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Nephelometric Determination of Elastase Activity and Method for Elastoproteolytic Measurements

BY ILONA BANGA, J. BALÓ AND MAGDOLNA HORVÁTH

*The First Department of Pathological Anatomy and Experimental Cancer Research
Medical University, Budapest*

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Measurements on the pancreatic elastase activity are performed by different methods in different laboratories. Baló & Banga (1950) and Banga (1952) suggested a gravimetric method in which the fraction of insoluble elastin that has not been dissolved by the enzyme is determined. The same method was used by Lewis, Williams & Brink (1956). Hall (1955) and Hall & Gardiner (1955) used the biuret reagent described by Sols (1947) in determining the elastin dissolved by the elastase. The Folin-phenol reagent (Lowry, Rosebrough, Farr & Randall, 1951) was applied by Grant & Robbins (1957) to measure the proteolytic dissolution of elastin. Sachar, Winter, Sicher & Frankel (1955) measured the colour liberated during the elastolytic action of elastase on elastin which was previously stained with orcein. Robert & Samuel (1957) measured the elastolytic dissolution of 'azoelastin' by a spectrophotometric method and gave some data for the kinetics of elastolysis.

In this paper we describe a method by which the activity of different elastase preparations obtained in different laboratories may be compared. The

need for such a method is emphasized by the fact that the mechanism of elastolysis is far from being uniform and well understood.

The enzymic dissolution of elastin, i.e. elastolysis, is not a mere proteolysis. Banga (1951) stressed that no free amino groups are demonstrable by the formol method or the Van Slyke determination during the dissolution process. Hall, Reed & Tunbridge (1952) and Hall (1953) believe that a polysaccharide and a sulphate group are liberated. Hall & Gardiner (1955) performed quantitative measurements on the amount of liberated acid during elastolysis and considered the acid, in accordance with the original assumption of Hall *et al.* (1952), to be an organically bound sulphate. Since they held the view that the sulphate ion would be bound to mucopolysaccharide, they conceived the elastolysis to be mucolysis. Partridge & Davis (1955), on the other hand, demonstrated the formation of free α -amino groups with the fluorodinitrobenzene technique. This led them to suppose that elastase or one of its components is a proteolytic enzyme. These authors found no more

than 0.3 % of carbohydrate in the purified elastin, and their experiments showed that the amount of amino sugars did not exceed 0.1 %. Lansing, Rosenthal, Alex & Dempsey (1952) demonstrated liberation of lipid during elastolysis. According to Labella (1957) the substance manifesting itself after the periodic acid-Schiff reaction must be lipoprotein. Banga & Schuler (1953) used several methods for demonstrating the appearance in the elastolysate of reducing substances which they measured with the Hagedorn-Jensen method. Baló & Banga (1954) measured the reducing substance liberated during elastolysis from its consumption of bromine and showed that Schiff's reagent and Ehrlich's *p*-dimethylaminobenzaldehyde brought about red and purple staining respectively.

All these studies suggest that elastase does not have a single and uniform mechanism of action. This is clear also from the experiments in which we failed to correlate the results of our gravimetric method with quantitative determinations of dissolved protein measured in the supernatant. The need for a quick and reliable method to replace the laborious gravimetric procedure induced us to elaborate our nephelometric method described below.

It seemed to be necessary to make a series of determinations with the same substrate and the same enzyme, with the different methods. Three methods were employed and they were applied in a single experiment so that the changes during elastolysis were determined under uniform experimental conditions. A photometer was used in the nephelometric technique for the measurement of the changes in the extinction of elastin granules. The insoluble fraction of elastin was determined gravimetrically and the dissolved protein in the supernatant was measured with the Folin-phenol reagent (Lowry *et al.* 1951).

The elastase activity is greatly influenced by the salts of the different buffers and by the ionic strength. These factors have been considered with all three methods.

