphorus rendered inorganic was estimated as described above. For identification of nucleotide products, the total reaction mixture was treated with about 0.1 ml of carefully washed Amberlite IR-120 (pyridinium form) resin to remove the divalent ions, the resin was washed three times with water, and the reaction mixture and washings were lyophilized. The lyophylate was redissolved in 0.1 ml of water and the solution chromatographed on paper. (2) Alternatively, the treatment with phosphomonoesterase was omitted and the samples, after boiling for 2 minutes, were chromatographed directly by column chromatography as described below.

(c) The incubation mixture for polynucleotide degradation used most generally contained approximately 0.3–0.6 μmoles of the polynucleotide in 0.1 ml of water, 0.05 ml of 0.5 M Tris-acetate (pH 7.4)

[1] With one exception, the system of abbreviation is that currently used in the *Journal of Biological Chemistry* and previously defined in earlier papers of this series (see, for example, Khorana and Vizsolyi, 1961). Thus the single letters A, T, G, and C represent the nucleosides of, respectively, adenine, thymine, guanine, and cytosine. The letter "p" to the left of the nucleoside initial indicates a 5′-phosphomonoester group and the same letter to the right indicates a 3′-phosphomonoester group. Thus, in going from left to right a polynucleotide chain is specified in the C_5'–C_3' direction. In the present manuscript, the 2′-deoxyribopolynucleotides are distinguished from the ribopolynucleotides by the letters "de-" before the total abbreviation. For diagrammatic representation of oligo- and polynucleotides see Khorana (1960).

buffer, 0.1 ml of 1 M manganese acetate solution, and 0.5 mg of the enzyme in 0.05 ml of water. The incubation was carried out at 37° for varying times up to 4 hours. The reaction was stopped by boiling for 2 minutes. After addition of 1 ml of water and centrifugation, the clear supernatant and a water wash (0.5 ml) were applied to the top of a standardized diethylaminoethyl (DEAE)-cellulose (carbonate) column (see below).

Chromatography.—The products of degradation were separated and characterized mainly by chromatography on a DEAE-cellulose (carbonate) column. A standard column (10 × 0.6 cm diameter) was used, and it was prepared by packing a thin slurry of the anion exchanger under pressure and converting the exchanger to the carbonate form by washing with 2 M ammonium carbonate and then with water. The column was standardized for position of elution of homologous oligonucleotides by use of a synthetic mixture containing about 5 $O.D._{257}$ units of thymidine mono- to decanucleotides. The elution was carried out with use of a linear gradient (0 → 0.4 M) of triethylammonium bicarbonate (pH 7.5). The mixing vessel contained 200 ml of water and the reservoir an equal volume of 0.4 M triethylammonium bicarbonate. Two-ml fractions were collected every 6 minutes. For typical elution patterns see under Results.

For identification of different peaks obtained from the enzymic degradation experiments, the peaks were pooled and the salt removed by repeated evaporation of an aqueous solution *in vacuo*. The residual material was passed through a short Dowex-50 ion exchange resin column in the ammonium form, and the eluate and washings were lyophilized. The residue was identified by paper chromatography alongside authentic markers.

Paper chromatography was used for identification as well as for following directly the products of enzymic degradation in some experiments. Paper chromatography was performed by the descending technique, mostly with Whatman No. 40 double acid-washed paper. The solvent systems used were: isopropyl alcohol–concd. ammonia–water (7:1:2), Solvent A; 95% ethyl alcohol–1 M ammonium acetate (pH 7.5) (5:2), Solvent B; isobutyric acid–1 M ammonia–0.1 M disodium EDTA (100:60:1.6), Solvent C; and *n*-propyl alcohol–water–concd. ammonia (55:35:10), Solvent D. Paper electrophoresis was performed with an apparatus similar to that described by Markham and Smith (1952). The medium used was 1 M acetic acid.

