THE MAINTENANCE OF PROSTATIC ACID PHOSPHATASE ACTIVITY BY PROTEINS AND POLYAMINES

by

GRACE M. JEFFREE

University of Bristol, Pathology Research Laboratory, Bristol (England)

Saline dilutions of seminal fluid, or of extracts of prostate, often show markedly lower acid phosphatase activity than comparable dilutions in blood serum or serum derivatives[1], particularly when the original specimen has been stored for some time in the refrigerator. A similar effect was found with a number of proteins and polypeptides, and with a variety of amino-compounds[2]. The effect was at first thought to indicate activation of prostatic acid phosphatase by these substances, since the curve of phosphatase activity with time is linear both when full activity is shown with their addition, or minimal activity in their absence. However, it was found that their addition after the start of incubation failed to restore activity; and it was concluded that they acted by protecting the enzyme from surface denaturation, which was found by LONDON AND HUDSON[3] for highly purified extracts. A large measure of protection may be obtained by overlaying the incubation mixture with petroleum ether. Factors conducive to denaturation are under investigation.

EXPERIMENTAL METHODS

Sources of prostatic phosphatase

Specimens of seminal fluid which had been submitted for the investigation of impaired fertility were obtained from the pathological laboratory of the Bristol General Hospital, and stored in the refrigerator until required. Some were considered to be completely normal; others showed oligospermia or even azoospermia. For some experiments, saline extracts of human prostate, obtained from fresh operation specimens, were used. Portions of the gland were allowed to stand in the refrigerator in about ten times their weight of physiological saline. After a day or more the saline was decanted and used as a source of acid phosphatase.

Initial dilutions were made into physiological saline. These were approximately 1/1,000 of seminal plasma, and from 1/10 to 1/200 of the original saline extract of prostate. Further dilutions of 1/5 to 1/20 were made, either again into physiological saline, or into the solution under investigation. In later work with known substances, these were added directly to the substrate mixture, and saline dilutions were used throughout.

Acid phosphatase estimations

These were carried out with phenyl phosphate as substrate by the GUTMAN AND GUTMAN[4] modification of the method of KING AND ARMSTRONG[5], or with *p*-nitrophenyl phosphate by the method of BESSEY, LOWRY AND BROCK[6], modified for the estimation of acid phosphatase by the use of either citrate or acetate buffer at pH 4.9; activity being stopped by the addition of the glycine-pyrophosphate buffer of HUGGINS AND TALALAY[7]. Enzyme dilutions were kept well below the limits of linear phosphatase activity; most experiments being performed with one hour incubations of specimens showing a maximum acid phosphatase activity in the region of 30 KING AND ARMSTRONG units per 100 ml, or 30 minute incubations of specimens with 50–60 units per 100 ml.

Estimations were performed in duplicate or triplicate, and readings were made on a Spekker Absorptiometer, using monochromatic light. For the blue colour developed with FOLIN AND CIOCALTEAU's reagent by liberated phenol, the mercury green wave-band at 5461 A was isolated

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by a combination of Ilford filters 625 (transmission 5100–5900 A) and 404 (peak transmission 5100–5500 A). For *p*-nitrophenol, the mercury violet band at 4360 A was isolated by Ilford filter 601 (transmission 3850–4750 A), with the supplementary filter 809, cutting out light between 3750 and 4150 A.

RESULTS

The effect of blood serum and proteins

Table I gives a comparison of acid phosphatase activities of four specimens of seminal fluid at different periods after ejaculation. Values estimated from saline dilutions drop towards zero, while those estimated from dilutions in serum extract remain close to the original level. This extract was the filtrate from coagulated blood serum boiled in four times its volume of physiological saline, and was as effective as fresh serum in maintaining acid phosphatase activity. A number of other biological fluids—boiled female urine, cerebrospinal fluid, and saline dilutions of boiled orange-juice or yeast extract (Marmite) were also active. The substances responsible were water-soluble, with very little solubility in organic solvents; they would stand evaporation to dryness on a boiling-water bath, but not incineration.

