Guanosine deaminase in human serum and tissue extracts – a reappraisal of the products

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ABSTRACT

Of the human salvage enzymes that deaminate ribonucleosides, two – cytidine deaminase and adenosine deaminase – have been found particularly useful for diagnostic purposes. In humans, no enzymes are present that can directly deaminate the bases of these ribonucleosides. Indeed, the only enzyme present that can directly deaminate a base is guanine deaminase, and the diagnostic usefulness of this enzyme has been well documented. The aim of this study is to identify the origin of the ammonia formed when human sera and tissue extracts are incubated with buffered guanosine, and to clarify whether the ammonia comes from the deamination of guanosine by guanosine deaminase or is produced as a result of deamination of guanine formed as a breakdown product of guanosine by purine nucleoside phosphorylase (PNP). Apparent deamination of guanosine by guanosine deaminase in human sera and tissue extracts was found to be due to two enzymes acting in tandem when the products of the reaction were examined by HPLC. The ribose was first removed from guanosine by PNP to form guanine, which was then deaminated to xanthine by guanine deaminase

KEY WORDS: Guanine deaminase. Guanosine deaminase. Liver diseases. Purine-nucleoside phosphorylase.

Introduction

Of the human salvage enzymes that deaminate ribonucleosides, two – cytidine deaminase[1-3] and adenosine deaminase[4-7] – have been found particularly useful for diagnostic purposes. In humans, no enzymes are present that can directly deaminate the bases of these ribonucleosides. Indeed, the only enzyme present that can directly deaminate a base is guanine deaminase, and the diagnostic usefulness of this enzyme has been well documented.[8]

Guanosine, the ribonucleoside formed from guanine, contains an amine group and if the deamination of cytidine and adenosine are taken as models then it would be expected that an enzyme, guanosine deaminase, should degrade guanosine to ammonia and xanthosine. Indeed, guanosine deaminase was first described by Jones[9] as long ago as 1911. Some years later, however, Kalckar[10] was unable to detect guanosine deaminase in pigs' liver, and it is this latter conclusion that is usually quoted in modern texts[11] and reviews.[12]

More recently, the enzyme was purified from bacterial origin[13] by a process utilising an ammonium sulphate precipitation step when the guanosine deaminase was contained in the 20-43% fraction which contained very little purine nucleoside phosphorylase (PNP). In addition, guanosine deaminase has been reported as present in rat liver.[14] The use of this enzyme has also been described in the monitoring of early pregnancy.[15] However, Georgelli,[16] using a radio labelling technique did not find guanosine deaminase in rat liver.

Both colorimetric[17,18] and high-performance liquid chromatography (HPLC)[19] methods have been described for the estimation of human serum guanosine deaminase. The colorimetric method is reported as being useful in the diagnosis of metastasising liver carcinoma and of infective hepatitis. The method is based on the estimation of the ammonia formed when serum is added to buffered guanosine (Fig. 1a). However, there is an alternative means by which ammonia may be formed and this is by an initial breakdown of guanosine to guanine by the enzyme PNP (Fig. 1b), followed by a deamination of the guanine to xanthine and ammonia (Fig. 1c). The last reaction is that reported by Kalckar[10] in 1947 using spectrophotometric methods.

Sherwood[19] presented a method of estimating guanosine deaminase by HPLC utilising the decrease of substrate and not the increase in the product, xanthosine, as a measure of enzyme activity. However, the author admitted that the performance characteristics of the method were under review. No further work has been reported on the estimation of guanosine deaminase by HPLC to date.

The aim of this study is to identify the origin of the ammonia formed when human sera and tissue extracts are incubated with buffered guanosine, and to clarify whether the ammonia comes from the deamination of guanosine by guanosine deaminase or is produced as a result of deamination of guanine formed as a breakdown product of guanosine by PNP.

Materials and methods

Reagents

All bases, nucleosides and inhibitors were obtained from the Sigma (Sigma- Aldrich Co Ltd., Fancy Rd Poole Doset BH12 4GH, UK). All other materials were obtained from BDH (VWR International, Poole, Dorset, UK).

