tation and freedom from turbidities often encountered in alternate procedures using acid-soluble tissue extracts. The typical Michaelis-Menten characteristics and kinetics, the orders of which may be manipulated as either zero or first order attest to the relatively uncomplicated action of the enzyme and substrate. However, first order kinetics were not ordinarily easily demonstrated since more than 3.0 ml of parotid saliva were required for the lowest practical amounts of substrate.

SUMMARY

The acid phosphomonoesterase of the human parotid was studied with sodium β-naphthylphosphate as substrate. The pH optimum was well defined between 4.5–4.7. The enzyme exhibited typical Michaelis-Menten behavior over a wide range of substrate concentrations. The K_m value for β-naphthylphosphate was $3.2 \cdot 10^{-4} M$, for sodium *p*-nitrophenyl phosphate was $4.5 \cdot 10^{-4} M$, and for disodium phenyl phosphate was $0.17 \cdot 10^{-4} M$. The enzyme was readily inhibited by fluoride, and L-tartrate, and less so by magnesium ion. Average values for four substrate concentrations gave an average activation energy of 7,300 cal/mole above 30° C. Below this transition temperature a value of 12,400 cal/mole was estimated. The energy of inactivation was 88,300 cal/mole. The properties of the parotid acid phosphomonoesterase resemble both the phosphomonoesterases of blood and prostate but with greater similarity to the latter.

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RIBONUCLEASE ACTIVITY AND CELLULAR GROWTH*

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INTRODUCTION

In a previous study on the nuclease activities in the human placenta a close correlation was found between the growing state of the tissue and the desoxyribonuclease activity in the homogenate[1,2]. This activity showed a considerable decrease during

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the development of the organ. On the other hand, no correlation between the growing state and the enzymic activity could be established for the ribonuclease (RNase).

In these experiments a purified commercial yeast ribonucleic acid (PNA) preparation was used as substrate. This nucleic acid had been prepared with rather drastic chemical methods, including the application of heat and alkali during the isolation procedure. This implies considerable degradation of the nucleic acid as demonstrated by LORING, FAIRLEY AND SEAGRAN[3].

It was pointed out that other results with respect to the correlation between the growth intensity of the tissue and RNase activity could probably be obtained by using a more appropriate substrate[1]. Recently, milder extraction methods for the preparation of PNA have been adopted. Chemical degradation has thereby been avoided, but enzymic hydrolysis during the isolation procedure has been shown to take place[4]. CRESTFIELD, SMITH AND ALLEN[4] have now elaborated a procedure for the preparation of PNA from yeast. Enzymic hydrolysis is here prevented. The authors state that a PNA is obtained that has fewer secondary phosphoryl dissociations and a higher percentage of phosphodiester bonds, which are susceptible to hydrolysis by RNase, than had previous samples.

The close interrelationship between the nucleic acids and the cellular growth processes has been repeatedly stressed[2,5,6,7,8]. The results on the desoxyribonuclease activity during the development of the human placenta[2] suggest the possibility of considering this enzyme as an indicator, perhaps even regulator, of the synthesis or degradation of desoxyribonucleic acid (DNA). The CRESTFIELD-SMITH-ALLEN[4] method for the preparation of PNA from yeast prompted a reinvestigation of the RNase activity during the development of the human placenta. The purpose of the present study was to find out if any positive correlation could be established between RNase activity and the growth intensity of the tissue.

MATERIAL AND METHODS

Human placentas from different stages of pregnancy were used and treated as described elsewhere[1].

A spectrophotometric method was elaborated for the determination of RNase activity in tissue homogenates. It was found that a final trichloracetic-acid (TCA) concentration of about 5 % had a negligible extinction at 290 mμ and that this concentration was appropriate for the precipitation of the tissue components and also for a quantitative precipitation of non-digested PNA. When the nucleic acid had been exposed to the action of tissue-RNase or to crystallized bovine pancreas RNase, a very fine turbidity was obtained with TCA. It was impossible to get a clear supernatant with an ordinary centrifuge (M.S.E. refrigerated centrifuge, 6000 $\times$ g for 30 minutes). The addition of lanthanum acetate[9], however, caused a heavy flocculation which was easily sedimented with a completely clear supernatant. In a model test crystalline bovine pancreas RNase (Worthington Biochemical Sales Co.) was used. It was found that the method is sensitive to $5 \cdot 10^{-4}$ μg of RNase under the present conditions.

PNA was prepared according to the method of CRESTFIELD, SMITH AND ALLEN[4]. Baker's yeast directly from the filter press was used.