TABLE I

ACID PHOSPHATASE ESTIMATIONS OF SEMINAL PLASMA FROM DILUTIONS IN SALINE, AND IN SALINE EXTRACT OF COAGULATED BLOOD SERUM

Specimen of seminal fluid	*Days since ejaculation*	*Dilution used for estimation*	*Estimated units of acid phosphatase per ml of seminal plasma*	
			Dilutions in saline	*Dilutions in serum extract*
1. Normal	1	1/ 5,000	5,400	8,720
	14	1/10,000	Nil	9,090
2. Oligospermia	0	1/20,000	620	7,720
	4	1/20,000	100	6,660
	8	1/20,000	340	6,500
	11	1/20,000	80	7,740
	18	1/20,000	Nil	8,420
3. Normal	1	1/20,000	1,028	2,900
	3	1/20,000	1,840	3,360
	9	1/20,000	Nil	2,900
4. Normal	2	1/20,000	1,960	1,940
	8	1/20,000	40	3,860

The discovery that boiling coagulated blood serum with *N*/10 NaOH increased its protective activity led to the investigation of proteins and polypeptides. Commercially pure gelatine, used as a 1% (w/v) solution for the final dilution of the enzyme preparation, or incorporated into the substrate mixture in a concentration of 50 mg per 100 ml, was as effective as blood serum preparations. Fig. 1 shows the curve of acid phosphatase values obtained with different concentrations of gelatine in the substrate mixture, using preparations which, in the absence of any addition, showed less than 10% activity. The drop in acid phosphatase activity with decreasing concentrations of gelatine is very gradual, and the concentration of gelatine is reduced to about 30 micrograms per 100 ml before the activity is halved.

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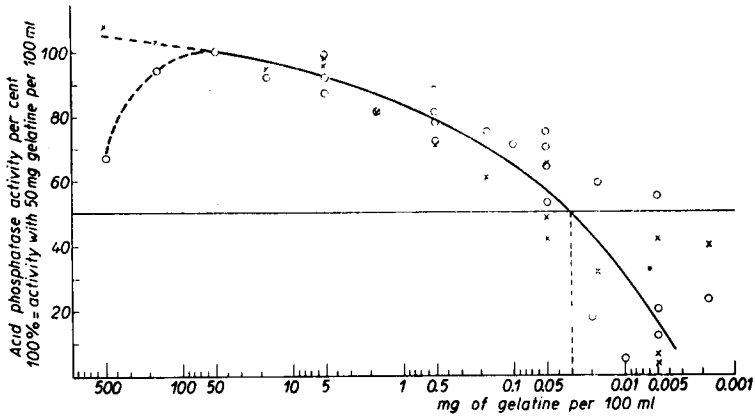

Fig. 1. Maintenance of prostatic acid phosphatase activity by gelatine. Acid phosphatase activity with different concentrations of gelatine in the incubation mixture, expressed as percentage of the activity with 50 mg gelatine per 100 ml. O Estimations with phenyl phosphate as substrate. × Estimations with *p*-nitrophenyl phosphate as substrate.

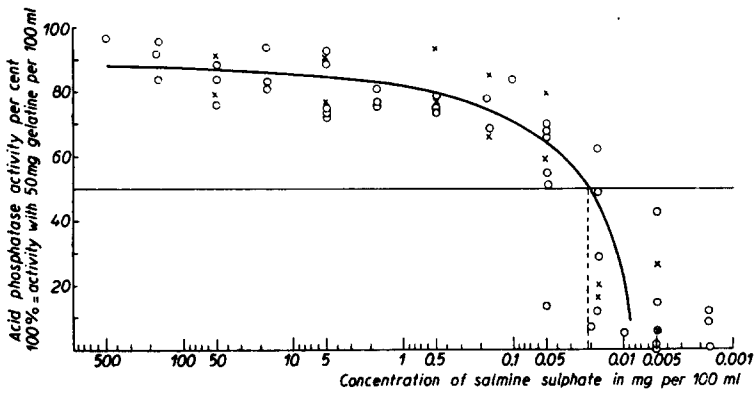

Fig. 2. Maintenance of prostatic acid phosphatase activity by salmine sulphate. Acid phosphatase activity with different concentrations of salmine sulphate, expressed as percentage of the activity with 50 mg gelatine per 100 ml. O Estimations in citrate buffer. × Estimations in acetate buffer.