EXPERIMENTAL

Substrate. Elastin was prepared from ox aorta. The aorta of young (1-year-old) animals was freed from the adventitia, minced and homogenized in an electric food-mixer (Kenwood Manufacturing Co., England) with water (0.5 ml./g. of mince) until a fine pulp was obtained. The water was then pressed out through a cloth, and 1 l. of 0.1 N-NaOH/kg. of the original wet wt. was added and the mixture was boiled for 45 min. After cooling and filtering through cheese-cloth the boiling with alkali was repeated in the same way as before. The residue was washed in water with the addition of a small amount of dilute HCl after filtration, until the washings became neutral and no salt remained.

It was then dehydrated by stirring five times with an excess of acetone and finally dried in air and ground flour-fine in a ball mill.

For a stock elastin suspension, 4 g. of the powdered material sifted through a 130-mesh sieve was ground by hand in a mortar for about 1 hr. while a total of 50 ml. of water was added dropwise. For the measurements the stock elastin was diluted with the appropriate buffer to 10 times its original volume. This suspension was so stable that its extinction did not vary within 30 sec. in the photometer owing to sedimentation.

Buffer solutions. Carbonate-HCl buffer, pH 8.8, *I* 0.1: this was prepared with 23 ml. of 0.1 M-Na₂CO₃ + 20 ml. of 0.1 N-HCl and 43 ml. of water.

Carbonate-HCl buffer, pH 8.8, *I* 0.05: the above-mentioned buffer was diluted 1:1 with water.

Veronal-acetate buffer, pH 8.8, *I* 0.1: 95 ml. of 0.1 M-veronal Na + 5 ml. of 0.1 N-HCl + 100 ml. of 0.1 M-sodium acetate.

Veronal-acetate, pH 8.8, *I* 0.025: 100 ml. of the veronal-acetate buffer, *I* 0.1, was diluted with water to 400 ml.

Veronal Na-HCl buffer: Michaelis (1930).

Glycine-NaOH buffer: Sørensen (1909).

Boric acid-borax: Holmes (1943).

From the above buffers used we recommend two buffers which do not inhibit elastoproteolysis: carbonate-HCl buffer, *I* 0.05, and veronal-acetate buffer, *I* 0.025.

Elastase. This was an adsorbed, eluted and further purified elastase preparation (213/F₃) described by Bagdy, Banga & Horváth (1959).

Gravimetric elastase determination. This differed from that described by Banga (1952) only in that the volume of the reaction mixture was altered according to requirements. For comparing the three methods, the gravimetric, the Folin-phenol (Lowry *et al.* 1951) and the nephelometric, we worked with 24 mg. of elastin and a total volume of 10 ml. Correspondingly, we used standard tubes of 100 mm. height and 14 mm. inner diameter, which had the same light-absorbing capacity and could be employed at the same time for nephelometric measurements. In preliminary experiments, which served to compare the gravimetric method with the Folin-phenol method, a volume of 5 ml. was used. Buffer concentration and the amount of elastase will be given with each individual experiment.

Nephelometric elastase determination. A single-cell photometer (Factory for Laboratory Equipment, Budapest) was used in the measurements. A standard curve was obtained by measurement of the extinction of suspension containing different elastin concentrations and was read with a red RG₁ colour filter with maximum transmission at 620–750 mμ (Fig. 1). The procedure of determination is as follows: five or six standard tubes were each filled with 3 ml. of diluted elastin suspension containing 24 mg. of elastin, and then buffer and water were added to reach 8 ml. The series was put into a water bath at 38° for 10 min. to preheat it. Another series of ordinary test tubes was filled with elastase solution of dilutions varying between 1.0 and 0.1 mg./2 ml., likewise preheated. The first tube served as a control containing no elastase, only the proper solvent. A volume (2 ml.) from each tube containing elastase was pipetted into the corresponding elastin tube after an interval of 1 min. Immediately after shaking, the extinction in the spectrophotometer was read, which needed 15 sec. This value was considered as the starting point.

With shaking of the tubes at 1 min. intervals, the incubation was carried on for 15 min., whereafter the extinction of the solutions was measured. The results were read from the elastin standard curve presented in Fig. 1.

After finishing the nephelometric readings the tubes served for the Folin-phenol and gravimetric determinations. The tubes were cooled in ice-water and centrifuged. The protein determination was carried out on the supernatant by the Folin-phenol method and the amount of precipitate was determined gravimetrically after washing it with 5 ml. of absolute ethanol, centrifuging and drying at 110°.