Results

Activation by Metal Ions.—A number of previous workers have studied the activation of the enzyme by different divalent metal ions. In the present study, the absolute requirement for the presence of such ions was shown by the total inactivity of the enzyme against thymidine pentanucleotide after dialysis against EDTA. The rates obtained in the presence of 6×10^{-2} M concentration of manganese and magnesium ions for the degradation of de-pTpTpTpC and de-pTpTpTpTpC are shown in Figure 1. The rates were followed by determination of the increase in release of inorganic phosphate

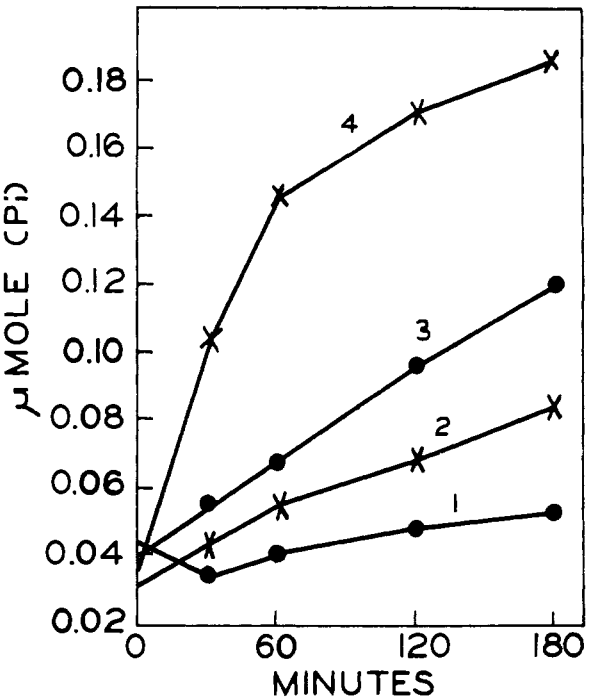

FIG. 1.—Comparison of the rates of degradation of de-pTpTpTpC and de-pTpTpTpTpC by pancreatic deoxyribonuclease in the presence of magnesium acetate and in the presence of manganese acetate. Incubation mixtures were set up as described in the text under Experimental [incubation mixture (b)]. After termination of the action of the pancreatic enzyme, incubation was carried out with prostatic phosphomonoesterase as in Experimental for the determination of the phosphomonoester groups released. Curve 1, de-pTpTpTpC in the presence of magnesium (6×10^{-2} M); Curve 2, de-pTpTpTpC in the presence of manganese (6×10^{-2} M); Curve 3, de-pTpTpTpTpC in the presence of magnesium (6×10^{-2} M); Curve 4, de-pTpTpTpTpC in the presence of manganese ions (6×10^{-2} M).

upon incubation with phosphomonoesterase, as the degradation with the deoxyribonuclease progressed. The results show that the rates were two to three times higher in the presence of manganese ions than with magnesium ions. The finding is similar to that made previously by Wiberg (1958) on effectiveness of these ions in enzymic activity against DNA.

Increase in Rate of Degradation as a Function of Chain Length.—Figure 2 shows the rates of degradation as a function of chain length. Again the rates were measured by removal of aliquots from the incubation mixture followed by treatment with phosphomonoesterase. As can be seen, the rate, under the standard conditions used, increased from de-pTpTpTpT to the thymidine hexanucleotide. The rate of attack on thymidine decanucleotide, although not followed along with the set in Figure 2, was even higher (see below). Figure 1 also shows the increase in rate of attack in going from de-pTpTpTpC to de-pTpTpTpTpC.

Degradation of Thymidine, Deoxycytidine, and Deoxyadenosine Tetranucleotides.—The mode of degradation of these three tetranucleotides bearing 5′-phosphate end-groups was identical in that the dinucleotide (de-pTpT in the case of de-pTpT-pTpT) was the most abundant product. In addi-