The curve for salmine sulphate is shown in Fig. 2. The concentration required to maintain half activity is very close to that for gelatine, being approximately 21 micrograms per 100 ml.

The concentration, A, required to maintain half the activity of prostatic acid phosphatase shown in the presence of 50 mg gelatine per 100 ml was taken as a standard of comparison between protective substances. Table II shows comparative values for some treated and untreated proteins and polypeptides. The values of A for untreated gelatine, blood albumin and the protamine sulphates are very close. The value for gelatine is altered to a varying extent by different methods of hydrolysis; peptic digestion causes no significant alteration; tryptic digestion increases it 300 times, and hydrolysis with 8N HCl destroys the protective activity altogether (in all cases, digestive enzymes were inactivated by heat, and acids and alkalis neutralized before estimations were carried out). By contrast, tryptic digestion of salmine sulphate increases the value of A only 7 times.

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TABLE II

MAINTENANCE OF PROSTATIC ACID PHOSPHATASE ACTIVITY BY PROTEINS AND POLYPEPTIDES

Substance	*Treatment*	*"A", Concentration required to maintain 50% activity*	
		Micrograms per 100 ml	*Approximate micromoles per litre*
Gelatine	Untreated	30	0.005
	3 h peptic digestion	35	
	3 h tryptic digestion	10,000	
	22 h papain digestion	600	
	2 h hydrolysis in *N*/1 NaOH	50,000	
	2 h hydrolysis in 8*N* HCl	(No activity at 50,000)	
Salmine sulphate	Untreated	21	0.02
	3 h tryptic digestion	140	
Clupeine sulphate	Untreated	20	0.02
Blood albumin (B.D.H.)	Untreated	50	0.007
"Tyrothricin" (Sharp & Dohme) Gramicidin 10–20% Tyrocidin 40–60%	Untreated	5	

Polyamines

Fig. 3 shows the curve for acid phosphatase activity in different concentrations of spermine. This gives considerably less protection than gelatine; *A* being *M*/30,000, or approximately 700 micrograms per 100 ml. Spermine was one of the most active amino-compounds investigated, as will be seen from Table III. Protective activity generally increases with increasing chain-length between terminal amino-groups, but the figure for N-(1-naphthyl)-ethylene diamine is anomalous.

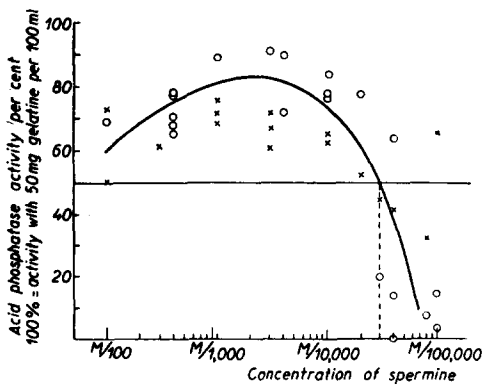

Fig. 3. Maintenance of prostatic acid phosphatase activity by spermine. Acid phosphatase activity with different concentrations of spermine, expressed as percentage of the activity with 50 mg gelatine per 100 ml. O Estimations with phenyl phosphate as substrate. × Estimations with *p*-nitrophenyl phosphate as substrate.

Amino-acids

The amino-acids investigated are listed in Table IV. Slight protection was found in three cases only, and could be ascribed entirely to the basic groups in the side-chain.