Determination of elastase activity with the Folin-phenol method. The supernatant obtained from gravimetric and nephelometric determinations was used. The standard curve obtained with elastin completely dissolved with elastase was used as a basis (Fig. 2).

Determination of elastase unit. The elastase activity is given as elastase unit (E.U.)/mg., which expresses the amount of elastin that can be dissolved by 1 mg. of elastase within 30 min. under the given experimental conditions. With the three methods described in this paper the time of incubation was 15 min. In each case initial speed was compared.

Errors. In a large number of experiments we found with all three methods a 10–15% error. The error has its origin in the elastin standard curves. Neither the Folin-phenol (Fig. 2) nor the nephelometric (Fig. 1) method gave a

Fig. 1. Standard curve relating elastin concentration to extinction for the nephelometric determination of elastase. A variable quantity of 0.8% elastin suspension was diluted to 10 ml. with carbonate-HCl buffer, I 0.05. The extinction values obtained with the different elastin concentrations are shown.

linear correlation of concentration with the extinction. The relationship is more nearly linear with low elastin concentrations and for this reason 24 mg. of elastin was used in the determinations.

RESULTS

Differences in the values for elastase activity obtained by the gravimetric and the Folin-phenol methods. According to different data in the literature elastolytic dissolution requires buffer of ionic strength 0.1 and an optimum pH value of 8.8. Table 1 contains the results of an experiment in which the results of activity determinations by the gravimetric and Folin-phenol method were compared. Different buffer solutions with the same ionic strength and pH were used for the experiments.

Fig. 2. Standard curve relating elastin concentration to Folin-phenol colour used for estimation of elastase. Elastin (100 mg.) was entirely dissolved with 1 mg. of elastase in a volume of 100 ml. of carbonate-HCl buffer (16 hr. incubation). The extinction values obtained with the different concentrations were measured with the Pulfrich photometer in a 0.5 cm. cuvette at λ 660 m μ .

Table 1. Activity of a preparation of elastase measured in different buffer solutions of the same ionic strength

Measurement was by the gravimetric and Folin-phenol methods. pH 8.8; I 0.1; total volume, 5 ml.; 20 mg. of dry powdered elastin; 0.25 mg. of elastase; incubated at 38° for 15 min. Buffers: see Methods.

Buffer	Activity (E.U./mg.)		Ratio (Gravimetric/Folin-phenol)
	Gravimetric	Folin-phenol	
Carbonate-HCl	95	90	1.05
Veronal-acetate	54	56	0.97
Veronal Na-HCl	51	32	1.59
Glycine-NaOH	66	33	2.00
Boric acid-borax	70	44	1.59

The E.U. values obtained show great differences with the different buffers even if the same method of determination is used. Lewis *et al.* (1956) have already noted this fact. The values show the greatest divergence if the two different determinations are carried out in the glycine buffer. The ratio of the values obtained by the two methods is shown in column 4 of Table 1. The values obtained with the Folin-phenol method were in part lower than those with the gravimetric procedure, so an influence of certain buffering salts on the Folin-phenol reaction was suspected. To decide this question the effect of the concentration of different buffers on the Folin-phenol reaction was examined. A 0.25% gelatin or elastin solution was used as standard protein solution to which the different buffering-salt solutions were added in 0.1 and 0.05 M-concentration respectively. Most of the buffering salts in 0.1 M-concentration were found to

have some inhibitory effect on the Folin-phenol reaction, but this inhibition never exceeded 10–15%. Glycine buffer, on the other hand, strongly inhibited the reaction, showing a 40% inhibition with 0.1 M and a 25% inhibition with 0.05 M. Thus the great difference in the E.U. values of Table 1, measured in glycine buffer by the gravimetric and Folin-phenol method respectively, is partly explained by this fact.