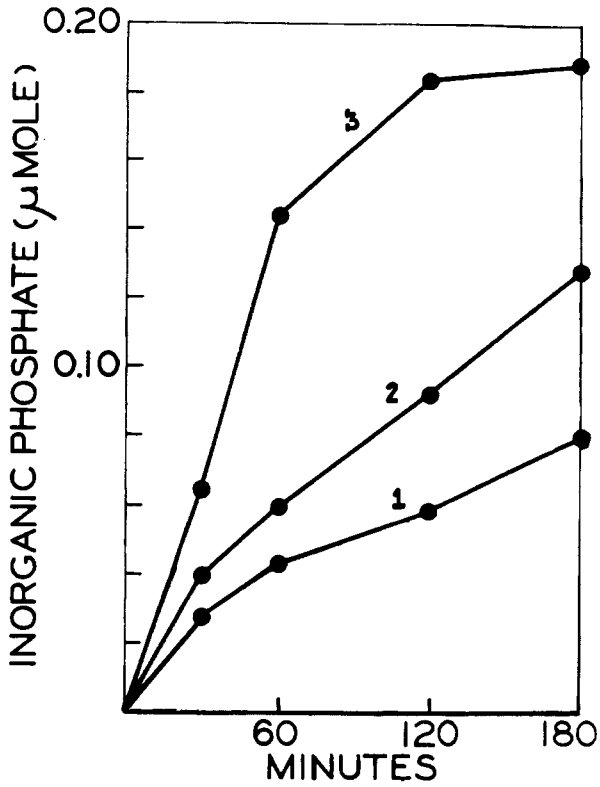

FIG. 2.—Rates of degradation of homologous thymidine oligonucleotides under identical conditions and with use of equimolar concentrations of oligonucleotides [incubation mixture (b) in Experimental]. Curve 1, de-pTpTpTpT; Curve 2, de-pTpTpTpTpT; Curve 3, de-pTpTpTpTpTpT. The curves show the rate of increase in the formation of inorganic phosphate, the inorganic phosphate formed at zero times (due to the terminal phosphomonoester groups in starting materials) having been subtracted.

tion, the trinucleotides and the corresponding mononucleotides were evidently produced in trace amounts. The result obtained with de-pTpTpTpT, as shown in Figure 3, is typical for this set of experiments. However, marked differences were noted in the rates of attack on the three tetranucleotides; thus after 4 hours the deoxycytidine tetranucleotide was attacked only to a slight extent, while the thymidine analogue was 83% degraded and the deoxyadenosine tetranucleotide was 89% degraded. (The rate differences with the pentanucleotides are described below.)

Degradation and Rates of Attack on Pentanucleotides Bearing 5'-Phosphomonoester Groups.—The pentanucleotides de-pTpTpTpTpT, de-pCpCpCpCpC, and de-pApApApApA were all cleaved so as to give mainly the di- and trinucleotides, with only traces of mono- and tetranucleotides. The elution pattern obtained on chromatography of the products from deoxyadenosine pentanucleotide is typical for this set of experiments and is shown in Figure 4. The di- and trinucleotides were identified by subsequent paper chromatography along with authentic markers.

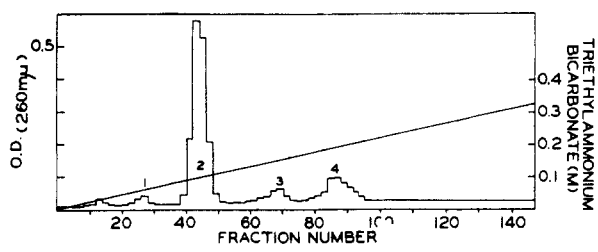

FIG. 3.—Degradation of thymidine tetranucleotide (de-pTpTpTpTpT). The conditions used were those of incubation mixture (c) in Experimental. Chromatography on a DEAE-cellulose column was performed as in text. The peak numbers 1–4 correspond, respectively, to the mono-, di-, tri-, and tetranucleotides.

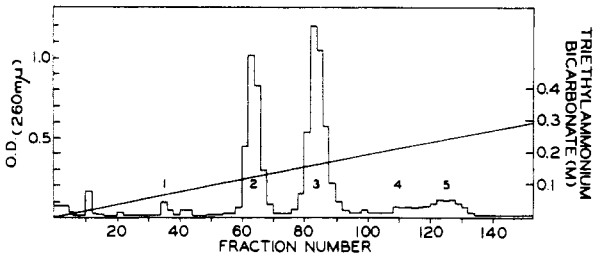

FIG. 4.—Products of degradation of deoxyadenosine pentanucleotide (de-pApApApApA). Incubation conditions were as described under (c) in Experimental, and for column chromatography a DEAE-cellulose column was used under the standard conditions. Peak numbers from left to right correspond, respectively, to the mono- to pentanucleotides.