The RNase-activity determinations were run at several levels of tissue concentration in the range 2 mg to 3.5 mg of total tissue-nitrogen per ml homogenate. Tris (Sigma) buffer at pH 7.7 and 2.5 mg of PNA were used. The total volume of the incubation mixture was 3.5 ml. The incubation time was 15 minutes, when not stated otherwise, and the reaction was stopped by adding 3.0 ml of 10 % (w/v) TCA, containing 0.5 % (w/v) of lanthanum acetate (The British Drug Houses). After standing at $+4^\circ$ C for 10 to 15 minutes the tubes were centrifuged for 10 minutes at 3000 $\times$ g and the supernatant read at 290 mμ. Appropriate tissue and substrate blanks were run. The activity is expressed as increase in optical density per 15 minutes per tissue unit.

Tissue-PNA and DNA phosphorus was determined as described earlier[10,11].

The spectrophotometric determinations were performed in a Beckman quartz spectrophotometer, using a 1 cm cell. The pH values were electrometrically controlled with a Radiometer pH-meter 3.

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DISCUSSION OF RESULTS

In Fig. 1 the time-activity curve for placenta RNase in tissue homogenates is depicted. After a linear increase there is a flattening of the curve. The result demonstrates that the degradation of PNA by this enzyme follows the kinetics of a first-order reaction.

The relationship between homogenate concentration and enzymic activity was established and some representative curves from placentas of different ages are given in Fig. 2. The results contrast with those obtained earlier with commercial PNA[1].

The S-shape of the curves gives rise to difficulties in giving a numerical expression for the RNase activity in the different placenta homogenates. It is necessary for the sake of comparison to express the activity at a certain point on the concentration axis as shown in Fig. 2. It has been demonstrated that the DNA content per nucleus is approximately constant during the development of the human placenta[2]. As basis of reference a tissue unit containing 10 μg of DNA phosphorus was chosen. This amount of tissue thus contains the same amount of nuclei in the different samples. Table I shows the values obtained during the development of this organ. There is a considerable decrease with aging.

The configuration of the homogenate concentration-activity curves points to the presence of some factor modifying the activity of the RNase. Accumulated evidence has revealed the presence of a RNase inhibitor in several different animal tissues. PIROTTE AND DESREUX[12] found in studies on liver tissue that part of the tissue RNase was bound to an inhibitor, and that a considerable reactivation was effected with salts and sulphuric acid. Recently ROTH[13] has studied the properties and distribution

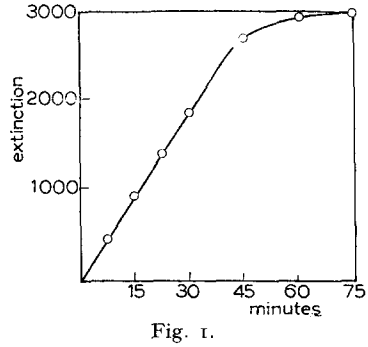

Fig. 1.

Fig. 1. Rate of formation of acid-soluble, ultraviolet-absorbing degradation products by incubation of PNA with placenta homogenate.

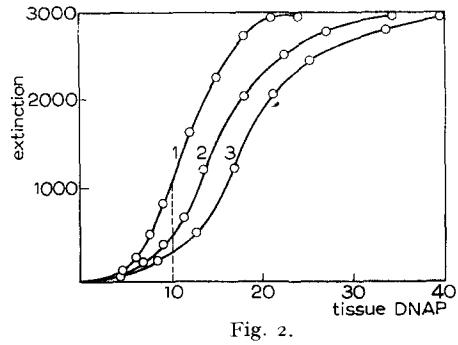

Fig. 2.

Fig. 2. Relationship between homogenate concentration (expressed as tissue DNA phosphorus) and formation of acid-soluble, ultraviolet-absorbing degradation products from PNA. Curve 1 is a 14-week-old placenta, curve 2 a 21-week-old placenta and curve 3 a 40-week-old placenta.

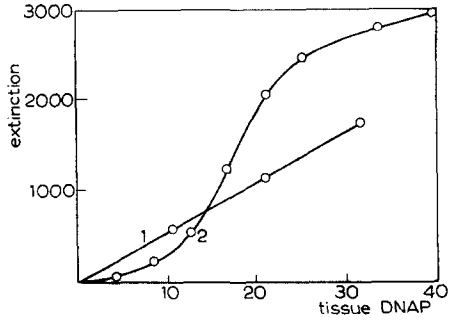

Fig. 3.

Fig. 3. Degradation of PNA by placenta homogenate with (curve 1) and without (curve 2) *p*-chloromercuribenzoate in the incubation mixture. For further details, see text.

