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BRAIN GUANINE DEAMINASE: PURIFICATION, PROPERTIES AND REGIONAL DISTRIBUTION

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SUMMARY

1. Brain guanine deaminase (guanine aminohydrolase, EC 3.5.4.3) has been purified 80–100-fold by a combination of ammonium sulphate fractionation, calcium phosphate gel treatment and DEAE-cellulose fractionation.

2. The rat-brain enzyme showed optimum activity at two values of pH, namely, 6.6 and 9.0 (for sheep brain the corresponding values were pH 6.5 and 8.5); while the liver enzyme manifested a single peak of optimum activity at pH 9.0.

3. The two activities were not resolved during the purification procedure. The rate of denaturation of the two activities by heat was also the same, suggesting the presence of the two activities on the same protein molecule.

4. The Michaelis–Menten constant for guanine was found to be very similar at both pH values: $1.92 \cdot 10^{-5}$ M at pH 6.6 and $1.98 \cdot 10^{-5}$ M at pH 9.0.

5. The enzyme had a high specificity of action for guanine, but exhibited no activity on guanosine, guanylic acid, adenine, adenosine and adenylic acid.

6. The enzyme action has been studied on a number of guanine analogues. 2-Aminopurine was not attacked by the enzyme at either pH value. Isoguanine was very slightly deaminated at pH 6.6 and 2-amino-6-mercaptopurine was deaminated to a small extent at both pH values. The results suggest the requirement of oxygen function at position 6 of the purine ring. The replacement of C at position 8 in the purine ring by N further potentiated the enzyme action at pH 6.6.

7. The distribution of the enzyme activity in various parts of the central and peripheral nervous system of monkeys has been studied. The enzyme activity was highest in the thalamus and was fairly abundant in most of the cerebral-cortex regions. No activity could be detected in the cerebellum or the optic nerve. The corpus callosum also had a very low activity.

INTRODUCTION

Guanine deaminase (or guanase) (guanine aminohydrolase, EC 3.5.4.3) catalyses the transformation of free guanine to xanthine and ammonia. TALWAR *et al.*[1] observed in rats that the activity of this enzyme was twice as high in the brain as in the liver

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and higher than in most other organs. The enzyme was seen to be localised in the supernatant (54 000 × *g*) fraction and exhibited some differences as regards pH of optimum activity when compared with the liver enzyme.

The present paper describes the partial purification of this enzyme from rat- and sheep-brain tissues. The properties and regional distribution of the enzyme have also been studied. Part of this work has been presented elsewhere[2].

MATERIALS AND METHODS

Albino rats were killed by decapitation. Blood clots were removed from the brains by rinsing with distilled water at 2–4°. Brain tissue was pooled and stored at −15°. Sheep brains obtained immediately after the death of animals were collected in polyethylene bags, brought to laboratory and stored after washing.

Rhesus monkeys were used in the study of regional distribution of the enzyme activity in brain. The skull was opened under hypothermia according to the method described earlier[3]. Brain regions were taken out and collected in small weighing bottles. The regions were rinsed with cold distilled water and excess water was removed with filter paper. The tissues were kept frozen before homogenisation.

Calcium phosphate gel was prepared according to KEILIN AND HARTREE[4].

DEAE-cellulose was obtained from M/S Serva Entwicklungslabor. Before use it was washed several times with buffer.

Guanine, 8-azaguanine, xanthine and adenine were obtained from California Biochemical Research and AMP, GMP, adenosine, guanosine were from Nutritional Biochemical Corporation. 2-Aminopurine (nitrate salt), isoguanine sulphate and 2-amino-6-mercaptopurine were supplied by Sigma Chemical Co.

The stock solutions of all bases, nucleosides and nucleotides were 1 mM. The stock solutions were prepared in glass-distilled water, dilute NaOH being used to facilitate the dissolution of bases and nucleosides.