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TABLE III

MAINTENANCE OF PROSTATIC ACID PHOSPHATASE ACTIVITY BY AMINO-COMPOUNDS

Substance	*"A", Concentration required to maintain 50% activity Millimoles per litre*
Ethylamine, $CH_3 \cdot CH_2 \cdot NH_2$	143
Hydrazine, $NH_2 \cdot NH_2$	111
Ethylene diamine, $NH_2 \cdot (CH_2)_2 \cdot NH_2$	7.1
Propylene diamine, $NH_2 \cdot CH_2 \cdot CH(CH_3) \cdot NH_2$	7.7
N-(1-naphthyl)-ethylene diamine, $C_{10}H_7 \cdot NH \cdot (CH_2)_2 \cdot NH_2$	0.09
Trimethylene diamine, $NH_2 \cdot (CH_2)_3 \cdot NH_2$	11
Tetramethylene diamine, $NH_2 \cdot (CH_2)_4 \cdot NH_2$	6.7
Pentamethylene diamine, $NH_2 \cdot (CH_2)_5 \cdot NH_2$	4.5
Hexamethylene diamine, $NH_2 \cdot (CH_2)_6 \cdot NH_2$	0.19
Spermidine, $NH_2 \cdot (CH_2)_3 \cdot NH \cdot (CH_2)_4 \cdot NH_2$	0.15
Decamethylene diamine, $NH_2 \cdot (CH_2)_{10} \cdot NH_2$	0.08
Spermine, $NH_2 \cdot (CH_2)_3 \cdot NH \cdot (CH_2)_4 \cdot NH \cdot (CH_2)_3 \cdot NH_2$	0.03
Synthalin A, $NH_2 \cdot (NH:)C \cdot NH \cdot (CH_2)_{10} \cdot NH \cdot C(:NH) \cdot NH_2$	0.015

Ornithine shows a trace of activity, but less than that of ethylamine. Arginine and creatine, each with a guanidino group, have values of A about $M/17$, whereas that for guanidine is $M/20$. Neither tryptophan nor histidine shows any activity, but tryptamine and histamine, each with two amino-groups separated by four carbon atoms, show activities very close to that of tetramethylene diamine.

Amides

The few amides investigated are shown in Table V. No activity was found with any of these. Uridylic acid was included on account of the report by LORA-TAMAYO AND ALVAREZ[8] of its presence as a co-enzyme of kidney alkaline phosphatase.

Modification of amino-groups

Acetylation. Treatment of decamethylene diamine in ethanol with excess of acetic anhydride produced decamethylene diacetamide, $CH_3 \cdot CO \cdot NH \cdot (CH_2)_{10} \cdot NH \cdot CO \cdot CH_3$, as a white crystalline substance, M.P. 131°.5 (M.P. 131°, OFFE[9]; 130°, LINSTEAD, SHEPHARD AND WEEDON[10]). This was completely without protective activity at the limit of solubility, which was slightly less than $M/100$, whereas A for the original diamine is $M/11{,}500$.

Spermine was similarly treated with acetic anhydride, and the oily product was without activity at a concentration of $M/400$, though A for spermine is $M/30{,}000$. A portion was hydrolysed by refluxing for 30 minutes in 10% (v/v) concentrated hydrochloric acid, and again tested after neutralization. 64% acid phosphatase activity was then found at a concentration of $M/400$, so that some protective activity was restored by hydrolysis.

Treatment with nitrous acid. Treatment of primary aliphatic amines with nitrous acid destroys the amino group with the evolution of nitrogen and the production of the corresponding hydroxy compound, or of a mixture of olefins and corresponding alcohols[11].

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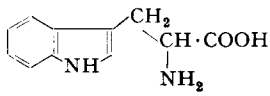
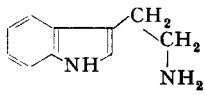
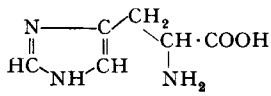
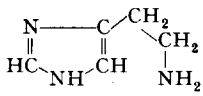
TABLE IV