A further parallel experiment was carried out on the rates of attack on the above pentanucleotides. The standard incubation conditions [(c), above] were used, and the incubation was performed for only 5 minutes. Analysis of the products from each reaction mixture by standard column chromatography showed that deoxycytidine pentanucleotide was degraded to the extent of 13% and the thymidine analogue to the extent of 25%, while the deoxyadenosine pentanucleotide was degraded 58%. The result thus showed a distinctly higher rate for the cleavage of the diester bonds in the adenine nucleotide polymer.

Degradation of the Pentanucleotide de-pTpTpTpTpTpC.—While the experiments reported above demonstrated that the enzyme attacks at the interior of the oligonucleotide chain (endonuclease action), an experiment carried out with de-pTpTpTpTpC, in which the two ends of the molecule are distinguished, showed that the attack occurs simultaneously at multiple internal points in the molecule. Thus the products of degradation were those shown in Figure 5. In this experiment the incubation was performed as described under (a), above, and the products were identified by paper chromatography in Solvent A and by paper electrophoresis at acidic pH after removal of the terminal phosphate groups with phosphomonoesterase. The products thus formed were de-TpC, de-TpT, de-TpTpC, and de-TpTpT, all of which were identified by comparison with authentic markers. Although no quantitative measurements of the products formed by attack at the two internal points (Fig. 5) have been carried out, the yields were such as to indicate approximately equal probability of attack at the two sites.

In view of the greater ease of degradation of deoxyadenosine pentanucleotide than of pyrimidine pentanucleotides, the study of a pentanucleotide

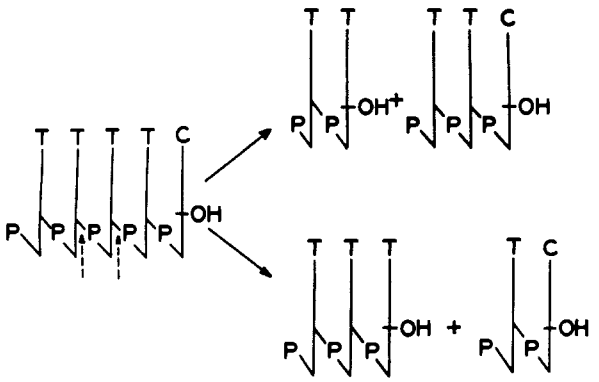

FIG. 5.—Schematic representation of the mode of action of pancreatic deoxyribonuclease on the pentanucleotide de-pTpTpTpTpC. Points of attack are shown by the arrows.

containing a terminal purine nucleotide should be interesting.

Degradation of Thymidine Pentanucleotide Bearing a 3'-Phosphomonoester Group (de-TpTpTpTpTp).—Incubation of this substrate was carried out under the conditions described in (a), above, and the mixture was directly chromatographed in Solvent A. Four ultraviolet absorbing bands were noted; one strong band at the origin and three with increasing order of R_F which corresponded to de-TpTpTpT, de-TpTpT, and de-TpT. The band at the origin, consisting of de-pTp and homologues, was eluted and treated with an excess of the phosphomonoesterase preparation. The products thus formed, which again were identified by paper chromatography, were de-TpTpT, de-TpT, and thymidine. From the sum of these results, it is clear that degradation occurred at multiple points, as shown in Figure 6.

Degradation of Hexanucleotides and Thymidine Decanucleotide.—That attack occurs internally at multiple points was also shown by the study of thymidine and deoxyadenosine hexanucleotides bearing 5'-phosphomonoester groups. At a time