The method used for enzyme assay was a modification of the procedure suggested by ROUSH AND NORRIS[5]. The decrease in absorbancy at 248 mμ, the absorption maxima for guanine at pH 1, was taken as a measure of substrate metabolised. The incubation mixture consisted of 2.4 ml of 0.1 M Tris–acetic acid buffer of appropriate pH, 0.5 ml of 1 mM guanine solution and 0.1 ml of enzyme preparation in a total volume of 3 ml. When the activity of a particular fraction was high, the enzyme preparation was suitably diluted with cold distilled water. The enzyme solution was pre-incubated in buffer at the appropriate pH at 37° for 5–10 min and the reaction started by the addition of 0.5 ml of 1 mM solution of substrate which was previously brought to 37°. After 5 min incubation, the reaction was stopped by the addition of 1 ml of 10% (w/v) perchloric acid. For each experiment a blank was taken, in which case perchloric acid was added, prior to the addition of substrate. After centrifugation, the absorbancy of the supernatant was read in a Hilger Uvispeck spectrophotometer at 248 mμ. In some experiments the absorbancy of the supernatant was also read at 282 mμ. At this wavelength, though the absorption of guanine is lower than at 248 mμ, the absorption due to xanthine is negligibly small. The results obtained by measurement at either wavelength were parallel.

A unit of enzyme activity was that amount of enzyme which caused a decrease

of 0.01 in absorbancy at 248 mμ (or 282 mμ) after 5 min incubation at 37° under the conditions of assay.

Enzyme action on guanine analogues was studied by the estimation of ammonia produced after a period of 30 min incubation at 37°. Two types of buffers were used depending upon the pH at which the reaction was studied. For pH 9.0 boric acid–sodium borate buffer (0.1 M) was used and for pH 6.6 sodium dihydrogen phosphate–disodium monohydrogen phosphate buffer 0.1 M. The reaction was stopped as described above. After centrifugation, 2 ml of the supernatant was drawn, neutralised with dilute alkali and made up to 3.5 ml with distilled water. Ammonia content was estimated by the method described by FAWCETT AND SCOTT[6]. The colour is developed by the addition of 1.0 ml of 2.5% sodium phenate, 1.5 ml of 0.01% sodium nitroprusside and 1.5 ml of 0.02 M sodium hypochlorite solution. The readings were taken against reagents blank at 620 mμ in a Hilger Uvispeck spectrophotometer. The quantity of ammonia was deduced from the calibration graph obtained with a standard solution of ammonium sulphate.

The protein content of enzyme preparation was estimated by the method of WARBURG AND CHRISTIAN[7]. In homogenates for which the 280/260 ratio was below 0.7, proteins were estimated by the biuret method[8].

Purification of enzyme from rat brain

All procedures were conducted at 0–4°, unless indicated otherwise.

Extraction. 150 g of frozen rat-brain tissue was homogenised in 800 ml solution of 2 mM KCl and 2 mM $NaHCO_3$ in a Waring blendor for 2 min. After homogenisation the volume was brought to 1200 ml by a further addition of the salt solution. The homogenate was stirred for 20 min and centrifuged in a refrigerated centrifuge at 10 000 × *g* for 20 min. The reddish cloudy supernatant contained 8.1 g of protein in a volume of 900 ml (Fraction I).

First ammonium sulphate fractionation. Fraction I was brought to 45% saturation by the slow addition of 242 g of solid ammonium sulphate and the mixture was stirred for 20 min. The pH was between 5.5 and 6. No adjustment of pH was made during fractionation. After centrifugation at 10 000 × *g* for 15 min the supernatant was collected and brought to 70% saturation by the addition of 148 g of ammonium sulphate. The mixture was stirred for 20 min and centrifuged as above. The precipitate was collected and dissolved in 50 ml of cold distilled water. The solution was dialysed for 20 h against 3 l of distilled water, with a change of water after 10 h and subsequently against 1 l of distilled water for 4 h. The solution was then centrifuged at 10 000 × *g* for 10 min. The yellowish clear solution contained 0.87 g of protein in a volume of 55 ml (Fraction II).

Calcium phosphate gel treatment. 100 ml of gel suspension (27.5 mg/ml) was centrifuged. The gel was washed twice with excess of 0.025 M phosphate buffer (pH 6.5) and 6 ml of 0.25 M phosphate buffer (pH 6.5) were added to 55 ml of Fraction II to bring the pH to 6.5 and the phosphate ion concentration to 0.025 M. The protein solution was then added to 2.75 g of the gel (the gel–protein ratio being 3:1). The mixture was stirred for 20 min and centrifuged at 8000 × *g* for 10 min. Batch elutions were made successively with 0.1 M and 0.25 M phosphate buffers (pH 6.5). This was followed by elution with 0.25 M phosphate buffer (pH 7.5) and the final