MAINTENANCE OF PROSTATIC ACID PHOSPHATASE ACTIVITY BY AMINO-ACIDS AND RELATED COMPOUNDS

Substance	*"A", Concentration required to maintain 50% activity; in millimoles per litre*
Ornithine, $NH_2 \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	Above 250
Ethylamine, $NH_2 \cdot CH_2 \cdot CH_3$	143
Tetramethylene diamine, $NH_2 \cdot (CH_2)_4 \cdot NH_2$	6.7
Lysine, $NH_2 \cdot (CH_2)_4 \cdot CH(NH_2) \cdot COOH$	(No activity at 100)
Pentamethylene diamine, $NH_2 \cdot (CH_2)_5 \cdot NH_2$	4.5
Arginine, $NH_2 \cdot C(:NH) \cdot NH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	59
Creatine, $NH_2 \cdot C(:NH)N(CH_3)CH_2 \cdot COOH$	59
Guanidine, $NH_2 \cdot C(:NH) \cdot NH_2$	50
Sarcosine, $CH_3 \cdot NH \cdot CH_2 \cdot COOH$	(No activity at 100)
Citrulline, $NH_2 \cdot CO \cdot NH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$	(No activity at 100)
Asparagine, $NH_2 \cdot CO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	(No activity at 100)
Tryptophan 	(No activity at 10)
Tryptamine 	7.2
Histidine 	(No activity at 100)
Histamine 	6.2
Tyrosine, $HO \cdot C_6H_4 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	(No activity at 10)
Serine, $HO \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	(No activity at 100)
Glycine, $NH_2 \cdot CH_2 \cdot COOH$	(No activity at 1,000)
Cystine, $COOH \cdot CH(NH_2) \cdot CH_2 \cdot S \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	(No activity at 1)
Cysteine, $HS \cdot CH_2 \cdot CH(NH_2) \cdot COOH$	(No activity at 1)

Trimethylene diamine and decamethylene diamine were treated with sodium nitrite in 50% (v/v) acetic acid, in ice-cooled tubes. Gas was evolved in both cases, and with decamethylene diamine an oily layer, probably consisting mainly of decane or decanol, formed above the aqueous phase. Only a trace of residual activity was found with the aqueous phase from either reaction, and none with an emulsion of this oily product.

Treatment of spermine with sodium nitrite in *N* acetic acid resulted in a slow evolution of gas and the development of a golden-brown colour, suggesting the production of nitrosamines from the secondary amino-groups. The product was fully

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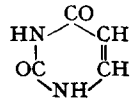
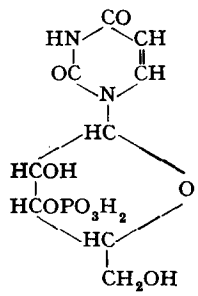
TABLE V

AMIDES AND RELATED COMPOUNDS FOUND INEFFECTIVE IN MAINTAINING PROSTATIC ACID PHOSPHATASE ACTIVITY

Substance	*Greatest concentration tested: in millimoles per litre*
Malonamide, $NH_2 \cdot CO \cdot CH_2 \cdot CO \cdot NH_2$	1,000
Urea, $NH_2 \cdot CO \cdot NH_2$	167
Thiourea, $NH_2 \cdot CS \cdot NH_2$	1,000
Uracil, 	50
Uridylic acid, 	10

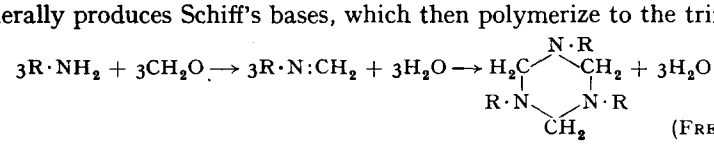
active at a concentration of $M/400$, but quite inactive at $M/4{,}000$, whereas A for spermine is $M/30{,}000$.

Treatment of salmine sulphate with nitrous acid, even in the presence of mineral acid, still left a highly active substance; but it was found on estimation of the arginine present by the method of ALBANESE AND FRANKSTON[12] that less than half the reactive groups had been destroyed, so that activity was still associated with the presence of intact amino-groups.