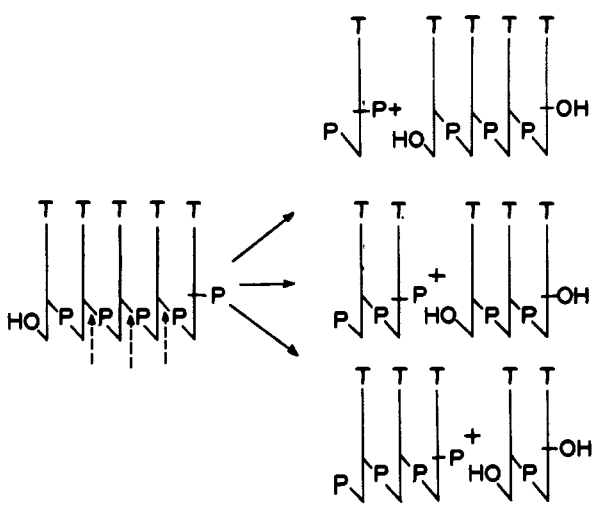

FIG. 6.—Schematic representation of the mode of action of pancreatic deoxyribonuclease on the pentanucleotide de-TpTpTpTpTp, which bears a 3'-phosphomonoester end group. The points of attack are indicated by the arrows.

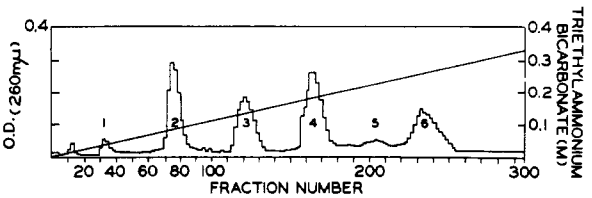

FIG. 7.—Products of partial degradation of deoxyadenosine hexanucleotide (de-pA[pA]$_4$pA). Incubation conditions were as described under (c) in Experimental. Column chromatography was performed under the standard conditions.

interval [incubation conditions (c)] when partial degradation of the starting material had occurred, the products were identified by column chromatography to be di-, tri-, and tetranucleotides (Fig. 7). With a longer period, the tetranucleotide was further degraded so that the ultimate products were di- and trinucleotides.

Finally, as an example of a larger polynucleotide, the mode of degradation of thymidine decanucleotide de-pT[pT]$_8$pT was examined. The results of partial degradation are shown in Figure 8. The

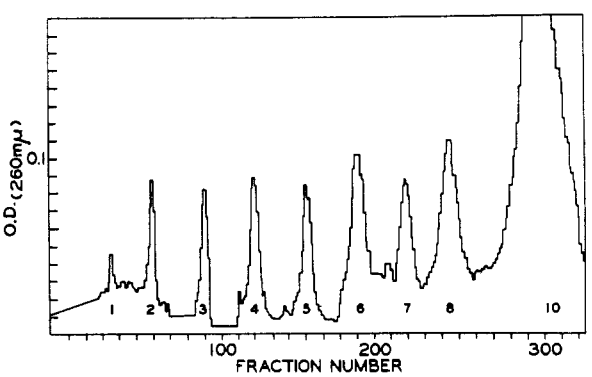

FIG. 8.—Products of partial degradation of thymidine decanucleotide (de-pT[pT]$_8$pT). The incubation mixture used was (c) as described in Experimental. Incubation was for 5 minutes. The peak numbers correspond to the chain length of the oligonucleotides.

presence of a small amount of mononucleotide was again noted, and the result is in agreement with results on degradation of DNA. Furthermore, the results indicate that the attack is random at multiple points rather than preferentially beginning near one or the other end of a long chain.

Inertness of Thymidylyl-(3' → 5')-deoxyadenylyl-(3' → 5')-deoxycytidine (de-TpApC).—Under the conditions described in (a), above, the compound de-TpApC was found to be completely resistant.

DISCUSSION

In discussing the results of degradation of short-chain polynucleotides it should be emphasized that the two-stranded macromolecular DNA is the best substrate[2] for the enzyme and that in relation to the amounts of the enzyme needed for an attack on DNA the amounts necessary for degradation of the present series of compounds and of the oligonucleotides studied by Laskowski and co-workers (Potter *et al.*, 1958; Vanecko and Laskowski, 1961)

[2] For references see Laskowski (1961), p. 135.

are very much larger. The question whether the same enzyme is involved in the initial stages of degradation of the native DNA on the one hand and the degradation of short-chain polynucleotides on the other must be left open.[3] We prefer, however, to assume at this time that the same enzyme is involved in the activity both against DNA and against short-chain polynucleotides. The preference is based on the observations reported in this paper: namely (1) the similarity of the metal ion activation demonstrated here to that found previously for DNA degradation; (2) the increase in susceptibility with increase in chain length of the substrate; and (3) the mode of action of the enzyme on the compounds studied. Thus if a second activity is present, it must also be an "endonuclease" and not any of the known types of phosphodiesterases (Khorana, 1961), which according to the generally accepted terminology form mononucleotides as the products of degradation.