elution was made with 1 M KCl (pH 7.5). In each case 75 ml of buffer was used (except in the elution step with 0.25 M phosphate buffer at pH 6.5, where 150 ml of eluate was used). In each case the gel and eluent were stirred and the suspension was centrifuged at 8000 × *g* for 10 min. The eluate of 0.25 M phosphate buffer (pH 6.5) contained the major portion of the enzyme activity. This fraction contained 0.24 g of protein in 150 ml (Fraction III). The gel treatment did not give rise to a marked enrichment of the enzyme fraction, but its inclusion at this stage increased the efficacy of subsequent steps in the fractionation.

Second ammonium sulphate fractionation. 150 ml of saturated ammonium sulphate solution (saturated at 4° at pH 6.0) were added to the Fraction III with constant stirring. After 15 min, the mixture was centrifuged at 10 000 × *g* for 15 min. The supernatant was collected and again 150 ml of the salt solution were added and stirred for 15 min. After centrifugation it was dissolved in 10 ml of cold distilled water and dialysed for 20 h against 1.5 l of distilled water, water being changed after 10 h. The solution was centrifuged at 10 000 × *g* for 10 min. The protein content was 0.13 g in a total volume of 10.5 ml (Fraction IV).

DEAE-cellulose treatment. 540 mg of DEAE-cellulose, washed and equilibrated with 0.025 M phosphate buffer (pH 6.5), was stirred with Fraction IV, which was previously brought to 0.025 M with respect to phosphate. After slow stirring for 20 min, the suspension was centrifuged at 5000 × *g* for 15 min. The supernatant was discarded as the activity was almost completely adsorbed to DEAE-cellulose. This activity was subsequently eluted by a batch-wise elution procedure. The elutions were in the first instance carried with 15-ml volumes of successively, 0.05 M, 0.1 M and 0.2 M phosphate buffers (pH 6.5) followed by 15 ml of 0.25 M phosphate buffer (pH 7.5). The residual protein could be eluted with 1 M KCl (pH 7.5). The major portion of the activity was eluted with 0.1 M buffer (pH 6.5). It was extensively dialysed against distilled water and centrifuged again at 5000 × *g* for 15 min. This fraction contained 25 mg of protein in a total volume of 15 ml (Fraction V). The specific activity of the fraction was 265 and the yield was 5%. The fraction was stored at 4°.

Purification from sheep brain

1.36 kg of sheep-brain tissue was homogenised in three batches in a solution of

TABLE I

PURIFICATION OF GUANINE DEAMINASE FROM RAT BRAIN

Steps	*Volume (ml)*	*Total units enzyme activity*	*Total protein*	*Specific activity (units/mg protein)*	*Yield (%)*
Homogenate	1200	132 000	38.4 g	3.4	100
Supernatant	900	116 100	8.1 g	14.3	88
First ammonium sulphate fractionation	55	52 250	0.97 g	54.0	40
Calcium phosphate gel treatment	150	21 150	0.24 g	88.0	16
Second ammonium sulphate fractionation	10.5	12 200	0.13 g	93.0	9
DEAE-cellulose treatment	15	6 750	25 mg	265.0	5

KCl and $NaHCO_3$ (each 2 mM). The volume of total homogenate was 6.8 l. The remaining procedure was the same as described for the rat brain.

The fraction thus obtained contained 158 mg of protein in a volume of 66 ml (2.4 mg of protein/ml). The specific activity of the enzyme was 100. The purification achieved was 100-fold. The total yield was 6%. The purified enzyme was divided in 3-ml aliquots in small tubes and stored at −15°.

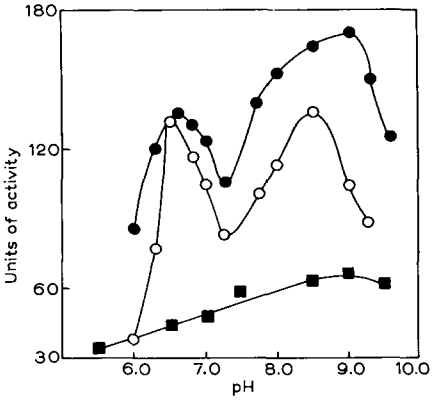

Fig. 1. Effect of pH on the activity of guanine deaminase. ●—●, purified rat brain enzyme, 0.1 ml (60 μg protein); ○—○, purified sheep brain enzyme, 0.1 ml (50 μg protein) and ■—■, 0.1 ml rat liver supernatant (17 000 × *g*, 30 min) from 10% homogenate. Tris–acetate 0.1 M buffer was used in the pH range 6.0 to 9.5; acetate 0.1 M buffer was used for pH 5.5. Assay conditions are described in the text.