Benzylation. Solutions of trimethylene diamine and spermine in ethanol were treated with excess benzyl chloride. The crystalline products were washed with ethanol, dried and dissolved in the incubation mixture. In both cases, the product was as active as the original substance, and assuming the dibenzyl derivative to have been formed, even slightly more so.

Dinitrophenyl derivatives. Solutions of trimethylene diamine and spermine in ethanol were treated with excess 1-chloro-2,4-dinitrobenzene. The yellow products were washed with ethanol, dried and tested for activity. The trimethylene diamine derivative was too insoluble for full comparison, but was probably as active as the parent substance. Assuming the reaction with spermine to have produced the di-2,4-dinitrophenyl derivative, this was slightly more active than spermine, with A in the region of $M/60{,}000$ instead of $M/30{,}000$.

Treatment with formaldehyde. The reaction of formaldehyde with primary amines generally produces Schiff's bases, which then polymerize to the trimeric form:



(FRENCH AND EDSALL[13])

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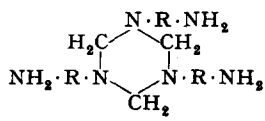
TABLE VI

MAINTENANCE OF PROSTATIC ACID PHOSPHATASE ACTIVITY BY UNTREATED SUBSTANCES AND THEIR FORMALDEHYDE DERIVATIVES

Substance	Concentration required to maintain 50% activity		A.1/A.2
	Untreated (A.1)	Formaldehyde treated (A.2)	
	Concentration in millimoles/litre		
Ethylene diamine	7.1	2.5	2.8
Hexamethylene diamine	0.19	0.006	30.0
Decamethylene diamine	0.08	0.02	4.0
Spermine	0.03	0.04	0.75
	Concentration in micrograms/100 ml		
Salmine sulphate	21	21	1.0
Gelatine	30	30	1.0

Ethylene diamine, hexamethylene diamine and decamethylene diamine, in aqueous solution, were treated with excess formaldehyde solution, and the original pH restored by the addition of *N* NaOH. Ethylene and decamethylene diamines required approximately 1 mole NaOH to 1 mole diamine, suggesting a reaction similar to that given above, with the production of a trimeric form:



For hexamethylene diamine, slightly over 1 mole of NaOH was required to two of the diamine. This suggests that the main reaction may be as follows:

$$2NH_2(CH_2)_6 \cdot NH_2 + CH_2O \longrightarrow NH_2 \cdot (CH_2)_6 \cdot NH \cdot CH_2 \cdot NH \cdot (CH_2)_6 \cdot NH_2 + H_2O$$

with subsidiary reactions forming longer chains or cyclic compounds.

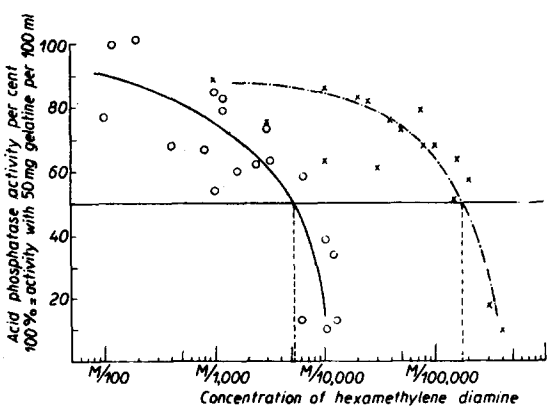


Fig. 4. Formaldehyde treatment of hexamethylene diamine. Prostatic acid phosphatase activity with different concentrations of hexamethylene diamine, expressed as percentage of the activity with 50 mg gelatine per 100 ml. O Untreated hexamethylene diamine. × Formaldehyde-treated hexamethylene diamine.

Though a similar reaction was expected with spermine, no change of pH (to Universal Indicator) was found on treating an aqueous solution with formaldehyde, nor again with gelatine or salmine sulphate. This is not so surprising, since the reaction of formaldehyde with proteins involves mainly the ε-amino groups of lysine, rather than the guanidino groups of arginine[13].