If, then, the same enzyme is involved in the initial phase of degradation of the macromolecular DNA and in the later stages when short chains of presumably single-stranded polynucleotides are present, the possibility should be considered that the differences in rates are due to the requirement by the enzyme of a particular configuration (helical?) in the substrate. It seems to be well established now that the enzyme attacks DNA as it exists in the double-stranded helical coil and that the collapse of this structure occurs only after a sufficient number of bonds has been broken (see, *e.g.*, Schumaker *et al.*, 1956; Thomas, 1956). The poorness of the short-chain polynucleotides as substrates could then be explained by their attainment to only a very small degree of helical configuration. In any event, the questions that must be asked concerning the attack of the enzyme on the native DNA are: What is the mode of attachment of the enzyme to the macromolecule? In addition to the binding at the backbone of phosphodiester bonds, are the hydrogen-bonded purines and pyrimidines also involved in the binding?

Under the standard conditions used in the present work, di- and trinucleotides bearing 5′-phosphomonoester end-groups were resistant, while the tetranucleotides and higher polymers of the same general structure were all attacked in an identical manner. Deoxythymidylyl-(3′ → 5′)-deoxyadenylyl-(3′ → 5′)-deoxycytidine, which contains only two phosphodiester bonds but three different bases, was also resistant. The resistance of dinucleotides to further attack by the enzyme has also been shown previously (Potter *et al.*, 1958). The rate of attack from tetranucleotide onward increased with increase in chain length. From these results, it appears that the primary requirement for the enzyme to attack a given substrate is minimal chain length. Once the requirement of the chain length is fulfilled, the enzyme may attack simultaneously at multiple points. Thus the binding of the enzyme for effective cleavage of a phosphodiester bond can occur equally well in de-pTpTpTpTpC at two sites, giving de-pTpT + de-pTpTpC and de-pTpTpT + de-pTpC as the products. The same conclusion was abundantly clear from the results of degradation of a number of other substrates tested.

It may also be noted that although the attack is preferentially directed toward the internal sites, that is, at least one internucleotide bond removed from either end of a substrate, the attack is also possible to a very minor extent at a terminal bond. This was evident from several of the experiments reported here (*e.g.*, Figures 3 and 7). The result provides a good model for the previously established formation of about 1% of mononucleotides during the degradation of DNA by this enzyme.

The results of attack on the pentanucleotide de-TpTpTpTpTp, which bears a 3′-phosphate end group, showed another feature. In addition to cleavages at "internal" points, the attack occurred to a significant extent at the internucleotide bond next to the 3′-phosphomonoester group. Potter *et al.* (1958) have previously studied the degradation of de-ApApTp and mixtures of oligonucleotides of the same general type (Vanecko and Laskowski, 1961). Degradation of de-ApApTp occurred, giving de-ApA and de-pTp. From all these results and the above-described resistance of the trinucleotides bearing 5′-phosphomonoester groups, it appears that the 3′-phosphomonoester end-group can serve as a phosphodiester bond. In other words, the 3′-phosphate end-group provides one of the binding sites equivalent, say, to that of the boldface phosphodiester bond in de-pTpTpT**pT**. Because of this behavior the oligonucleotides bearing 3′-phosphate end-groups are considered atypical substrates for this enzyme, and caution is necessary in drawing conclusions regarding substrate specificity of this enzyme from experiments using such compounds as substrates (*cf.* Vanecko and Laskowski, 1961).