RESULTS

A resumé of the purification procedure for rat-brain tissue is given in Table I. An 80-fold purification is obtained with an overall recovery of 5% of original activity.

Optimal pH

Guanine deaminase from brain tissue exhibits optimal activity at two pH values. The enzyme from rat brain shows maximum activity at pH 6.6 and 9.0; while that

TABLE II

SPECIFIC ACTIVITIES AT TWO OPTIMAL pH VALUES AT VARIOUS STAGES OF ENZYME PURIFICATION PROCEDURE

	Rat brain			*Sheep brain*		
	Sp. act. pH 6.6	*Sp. act. pH 9.0*	*Ratio of sp. act. pH 9.0/6.6*	*Sp. act. pH 6.5*	*Sp. act. pH 8.5*	*Ratio of sp. act. pH 8.5/6.5*
Supernatant	12.5	14.3	1.1	4.1	4.1	1.0
First ammonium sulphate fractionation	42.0	54.0	1.3	13.0	13.1	1.0
Calcium phosphate gel treatment	76.0	88.0	1.2	25.0	25.0	1.0
Second ammonium sulphate fractionation	83.2	93.0	1.1	57.0	62.7	1.1
DEAE-cellulose treatment	208.0	265.0	1.3	260.0	272.0	1.0

from sheep brain shows activity peaks at pH 6.5 and 8.5 (Fig. 1). The liver enzyme on the other hand has only a maximum at pH 9.0.

An attempt was made to dissociate the two pH activities, but the ratio of their specific activities remained unchanged at all stages of the purification procedure (Table II).

Heat inactivation of enzyme

Heat-inactivation experiments were conducted on purified enzyme preparation with a view to investigate whether both pH activities underwent a parallel denaturation or whether the rates of denaturation were different for the two activities. For this purpose two sets of experiments were performed. The enzyme was incubated in Tris–acetate buffer at pH 6.6 in Series I (Fig. 2, Curve A) and pH 9.0 in Series II

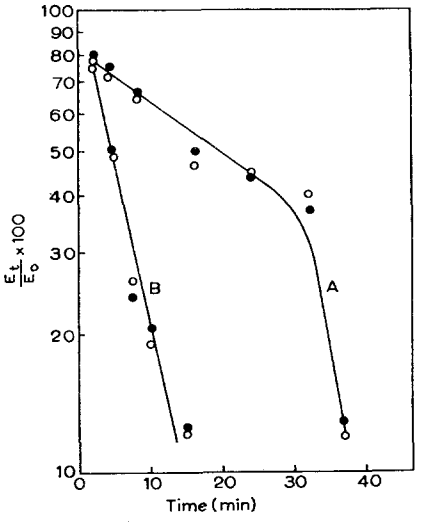

Fig. 2. Heat-inactivation kinetics. Purified rat brain enzyme was heated at 62.5° ± 0.2 in either pH 6.6 (Curve A) or in pH 9.0 (Curve B) Tris–acetate buffer 0.05 M. At varying time intervals aliquots were removed and the residual pH 6.6 (●) and pH 9.0 (○) activity of the enzyme assayed. E_t: activity at time intervals denoted on the abscissa. E_o: initial activity of the enzyme before heating. Ordinates are on a log scale.

(Fig. 2, Curve B). The stock enzyme solution was heated in the prescribed pH buffers at 62.5°. At varying intervals of time, aliquots were removed and assayed for residual guanine deaminase activity. Each aliquot removed was assayed for both pH-6.6 and pH-9.0 activity. Results are presented in form of a semilogarithmic plot in Fig. 2. Curve A gives the results of heating the enzyme at pH 6.6 and Curve B of heating at pH 9.0. In each case, the residual guanine deaminase activity of the enzyme at the two pH's of reaction was decreased in an equivalent proportion.