Table VI gives activity before and after formaldehyde treatment. Spermine shows a slight decrease of protective activity, and salmine sulphate and gelatine show no alteration; but there is an increase of activity of the diamines, in spite of the fact that molarities are

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expressed in terms of the original substance, so that, if a trimer has been formed, the actual molarity would be only 1/3 of that stated. Fig. 4 shows the curve of acid phosphatase activity with hexamethylene diamine before and after treatment with formaldehyde.

DISCUSSION

Substances found to maintain the activity of prostatic acid phosphatase in high dilution fall into the two groups of proteins or polypeptides, and amino-compounds, particularly long-chain polyamines. It is supposed that the effect is due to protection from surface denaturation on extreme dilution since addition of these substances after the start of incubation fails to restore activity; irreversible inhibition of the enzyme occurs very rapidly on dilution in the incubation mixture. Moreover, incubation beneath a layer of petroleum ether may maintain a high level of phosphatase activity (often 50–80%) without the addition of any protective substance, and similar high values may be obtained by incubation in polythene tubes. Traces of anionic detergents increase the destruction of the enzyme. When petroleum ether is used, higher concentrations of detergent are required for inactivation; and markedly greater concentrations in the presence of protective substances.

Though ethylamine gives slight protection, ethylene diamine is twenty times as effective, and the effect increases with the chain-length between terminal amino-groups, as shown in Fig. 5, whether the chain is composed of carbon atoms only, in the diamines, or of both carbon and nitrogen atoms, in spermine, spermidine and Synthalin A. It seems probable that protection by proteins and polypeptides is related to the amino-groups of basic side-chains, which are linked through the polypeptide chain. This would explain the destruction of the protective activity of gelatine on acid hydrolysis, and the different effects of enzymic digestion.

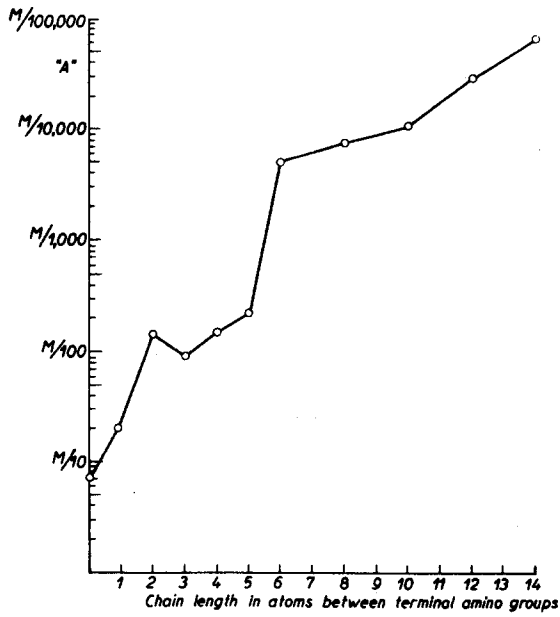

Fig. 5. Chain length and protective activity. Chain-length of amino-compounds plotted against the concentration, *A*, required to maintain 50% activity of prostatic acid phosphatase. Chain-length 0, hydrazine; 1, guanidine; 8, spermidine; 12, spermine; 14, Synthalin A. Other points, diamines.

Peptic digestion attacks proteins both by a random breaking of peptide bonds[14], and by specific attack on those involving the amino-groups of aromatic amino-acids[15]. These are comparatively few in gelatine; BOWES AND KENTEN[16] suggest 3 tyrosine and 10 phenylalanine residues out of a total of 419 in collagen, but 20 arginine and 12 lysine residues. If all the specific linkages were broken, it would divide the molecule into 14

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fragments, with a mean length of 30 residues, and an average of 2.36 basic residues.