While the mode of attack on the adenine and pyrimidine pentanucleotides of analogous structure was identical, the significant observation was made that the adenine pentanucleotide was attacked at about twice the rate at which the pyrimidine counterparts were attacked. Because of the endonuclease action, which by definition requires a segment of an oligonucleotide chain, it cannot be predicted what the results would be in various penta- or higher mixed polynucleotides. In the previous discussions of the question of the preferential specificity toward the heterocyclic bases (Laskowski, 1961), great significance has been attributed to the finding that in the dinucleotides found in the digests of DNA, the dinucleotides of the type de-*p*-purine nucleoside-*p*-pyrimidine nucleoside (*e.g.*, de-pApT) are largely absent, but those of the type de-*p*-pyrimidine nucleoside-*p*-purine nucleoside are abundant. The conclusion has been drawn that the former type of internucleotide bond is preferentially cleaved. It is our view that it may be quite erro-

[3] Hurst and Findlay (1960) have postulated the presence of a phosphodiesterase (oligonucleotidase) in the crystalline preparations of deoxyribonuclease. Their evidence in favor of this postulate is based merely on the effects observed on activity in the presence of different metal ions and EDTA and does not appear to be conclusive. Because of the extreme complexity of the total reactions starting with DNA and leading up to a complex array of degradation products, varied effects on the kinetics in the presence of different amounts of metal ions and chelating agents may be expected and are difficult to interpret.

neous to discuss the mode of action of endonucleases in terms of single internucleotide bonds. From the present results, a stretch of at least three internucleotide bonds appears to be necessary for the enzyme action to occur. In polynucleotides of chain length longer than tetranucleotide, multiple possibilities for degradation by the enzyme will exist, and the preference for attachment at certain segments and consequent degradation may be determined by the total sequence of bases in those segments. Quantitative definition of preferential specificity should be possible only from study of a variety of oligonucleotides in which initially a single base is varied. Compounds of the type de-pTpTpTpApT, de-pTpTpApTpT, and de-pTpApTpTpT are being therefore prepared in this laboratory, and the study of quantitative mode of degradation of these substrates should be informative.

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Studies on the Mechanism of Action of Monoamine Oxidase: Metabolism of *N,N*-Dimethyltryptamine and *N,N*-Dimethyltryptamine-*N*-Oxide

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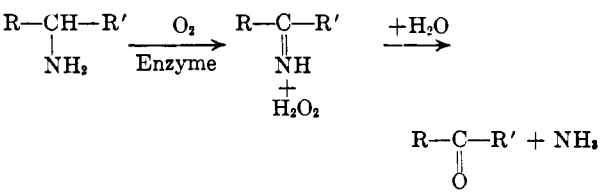
N,N-Dimethyltryptamine and *N,N*-dimethyltryptamine-*N*-oxide were found to be metabolized by a solubilized and partially purified monoamine oxidase preparation from guinea pig liver mitochondria. In contrast to the rate of reaction with other substrates for this enzyme, the rate of reaction with the *N*-oxide is highest under anaerobic conditions. The possibility that monoamine oxidase is an oxygenase and the *N*-oxide an intermediate in the deamination of *N,N*-dimethyltryptamine was investigated with H_2O^{18} and tritium-labeled dimethyltryptamine. The results of these experiments indicate that the *N*-oxide, although a unique substrate, is not an intermediate in the deamination of *N,N*-dimethylamines by monoamine oxidase. It appears that the deamination of these *N,N*-dimethylamines proceeds through an imino compound as in other enzymatically catalyzed deaminations.

Of the four most widely studied enzymes catalyzing oxidative deaminations, monoamine oxidase is the only one which catalyzes the deamination of *N,N*-dimethylamines. The other enzymes, D- and L-amino acid oxidase (Bender and Krebs, 1950) and diamine oxidase (Zeller *et al.*, 1956) require at least one hydrogen atom on the amino group of the substrate for activity. All of these enzymes have a common feature in that the carbon bearing the amino group is converted to a carbonyl function, an aldehyde in the case of the amine oxidases and an α-keto group in the case of the amino acid oxidases. Peroxide and ammonia are also formed.

The general mechanism of action of these enzymes has been described by the following equation (Krebs, 1951; Zeller, 1951):



where R′ may be a carboxyl in the case of the amino acid oxidases, or a hydrogen in the case of the amine