Michaelis constant for guanine

The effect of increasing substrate concentration on the reaction velocity at pH 6.6

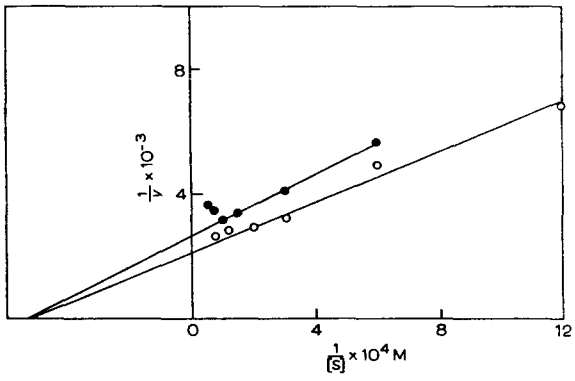

Fig. 3. Effect of substrate concentration on the rate of enzyme reaction. The reciprocals of guanine concentrations used in each assay are represented on the abscissa against the reciprocals of the corresponding rates of reaction. The purified rat brain enzyme preparation used in each case contained 58 μg protein. ●—●, reaction rate at pH 6.6; ○—○, at pH 9.0.

and pH 9.0 is represented on a Lineweaver–Burk plot in Fig. 3. The K_m values for guanine were $1.92 \cdot 10^{-5}$ M at pH 6.6 and $1.98 \cdot 10^{-5}$ M at pH 9.0.

Substrate specificity

The purified enzyme did not deaminate guanosine, guanylic acid, adenine, adenosine or adenylic acid at either of the pH optima of the guanine deaminase reaction. The assays for these deaminase activities were conducted as described for guanine; with different substrates the absorbancies were measured at the wavelengths denoted in Table III. No measurable differences in the absorbancies were

TABLE III

WAVELENGTHS OF MAXIMUM ABSORPTION FOR DIFFERENT SUBSTRATES AND THEIR DEAMINATED PRODUCTS

Substrate	*Wavelength in mμ of maximum absorption*	
	Substrate	*Deaminated product*
Guanosine	255	—
Guanylic acid	255	—
Adenine	265	249
Adenosine	257	249
Adenylic acid	258	249

observed with respect to blanks. This was taken as an indication of the non-reactivity of the enzyme on these substrates.

The enzyme was also unable to oxidise xanthine at pH 6.6 and pH 9.0.

The enzyme deaminated guanine specifically, which was confirmed by (i) depletion of guanine (ii) appearance of a xanthine spot chromatographically (iii) production of ammonia.

Action on substrate analogues

Enzyme activity on related aminopurines, *viz.* 2-aminopurine, 2-amino-6-mercaptopurine, isoguanine and 8-azaguanine was studied and is represented in Table IV.

TABLE IV

ACTION OF GUANINE DEAMINASE ON GUANINE AND RELATED AMINOPURINES

The reaction was performed at pH 6.6 as well as at pH 9.0. Purified rat-brain enzyme, 0.1 ml (85 μg protein) was used for the reaction. The incubations were carried out as described for the study of guanine analogues. Estimation of ammonia is described in the text. The quantity of ammonia produced when guanine was used as substrate is taken as 100, for comparative purposes.

Aminopurine	*pH 6.6*		*pH 9.0*	
	mμmoles of ammonia produced	*% deamination with respect to guanine*	*mμmoles of ammonia produced*	*% deamination with respect to guanine*
Guanine	200.0	100.0	255.9	100.0
2-Aminopurine	0.0	0.0	0.0	0.0
2-Amino-6-mercaptopurine	29.5	14.7	23.5	9.2
Isoguanine	11.7	5.8	0.0	0.0
8-Azaguanine	500.0	250.0	18.8	7.3

Regional distribution of the enzyme activity

While the activity in the whole brain was twice that of the liver, it was felt that in order to gauge the possible physiological function of the enzyme, a survey was necessary of its distribution in various parts of the central and peripheral nervous system. Table V summarises the results obtained in monkeys. The enzyme in this species also has optimum activity at two values of pH *i.e.*, pH 6.6 and pH 8.5, the same as observed with sheep brain.

TABLE V

REGIONAL DISTRIBUTION OF GUANINE DEAMINASE ACTIVITY IN MONKEY BRAIN

The brain regions were homogenised in cold distilled water to obtain a 10% homogenate. After centrifugation at 10 000 × *g* for 10 min, 0.1 ml of the supernatant was used for the assay of enzyme activity at pH 6.6. Specific activity (units of activity per mg protein) for regions tested are represented on a comparative scale, taking the specific activity of the thalamus as 100. The results are the mean values for three separate experiments. The same pattern of activity distribution was observed at pH 9.0.