pH-activity curves in different buffers at the same ionic strength (I 0.1). The dependence of activity on pH was determined by all three methods. Different buffers (I 0.1), and invariably 0.25 mg. of elastase, were used. Figs. 3–5 illustrate the changes in E.U./mg. Examination of the curves shows that neither the E.U. values obtained with the three different methods at a single pH nor the optimum pH values measured in the different buffers are the same, but that the pH optimum falls between

Fig. 3. pH-activity curve of elastase in different buffer solutions (I 0.1) measured by the gravimetric method. Powdered elastin (20 mg.) in 5 ml. of buffer was incubated with 0.25 mg. of elastase at 38° for 15 min. The activity of elastase related to pH is given in E.U./mg. Δ , Carbonate-HCl; $\blacktriangle$, veronal-acetate; $\square$, borate; $\bullet$, veronal Na; $\circ$, glycine.

Fig. 4. pH-activity curve of elastase in different buffer solutions (I 0.1) measured by the Folin-phenol method. Powdered elastin (20 mg.) in 5 ml. of buffer was incubated with 0.25 mg. of elastase at 38° for 15 min. The dissolved protein in the supernatant was measured by the Folin-phenol method and the activity related to pH is given in E.U./mg. Δ , Carbonate-HCl; $\blacktriangle$, veronal-acetate; $\square$, borate; $\bullet$, veronal Na; $\circ$, glycine.

8 and 9. There is a similar divergence in optimum pH values measured by the same method, but in different buffers.

Certain regularities can be discerned from the three curves. With all three methods the E.U.

Fig. 5. pH-activity curve of elastase in different buffer solutions (I 0.1) measured by the nephelometric method. Suspension containing 40 mg. of elastin in 10 ml. of buffer was incubated with 0.5 mg. of elastase at 38° for 15 min. The extinction values were measured with a single-cell photometer. The activity related to pH is given in E.U./mg. Δ , Carbonate-HCl; $\blacktriangle$, veronal-acetate; $\square$, borate; $\bullet$, veronal Na; $\circ$, glycine.

values are highest if a carbonate-HCl buffer is used. The next highest values are obtained in the glycine buffer when the gravimetric and nephelometric methods are used. With the Folin-phenol method the lowest values are obtained in glycine buffer because the latter inhibits the Folin-phenol protein determination. Values measured in borate buffer and in sodium barbiturate are likewise low. The veronal-acetate buffer, described in Methods, takes an intermediate place in these experiments.

All these methods show that at the same ionic strength each ion has a different effect on the inhibition of elastolysis. The carbonate ion seems to have the least influence.

Effect of diluted buffers on elastolysis. An attempt was made to overcome the inhibitory effect of buffering by the use of diluted buffers. This raised the danger, however, that a lack of buffering power would result in low E.U. values, since according to Hall & Gardiner (1955) a substance of acid character is liberated during elastolysis. Measurements before and after incubation with elastase were therefore carried out in the diluted buffers and showed the pH to have remained unchanged. With the elastase and aortic elastin used no liberation of the acid could be demonstrated. Table 2 presents the results which were obtained by the use of different diluted buffers of the same strength (0.025 M and pH 8.8). The E.U. values were determined with the three different methods in the same experiment.

The data in Table 2 show that the E.U. values measured with the three different methods in the carbonate-HCl, veronal-acetate and glycine buffer may be considered, within the limit of error, as approximately the same. The value obtained with the Folin-phenol method in the glycine buffer was lower, however, in this experiment too. The values in the veronal sodium and borate buffers also fail to show good agreement.

Table 3 shows the changes of E.U. values with dilution of the buffers carbonate-HCl and veronal-acetate. The data show the E.U. values to rise with lowered concentration of the buffers with all the

Table 2. Activity of a preparation of elastase in diluted buffer solutions measured by three methods

The suspension contained 24 mg. of elastin, 0.1 mg. of elastase. pH 8.8; incubated at 38° for 15 min. Buffers: carbonate-HCl, pH 8.8, I 0.05; 23 ml. of 0.1 M- Na_2CO_3 + 20 ml. of 0.1 N-HCl made up with water to 172 ml. Veronal-acetate, pH 8.8, I 0.025; 100 ml. of 0.1 M-veronal-acetate was diluted with water to 400 ml. Veronal Na-HCl (Michaelis, 1930) buffer was diluted four times. Glycine-NaOH (Sørensen, 1909); stock solution was diluted with water (1:4). Boric acid-borate (Holmes, 1943); stock solution was diluted with water (1:4).