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TABLE I

CHANGES IN RIBONUCLEASE ACTIVITY AND PNAP/DNAP QUOTIENTS DURING DEVELOPMENT OF HUMAN PLACENTA

Age in weeks	*Ribonuclease activity*	*PNAP/DNAP*
12	1.100	1.45
14	1.140	1.47
17	0.850	1.12
20	0.650	0.82
21	0.510	0.80
40	0.340	0.55
40	0.290	0.60
40	0.310	0.58

Ribonuclease activity expressed as increase in acid-soluble, ultraviolet-absorbing PNA-degradation products per 10 μg tissue DNAP per 15 minutes. PNAP = PNA phosphorus. DNAP = DNA phosphorus.

of a RNase inhibitor in several rat tissues. He found that the inhibitor was inactivated by *p*-chloromercuribenzoate (PCMB) and other sulfhydryl reagents. The addition of PCMB to the incubation mixture, according to ROTH[13], caused a considerable increase of the RNase activity.

PCMB in a final molar concentration of $4 \cdot 10^{-4}$, the concentration used by ROTH[13], was added to placenta homogenates and the results of a typical experiment are shown in Fig. 3. The effect is rather complex and difficult to interpret. With small amounts of tissue an activation was obtained and with increase of the homogenate concentration in the incubation mixture an inhibition of the activity was observed. The first part of this curve is in accordance with the results of ROTH[13]; the latter part, however, is in sharp contradiction.

When analyzing the effect of PCMB on the ability of the tissue homogenate to degrade PNA, the action of this reagent not only on a supposed inhibitor, but also on the enzyme must be considered. A definite evaluation cannot be made until the tissue RNase has been considerably purified. It may be mentioned, however, that several investigations concerning the effect of PCMB on crystalline RNase have been published. LEDOUX[14] studied this problem thoroughly and found that the effect varied considerably with the concentration of PCMB, the pH-value of the incubation mixture and the time of incubation. PCMB at a molar concentration of 10^{-4} at pH 8 caused an increase in activity when the incubation was carried on for 15 minutes. ROTH[13] found that $4 \cdot 10^{-4} M$ PCMB at pH 7.8 had no significant effect on the activity of crystalline RNase. He used an incubation period of 30 minutes. DICKMAN, AROSKAR AND KROPF[15], on the other hand, found a slight inhibition of pancreas RNase by $10^{-3} M$ PCMB, when incubating for 10 minutes.

Characteristically increased turnover rates have been found during the formation of proteins and polynucleotides in rapidly growing tissues such as regenerating bone-marrow and liver[6,7]. The results of this investigation point to a possible direct relationship between the RNase activity and the endocellular synthesis or degradation of PNA. As to the exact endocellular function of the RNase, the results of different investigations are somewhat contradictory. GROTH[16] added crystalline RNase to cultures of *Bacillus megaterium*. He found that the PNA content of the cultures was significantly reduced by the action of the enzyme and that this reduction caused

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a marked inhibition of the incorporation of labelled glycine into the bacterial protein. ROTH[17] observed an increased desoxyribonuclease activity when adding crystalline RNase to the incubation mixture of *Tetrahymena pyriformis* S. He interprets his results as suggesting a degradative function of RNase. MARKHAM AND STROMINGER[18], on the other hand, have brought forward experimental evidence lending support to the idea that the enzyme is concerned in the processes of synthesis and rearrangement of the ribonucleic acid.

During the period of logarithmic growth of the human placenta[8], when the average PNA content per cell is high and an increased turnover rate may be assumed, the present writer has found a high RNase activity. During the development of the organ a decreasing RNase activity is accompanied by a decreasing cellular PNA content. In an earlier paper the PNAP/DNAP quotients, which indicate the relative cellular PNA content, were shown to be a quantitative expression of the growth intensity of the tissue[2]. As is found in Table I, there is a close correlation between the RNase activity and these quotients.

SUMMARY

1. A spectrophotometric assay for ribonuclease is described. The method is sensitive to $5 \cdot 10^{-4}$ μg of crystalline pancreas RNase under the conditions described.

2. With this method the RNase activity in tissue homogenates from human placentas at different developmental stages was investigated at pH 7.7.

3. A positive correlation is established between the RNase activity and the growth intensity of the tissue. There is a considerable decrease in enzymic activity during the development.

4. The effect of *p*-chloromercuribenzoate on the ability of the tissue homogenate to degrade ribonucleic acid has been studied and the results are discussed.

5. The possible implications of the results bearing on the relationship between RNase activity and endocellular synthesis or degradation of PNA are discussed.

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