Regions	*Specific activity pH 6.6*	*Regions*	*Specific activity pH 6.6*
Thalamus	100.0	Lateral occipital cortex	
Temporal tip	79.5	(visual lobe)	28.2
Caudate nucleus	69.2	Corpus callosum	5.1
Cortex-8		Spinal cord fibers	2.6
(motor area)	53.8	Cerebellum	0.0
Hippocampus	53.8	Optic nerve and chiasma	0.0

DISCUSSION

Purine catabolism is normally localised in the liver, where the entire battery of enzymes converting adenine and guanine to uric acid (or subsequent oxidative products) is present. Guanine deaminase activity is, however, high in the brain[1]. This enzyme is particularly rich in the thalamus. The enzyme is well distributed in most of the cerebral cortex areas, while the cerebellum has no activity. The spinal cord and the nerve fibres are also devoid of guanine deaminase activity. The enzyme seems to be present mainly in the neuronal cells, as the corpus callosum region, populated with glial type of cells, shows low activity.

The possible function of this enzyme is not clear. It has been noted by THRELFALL[9] that guanine nucleotides constitute 18–20% of the total free nucleotides in the brain, whereas their proportion in muscle tissue is only 2–3% of the total nucleotides. HEALD[10] has observed that ^{32}P is incorporated in guanine nucleotides prior to its incorporation in ATP, in cerebral slices stimulated electrically. The guanine compounds seem to have an active turnover and may be performing an important role in the brain.

The high specificity of action of the enzyme reflects the possible formation of the free base in the course of some cellular function. The depletion of brain RNA content during convulsions[11–13], or strenuous exercise[14,15] has been reported by various workers. GEIGER[16] reported that the composition of nucleic acids in the cat brain alters during stimulation, the cytidine and adenine contents of nucleic acids increasing in proportion to the uridine and guanine contents. RAPPOPORT *et al.*[17] have described the existence of an active nucleoside phosphorylase in brain extracts. The experimental data is, however, insufficient to delineate the actual physiological function, if any, of guanine deaminase.

The purine product of guanine deaminase action is xanthine, for which the solubility in the physiological range of pH is higher than that of guanine. POLIS[18] has shown that xanthine increases the oxygen uptake, as well as the oxidative phosphorylation rate of brain mitochondria *in vitro*.

Several workers[17,19] have reported the absence of xanthine oxidase (EC 1.2.3.2) activity in the brain extract. The recyclization of purines into the nucleotide pool from xanthine could be expected in the brain by analogy with similar reactions in other systems[20–22].

The brain guanine deaminase differs from the liver enzyme as regards its pH-activity pattern. While the liver enzyme has an optimum activity at pH 9 (SCHMIDT[23] has earlier reported the pH optimum for liver guanine deaminase as 9.2), the brain enzyme manifests a double peak of optimum activity at pH 6.6 and 9.0 in rat, and at pH 6.5 and 8.5 in sheep brain. The ratio of the two activities remains the same at all stages of the purification procedure. The heat-inactivation studies demonstrate an equivalent rate of denaturation of the two activities. The two activities, therefore, seem to be linked with the same protein molecule in case of the brain enzyme. The present studies, however, are not sufficient for a conclusive statement on this point.

The enzyme has high specificity towards guanine among naturally occurring purines and their compounds, as no deaminase activity was detected when guanosine, guanylic acid, adenine, adenosine and adenylic acid were used as substrates under the conditions of assay. This high specificity of action is accompanied by a low

K_m value for guanine. The K_m values were observed to be $1.92 \cdot 10^{-5}$ at pH 6.6 and $1.98 \cdot 10^{-5}$ at pH 9.0; suggesting that the affinity of the enzyme to its substrate at the two values of the pH is similar.