Buffer	I	Activity (E.U./mg.)		
		Gravimetric	Folin-phenol	Nephelometric
Carbonate-HCl	0.05	130	120	130
Veronal-acetate	0.025	130	120	110
Veronal Na-HCl	0.025	95	90	88
Glycine-NaOH	0.025	120	76	115
Boric acid-borate	0.15	72	80	110

Table 3. *Change in measured activity of a preparation of elastase on dilution of the buffer solutions*

Measurement was by three methods in the same experiment. The suspension contained 24 mg. of elastin, 0.1 mg. of elastase. pH 8.8; incubated at 38° for 15 min.

Buffers: carbonate-HCl, pH 8.8, *I* 0.1, was prepared by adding 23 ml. of 0.1 M-Na₂CO₃ to 20 ml. of 0.1 N-HCl. The same buffer, *I* 0.05, was diluted twice, that of *I* 0.025 was diluted four times. Veronal-acetate buffer, *I* 0.1, pH 8.8; 95 ml. of 0.1 M-veronal Na + 5 ml. of 0.1 N-HCl + 100 ml. of 0.1 M-sodium acetate. The same buffer, *I* 0.05, was diluted twice, that of *I* 0.025 four times, that of *I* 0.012 eight times.

Buffer	<i>I</i>	Activity (E.U./mg.)		
		Gravimetric	Folin-phenol	Nephelometric
Carbonate-HCl	0.1	105 ± 10 (4)	95 ± 10 (4)	100 ± 10 (4)
Carbonate-HCl	0.05	140 ± 15 (4)	120 ± 10 (4)	150 ± 15 (4)
Carbonate-HCl	0.025	131 ± 13 (4)	100 ± 10 (4)	90 ± 10 (4)
Veronal-acetate	0.1	54 ± 10 (4)	56 ± 10 (4)	—
Veronal-acetate	0.05	50 ± 10 (4)	82 ± 10 (4)	80 ± 10 (4)
Veronal-acetate	0.025	140 ± 15 (4)	104 ± 10 (4)	100 ± 10 (4)
Veronal-acetate	0.012	130 ± 10 (4)	110 ± 10 (4)	100 ± 10 (4)

Table 4. *Change in measured activity with different elastase concentrations*

Carbonate-HCl buffer, pH 8.8, *I* 0.05; suspension containing 24 mg. of elastin, incubated at 38° for 15 min. Measurements were by three methods.

Method	Enzyme concn. (mg./10 ml.)		
	0.1	0.06	0.02
	Activity (E.U./mg.)		
Folin-phenol	144	140	150
Gravimetric	132	150	190
Nephelometric	160	332	450

three methods. With both buffers maximal E.U. values are obtained at 0.025 M, whereas further dilution results in a decrease of the E.U. values. These values show the dependence on buffer concentration in these two buffers. These had the least inhibitory effect on elastolysis. They also gave with all three methods results which were near to each other.

Changes in E.U. values with elastase concentration; a new effect on elastin demonstrable with nephelometric measurements. In the course of our experiments it turned out that the concentration of elastase also greatly influenced the E.U. values. Elastolysis is linear with elastase concentration only within very narrow limits, whichever of the three methods is used. Table 4 shows the results obtained with very low elastase concentration. The values at different low elastase concentrations in diluted carbonate-HCl buffer obtained with the Folin-phenol method were equal, within the limits of error, and they agreed with the values from the gravimetric determination. The E.U. value 190, that had been measured gravimetrically with 0.02 mg. of elastase, represented an exception. With such very low concentrations the error in the determination may exceed the usual 10–15 % and

may reach even 25–30 %. Taking this fact into consideration, the gravimetric method gives results similar to those of the Folin-phenol method with elastase concentrations of 0.02–0.1 mg./10 ml. The nephelometric method, on the other hand, is strikingly dependent on elastase concentration. Many careful experiments supported the above results. When the concentration of the elastase preparation was lower than 0.1 mg./10 ml. a prompt effect occurred which was manifested only when the nephelometric method was used. The transparency of elastin particles increases though we can find no dissolved protein corresponding to the apparent dissolution of elastin.

