PRESENCE OF TWO DIFFERENT DESOXYRIBONUCLEODEPOLYMERASES IN VEAL KIDNEY*

by

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The following abbreviations for animal desoxyribonucleodepolymerases[1], or DNases, will be used in this report. DNase I is desoxyribonuclease, the enzyme crystallized by KUNITZ[2] from pancreas, which has an optimum activity at about pH 7[2-5], requires magnesium[2-5] and is inhibited by citrate[2,5] and by an inhibitor present in tissues of vertebrates[6-9]. DNase II, observed by CATCHESIDE AND HOLMES[10] in spleen and by MAVER AND GRECO[11] in thymus, has a pH optimum at about 5 and is inhibited by magnesium[11].

Recently, it has been suggested[12] that DNase I is peculiar to pancreas, and that the enzyme concerned with desoxyribonucleic acid (DNA) metabolism within living animal cells is DNase II, for which no naturally occurring inhibitor is known. If this were true, it would be difficult to assign any function to the widely distributed inhibitor for DNase I.

Veal kidney, stored at —25° C after being obtained fresh from a slaughterhouse, was homogenized in a Waring blender with three volumes of distilled water. The suspension was strained through cheesecloth, the pH was adjusted to 3.0 with sulfuric acid, and the preparation was stored at 5° C for twenty-four hours. After the pH was adjusted to 7.0, it was found that DNase activity of the whole homogenate could be measured viscometrically by the method of LASKOWSKI AND SEIDEL[3], using DNA prepared according to KAY, SIMMONS AND DOUNCE[13]**. In the presence of 0.02 *M* $MgSO_4$, the pH-activity curve (A, Fig. 1) was characteristic of DNase I. When 0.01 *M* sodium citrate was added to inhibit DNase I, the pH-activity curve was characteristic of DNase II (curve B).

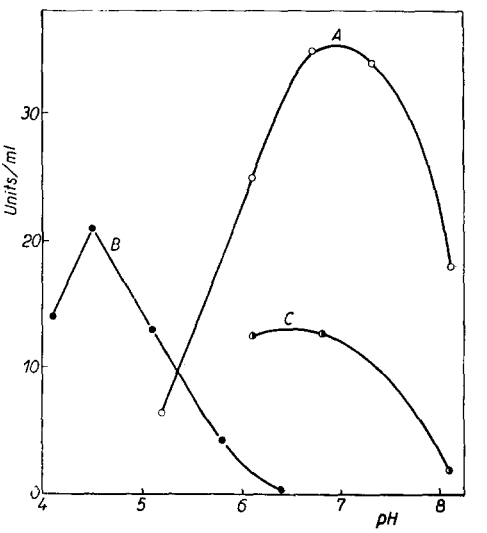

Fig. 1. Relation between pH and DNase activity of kidney homogenates expressed in viscosimetric units. Curve A, homogenate exposed to pH 3 for 24 hours, analyzed in presence of 0.02 *M* $MgSO_4$; curve B, homogenate exposed to pH 3 for 24 hours, analyzed in presence of 0.01 *M* citrate; curve C, homogenate not exposed to pH, 3 analyzed in presence of 0.02 *M* $MgSO_4$.

When the kidney homogenate was assayed directly in the presence of Mg, without being previously exposed to pH 3.0, curve C was obtained. Furthermore, the DNase I activity of acid-treated homogenate could be depressed by mixing it with a small portion of untreated homogenate. The loss of activity was apparently not caused by a proteolytic enzyme, since DNase I activity did not decline during incubation of mixtures of the two kinds of homogenates at 37° C for several hours. Quantitative assays of the inhibitor have not yet been attempted, and it is not possible at the present time to reconcile these findings with those of COOPER *et al.*[7], who failed to detect DNase I inhibitor in extracts of rat kidney made with distilled water. However, it may be suggested that kidney and other vertebrate tissues contain enzymes identical with, or similar to DNase I and DNase II, as well as an inhibitor for the former. Identification of DNase I in tissues other than pancreas may require use of methods designed to inactivate the inhibitor.

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THE INCORPORATION OF ^{32}P INTO THE NUCLEOTIDES OF RIBONUCLEIC ACID IN PIGEON PANCREAS SLICES

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It was reported in a preliminary note to this Journal[1] that when enzyme secretion was stimulated in pigeon pancreas slices by cholinergic drugs there was 50–100% increase in the rate of incorporation of ^{32}P into the nucleotides of ribonucleic acid (RNA). In this earlier study the nucleotides were isolated by paper chromatography of the SCHMIDT-THANNHAUSER ribonucleotide fraction[2] in 70% *iso*propanol-water-ammonia[3]. After chromatography the nucleotide spots were cut out, pooled and eluted. It was subsequently found that when the ribonucleotides in the SCHMIDT-THANNHAUSER fraction were separated by ionophoresis on paper, only the uridylic acid showed an increased specific activity (about 100%) in the stimulated pancreas. While this work was in progress DAVIDSON AND SMELLIE[4] reported that when the SCHMIDT-THANNHAUSER ribonucleotide fraction from liver was separated by ionophoresis on paper the uridylic acid was contaminated with an unknown phosphorus compound ("Substance D") immediately preceding it. We therefore cut out the area of paper immediately preceding uridylic acid, eluted with ammonia and assayed the eluate for radioactivity. The total radioactivity in the eluted material derived from stimulated pancreas slices was in some cases 800% higher than that from unstimulated slices. We have found that the incorporation of ^{32}P into those phospholipids which are extractable with ethanol and 3:1 hot ethanol-ether is 500–1500% greater in pancreas slices stimulated with cholinergic drugs than in unstimulated slices[5]. It seems possible therefore that the stimulated contaminating material in the chromatographed or ionophoresed SCHMIDT-THANNHAUSER RNA fraction is derived from phospholipids not extracted with neutral organic solvents—possibly inositol phospholipids[6].

It became clear that traces of phosphate substances whose phosphate turnover is so markedly stimulated with cholinergic drugs and which remain in the SCHMIDT-THANNHAUSER fraction could account for the 50–100% increases in the specific activities of the chromatographically isolated nucleotides of RNA from stimulated pancreas slices. It was thus imperative to rid the nucleotides of all contamination, and this was achieved by a procedure based on that of DAVIDSON AND SMELLIE[4], which could be adapted to quantities of pancreas tissue as low as 100 mg fresh weight. In this procedure the sodium nucleate is isolated by extraction with NaCl and precipitation with ethanol prior to its hydrolysis with alkali to free nucleotides. The free nucleotides are then separated by ionophoresis on paper. The following results were obtained using this improved technique.

When amylase synthesis was stimulated over 100% by the addition of an appropriate mixture of amino acids[7] there was about a 20–40% increase in the specific activities of the nucleotides of RNA (Table I). However, there was also a 20–40% increase in respiration and the specific activities

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