The enzyme action towards related aminopurines was studied to determine the structural group requirements for enzyme action. Activities at both pH optima were investigated for the analogues studied. It was found that 2-aminopurine is not deaminated at either pH value of maximum activity for guanine. Isoguanine (6-amino-2-oxypurine) is not deaminated at pH 9.0; the slight activity observed at pH 6.6 is of a negligible order. The 2-amino-6-mercaptopurine is deaminated to a small extent at both pH values. The results indicate that an oxygen function at position 6 of the purine ring is optimum for activity, because its absence in 2-amino-purine makes the latter inert to enzyme action. The replacement of the oxygen by a less negative atom (sulphur in the case of 2-amino-6-mercaptopurine), results in a marked decrease of activity. The respective positions of amino group and oxygen function on the purine ring seem to be a critical requirement for activity, because isoguanine does not serve as a substrate for this enzyme. The deaminating action of the enzyme is potentiated by replacement of C at position 8 by N (8-azaguanine). This effect is well marked at pH 6.6. ROUSH AND NORRIS[5] also observed that the liver enzyme deaminated 8-azaguanine maximally at pH 6.5. BROCKMAN *et al.*[24] have shown that in a system of purine ribonucleotide pyrophosphorylase, the conversion of 8-azaguanine to 8-azaguanylic acid is maximum at pH 6.5. Thus in all three systems described, the enzyme–8-azaguanine complex seems to be most suitably oriented for catalytic activity at a value of pH around 6.5.

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REFERENCES

1 G. P. TALWAR, B. K. GOEL, M. MANSOOR AND N. C. PANDA, *J. Neurochem.*, 8 (1961) 310.
2 M. MANSOOR, G. D. KALYANKAR AND G. P. TALWAR, *Acta Neurol. Scand.*, Suppl. 1, 38 (1962) 48.
3 G. P. TALWAR, B. K. ANAND, A. J. TOLANI AND N. C. PANDA, *Ind. J. Med. Res.*, 49 (1961) 710.
4 D. KEILIN AND E. F. HARTREE, *Proc. Roy. Soc. London, Ser. B*, 124 (1937–38) 397.
5 A. ROUSH AND E. R. NORRIS, *Arch. Biochem. Biophys.*, 29 (1950) 124.
6 J. K. FAWCETT AND J. E. SCOTT, *J. Clin. Path.*, 13 (1960) 156.
7 O. WARBURG AND W. CHRISTIAN, *Biochem. Z.*, 310 (1941) 384.
8 E. LYNE, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Academic Press, New York, Vol. 3, 1957, p. 450.
9 C. J. THRELFALL, *Biochem. J.*, 65 (1957) 694.
10 P. J. HEALD, *Biochem. J.*, 67 (1957) 529.
11 G. P. TALWAR, B. SADASIVUDU AND V. S. CHITRE, *Nature*, 191 (1961) 1007.
12 B. SADASIVUDU AND G. P. TALWAR, *Bull. Natl. Inst. Sci. India*, 19 (1962) 237.
13 V. S. CHITRE AND G. P. TALWAR, *Ind. J. Med. Res.*, 51 (1963) 80.
14 H. HYDEN, *Acta Physiol. Scand.*, 6 (1943) Suppl. 17.
15 R. VRBA AND J. FOLBERGROVA, *J. Neurochem.*, 4 (1959) 338.
16 A. GEIGER, in D. RICHTER, *Metabolism of the Nervous System*, Pergamon Press, London, 1957, p. 245.
17 D. A. RAPPOPORT, P. R. FRITZ, J. R. ALLEN AND A. MORACZEWSKI, *J. Neurochem.*, 6 (1960) 21.
18 D. POLIS, in S. S. KETTY AND J. ELKES, *Proc. 4th Intern. Neurochem. Symposium*, Varenna, Italy, 1961, p. 37.

[19] W. W. WESTERFIELD AND D. A. RICHERT, *Proc. Soc. Exptl. Biol. Med.*, 71 (1949) 181.
[20] M. FRIEDKIN, *J. Am. Chem. Soc.*, 74 (1952) 112.
[21] R. ABRAMS AND M. BENTLEY, *Arch. Biochem. Biophys.*, 58 (1955) 109.
[22] R. ABRAMS AND M. BENTLEY, *Arch. Biochem. Biophys.*, 79 (1959) 91.
[23] G. SCHMIDT, *Z. Physiol. Chem.*, 208 (1932) 185.
[24] R. W. BROCKMAN, C. S. DEBAVADI, P. STUTTS AND D. J. HUTCHISON, *J. Biol. Chem.*, 236 (1961) 1471.