The three methods can thus be regarded as equivalent and comparable only within certain limits. Their combined use is made objectionable by the difference in the ratio of the substrate-enzyme concentration even within one and the same method. Whereas the gravimetric and Folin-phenol method allowed an almost unrestricted alteration in the ratio of elastase to elastin, the nephelometric measurement required in our photometer an elastase:elastin ratio of 1:80–250.

Recommendation of a combined nephelometric and Folin-phenol method for measurement of the proteolytic activity of elastase. Based on a great many experiments, a combined method of the nephelometric and Folin-phenol measurements is recommended for routine procedures in determining the proteolytic activity of elastase. The conditions for applying this method are as follows. (i) A very fine suspension of elastin is required, so that no sedimentation takes place for at least 30 sec. after shaking. (ii) A ratio between the substrate and enzyme concentration is chosen in such a way—within the given volume (10 ml.) and within the given time of incubation (15 min.)—that a maximum of only 50 % of the substrate is dissolved after incubation. (iii) Only carbonate-HCl or veronal-acetate, described in Methods, should be

used in 0.025M-concentration at pH 8.8. (iv) Rapid ice-cooling should be used after incubation to stop the enzyme reaction, and the solution must also be centrifuged while cold. The protein content of the supernatant used for the Folin-phenol determination remains unchanged for some hours.

Our results show that this method is quick, can be performed easily in any laboratory and is applicable in comparing the activity of different elastase preparations.

In Fig. 6 we present the concentration curve of the 213/F₂ elastase preparation measured by the nephelometric and the Folin-phenol method. A linear slope is reached with 24 mg. of elastin in an elastase concentration of 0.1–0.3 mg./10 ml. Every 0.1 mg. increase in the amount of elastase brings about a 6–7 mg. increase in the dissolved elastin within 15 min. of incubation. Thus the activity computed from the three concentrations was, according to both methods, 120–140 E.U./mg.

DISCUSSION

Results presented in this paper support the view that determinations under strictly defined conditions are needed to clear up the confusion about measurement of the activity of elastase. An important requirement is that the elastolytic capacity of elastase preparations produced in different laboratories should be expressed in the same units. The protein dissolution accompanying elastin dissolution must be taken as a basis, because the

complete dissolution of elastin must still be regarded as proteolysis.

An enzyme preparation was used in our experiments which, according to our present knowledge, contains mainly the proteolytic component. Bagdy *et al.* (1959) describe the production and electrophoretic character of this enzyme. It is not a completely homogeneous enzyme preparation consisting of a single protein component, but the component responsible for elastoproteolytic activity has a high E.U. number and supports the assumption that this preparation is one of the most active elastoproteolytic enzymes so far described and is similar to the elastase described by Lewis *et al.* (1956).

The nephelometric method had a disadvantage which, however, was useful in revealing a hitherto unknown property of elastase preparations. We have observed that beside the proteolytic and mucolytic action of elastase, the enzyme has an effect which, at the onset of elastolysis, alters the transparency of elastin particles. The chemical reaction responsible for this effect has not yet been determined. Whether this effect is connected with the lipoprotein component of Lansing *et al.* (1952), which according to Labella (1957) makes up merely 0.5% of the elastin protein, has likewise not been determined.

SUMMARY

1. It has been shown that the different methods in the literature for the measurement of elastase activity fail to give comparable results. One of the main reasons of this discrepancy lies in the fact that certain buffer salts inhibit elastolysis. This inhibition manifests itself differently with the different methods.

2. With an ionic strength of 0.1, which has been accepted in the literature, the pH-activity curves in different buffers fail to show the same optimum pH values; the inhibitory effect of the ions of the buffering salts is apparent when results with either similar or different methods are compared.

3. A rapid and, within certain limits, sufficiently exact nephelometric method has been elaborated for the measurement of elastase activity.