The specific activity of trypsin is on the carboxyl groups of arginine and lysine[17,18]. Tryptic digestion should therefore split gelatine into peptides containing only one basic amino-acid residue, which would explain the great decrease in protective activity which occurs. Tryptic digestion of the protamines, however, is peculiar, owing to the high concentration of arginine residues in the molecule. WALDSCHMIDT-LEITZ[19] found clupean to be reduced by trypsin mainly to tripeptides, with terminal arginine residues enclosing a neutral residue. Such a tripeptide gives a longer chain between terminal amino-groups than any of the polyamines tested. If arginyl-seryl-arginine is taken as an example, the formula would be

$$NH_2 \cdot C(:NH) \cdot NH \cdot (CH_2 \cdot CH(NH_2)CO \cdot NH \cdot CH(CH_2OH) \cdot CO \cdot NH \cdot CH(COOH) \cdot (CH_2)_3 \cdot NH \cdot C(:NH)NH_2$$

There is a chain-length of 17 atoms between terminal amino-groups, and a molecular weight of 417 for the free base, or 573 for the sulphate. 140 micrograms per 100 ml, which is the value of A for the tryptic digest of salmine sulphate, represents a concentration of $M/367{,}000$, which is not an unreasonable figure for a polyamine with a separation of 17 atoms between terminal amino-groups.

It is thus reasonable to suppose that the protective activity of proteins and polypeptides is similar to that of long-chain polyamines. The activity of serum and other biological fluids can be ascribed to the proteins, polypeptides and amino-compounds present. The decreased activity of seminal plasma on keeping might result from either of two causes: the autolysis of proteins, or the precipitation of spermine in the form of spermine phosphate.

Destruction of amino-groups with nitrous acid, or their conversion to amide linkages by acetylation, destroys this protective activity, and the α-amino groups of amino-acids are likewise inactive; but the conversion of primary to secondary amines by the formation of benzyl or dinitrophenyl derivatives does not decrease activity, but rather increases it slightly.

Treatment with formaldehyde was carried out because of the high resistance of prostatic phosphatase to formaldehyde inhibition[20]. Formaldehyde treatment of gelatine and salmine sulphate, where little reaction was expected, and none was detected by the rather crude methods employed, had no effect on their protective activity. With spermine again, no reaction could be detected, and there was little effect on activity, which was very slightly reduced. With the diamines, the position was very different. The reactions produced a marked increase in acidity, requiring considerable amounts of alkali for neutralization; and with decamethylene diamine a spongy product quite unlike the original substance was formed. For ethylene and decamethylene diamines, neutralization figures indicate a monomolecular reaction, with the possible production of a cyclic trimer. With hexamethylene diamine, slightly over one equivalent of acid was liberated to two moles of diamine, and the production of a long-chain polymer seems more probable. In all three cases there is some increase in protective activity, but this is most marked in the last instance.

The protection of prostatic phosphatase by long-chain polyamines may be similar to the protection of urease and invertase by pentamidine from inhibition by Suramin, found by WILLS[21]. The negatively-charged surface of glass tubes may act in a similar fashion to the widely-spaced acidic groups of Suramin or of sodium

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dodecyl sulphate (SDS) micelles. Protection of prostatic phosphatase by gelatine, spermine and decamethylene diamine from irreversible inhibition by SDS has been found. Investigation of the effect of acidic groups on prostatic phosphatase is now in progress.

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SUMMARY

1. In the absence of protective substances, prostatic acid phosphatase in high dilution may be irreversibly inactivated, apparently by surface denaturation.

2. Enzyme activity is maintained in the presence of certain proteins, polypeptides and amino-compounds; particularly long-chain polyamines.

3. The protective effect of polyamines increases with the chain-length between terminal amino-groups.

4. The effect disappears with the destruction of amino-groups, or with their conversion to amides. The α-amino groups of amino-acids are ineffective.

5. Conversion of primary to secondary amines does not lessen the effect, and may even increase it.

6. Treatment with formaldehyde, with the formation of polymers, may increase the effect.

7. Complete hydrolysis of proteins destroys their protective action, and enzymic digestion reduces it to a variable extent. This can be explained on the assumption that the activity of proteins and polypeptides in this respect depends on the presence of basic side-chains, linked through the peptide chain to give an effect similar to that of the long-chain polyamines.

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