4. Conditions were examined under which the nephelometric and the Folin-phenol method can be combined to obtain good and reliable results in the determinations of proteolytic activity of elastase.

5. In the course of nephelometric studies a new feature of elastase activity was revealed. The elastase increased the transparency of elastin particles in a concentration too low for its activity to be measured by the gravimetric or the Folin-phenol method.

Fig. 6. Relationship of the amount of elastolysis to the concentration of an elastase preparation (213/F₂). Measured in veronal-acetate buffer (I 0.025) by the nephelometric and Folin-phenol methods. Elastin (24 mg.) in 10 ml. of buffer at 38° was incubated for 15 min. Ordinate shows the amount (mg.) of dissolved elastin. Abscissa shows the quantity of elastase used in 10 ml. of reaction mixture.

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The Degradation of Natural Polyamines and Diamines by Bacteria

By S. RAZIN, I. GERY AND U. BACHRACH

Department of Clinical Microbiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel

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The polyamines spermine and spermidine are known to be widely distributed in animal tissues (Rosenthal & Tabor, 1956), yeasts (Dudley & Rosenheim, 1925) and bacterial cells (Tabor, Rosenthal & Tabor, 1956). The biosynthesis of the natural polyamines is still obscure, although a pathway for their formation by some micro-organisms has recently been described (Tabor, Rosenthal & Tabor, 1957). The natural diamines putrescine, agmatine and cadaverine are known to be produced from amino acids by decarboxylation (Gale, 1946).

Little is known about the biological function of the natural polyamines. They have been shown to be growth factors for several micro-organisms (Herbst & Glinos, 1955; Martin, Pelczar & Hansen, 1952; Sneath, 1955; Traub, Mager & Grossowicz, 1955; Kihara & Snell, 1957). On the other hand, spermine and spermidine inhibit the growth of various bacteria (Rozansky, Bachrach & Grossowicz, 1954). The growth of *Mycobacterium tuberculosis* is inhibited by the degradation products of spermine or spermidine (Hirsch & Dubos, 1952).

Zeller (1938) studied the enzymic degradation of the natural polyamines by animal tissues; the enzymes were not purified and the oxidation products were not identified. Hirsch (1953*a, b*) found that sheep and bovine plasma contain an enzyme oxidizing spermine and spermidine. This enzyme was purified (Tabor, Tabor & Rosenthal, 1954), and its properties were studied (Werle & Roewer, 1954). However, the degradation pro-

ducts of the polyamines by this enzyme were not identified.

The oxidation of the natural polyamines by bacteria has not yet been thoroughly investigated. The only bacteria which have been found to metabolize these amines are *Pseudomonas aeruginosa* (Silverman & Evans, 1944) and *Mycobacterium smegmatis* (Roulet & Zeller, 1945). The diamines putrescine, agmatine and cadaverine are known to be oxidized by some bacteria (Gale, 1942). This study deals with the distribution of enzymes oxidizing the natural polyamines and diamines among bacteria. The mechanism of the oxidation is described.

MATERIALS AND METHODS

Organisms. The organisms used were obtained from the Diagnostic Laboratory of this department. Their identification was carried out according to Bergey (1948).

Media. The organisms were grown on brain-heart-infusion agar (Difco) or on an 'adaptation medium' composed of: yeast extract (Difco) 1 g.; K_2HPO_4 2 g.; KH_2PO_4 1 g.; $MgSO_4 \cdot 7H_2O$ 0.2 g.; amine for adaptation 3 g.; agar 15 g.; water 1 l.; final pH 7.2.

Cell suspensions and enzyme preparations. Bacteria grown on the above-mentioned media for 20 hr. at 37° were harvested, then washed twice with 0.85% NaCl soln. and kept at 4° until used.

Dried cell preparations were obtained by drying the cells over P_2O_5 at 0.3 mm. Hg (centrifugal freeze-drier, W. Edwards and Co.). Cell-free extracts were obtained by subjecting washed cell suspensions to the action of the Mickle tissue-disintegrator (Mickle, 1948) for 1 hr. with