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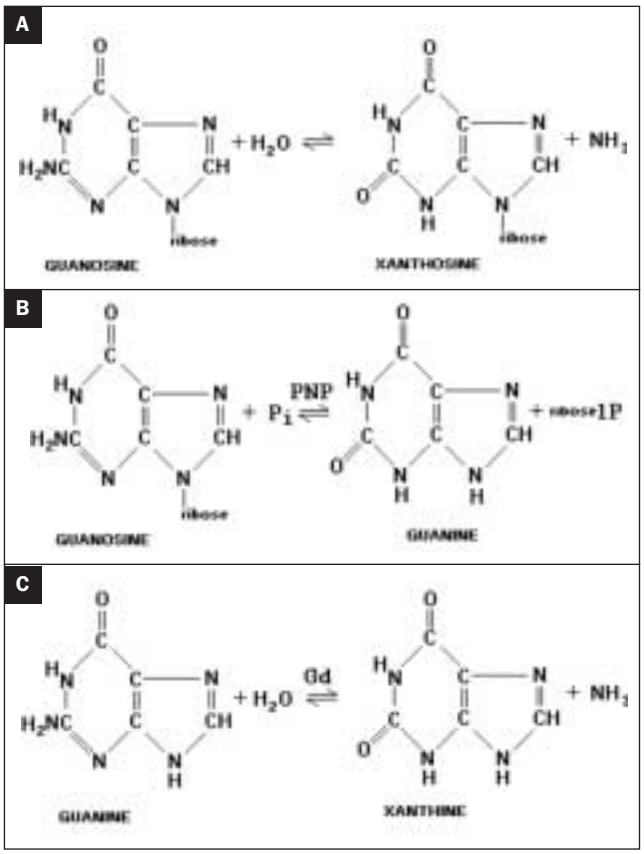

Fig. 1. (A) Formation of ammonia when serum is added to buffered guanosine, and (B) an alternative means by which ammonia may be formed by an initial breakdown of guanosine to guanine by the enzyme PNP, followed by (C) deamination of the guanine to xanthine and ammonia.

Specimen collection

Sera in excess of clinical requirements were used throughout. Normal samples from well-man and well-woman clinics and samples from patients, particular those suffering from liver metastasis and infective hepatitis, were collected. Normal liver, intestine, bone, kidney, bladder gall bladder and myocardium were obtained from fresh post-mortem tissue after routine histological examination.

Instrumentation

LKB/Pharmacia HPLC was used throughout (Amersham, Pharmacia Biotech, St Albans, Herts UK). This apparatus consisted of an LKB 2150 HPLC pump, an LKB 2158 uvicord SD spectrophotometer and an LKB 2210 potentiometric recorder. Samples were injected with a model 7125 Rheodyne syringe loading sample injector (Anachem, Luton, UK.). The valve was fitted with a 200 µL loop.

Tissue extraction

Approximately 1.0 g of each soft tissue was washed with isotonic sodium chloride to remove blood and, except for bone, homogenised with 10 mL of distilled water at room temperature. Bone extract was obtained by removing and discarding the bone marrow, then washing with distilled water. After drying, the bone was crushed in a pestle and mortar containing 10 mL of water. Extracts were centrifuged for 5 min at 1500 x*g* to remove debris.

Tissue protein estimation

The protein was estimated by measuring the turbidity formed with sulpho-salicylic acid[20] at 660 nm and compared with a standard protein solution obtained from Randox (Randox Laboratory, Diamond Road, Ardmore, Northern Ireland).

Colorimetric measurement of guanosine deaminase

Jones *et al.*,[17] assayed guanosine deaminase by estimating the ammonia liberated using the Berthelot reaction. Briefly, 0.1 mL serum or diluted tissue extract (1 in 400) was incubated with 0.15 mL guanosine (1.42 mmol/L) buffered at pH 6.8 for 4 h at 37°C. Phenol/nitroprusside solution (1.5 mL) was then added, followed by 1.5 mL of an alkaline hypochlorite solution. Serum blanks and reagent blanks were also prepared as described by the authors.

After incubation for a further 35 min at 37°C, the blue colour was measured at 625 nm and compared to dilutions of a 0.86 mmol/L (equivalent to 5.4 U/L) ammonium standard prepared by diluting a 17.2 mmol/L ammonia stock standard. The latter was prepared by dissolving 114 mg of anhydrous ammonium sulphate in 100 mL ammonia-free distilled water.

A unit of enzyme activity is defined as the amount required to produce 1 µmol of ammonia per min under the conditions described. In the case of tissue, enzyme activity was expressed as the equivalence of 1 unit to 1 µmol ammonia produced per min per 100 mg protein.

The published method was adhered to with the exception that instead of using a suspension of guanosine as a stock substrate, the stock guanosine substrate (28.4 mmol/L guanosine) was dissolved as follows: 80 mg of guanosine was dissolved in 8 mL 0.11 mol/L sodium hydroxide, and, after mixing, 1 mL ethyl alcohol was added. After further mixing, the solution was neutralised with 1 mL M HCl. Working substrate (1.42 mmol/L guanosine): 1 mL stock substrate was immediately added to 19 mL 0.066 mol/L phosphate buffer (pH 6.8). This was stable for 12 hours.

High-performance liquid chromatography

100 mmol/L ammonium acetate adjusted to pH 6.0 with 6 M HCl was used as the mobile phase. HPLC was set up using a mobile phase flow-rate of 1.2 mL/min. A stainless steel column, (100 mm x 4.6 mm) containing ODS-Hypersil (Jones Chromatography, Hengoed, Mid-Glamorgan, Wales) was used for the separation. The detection wavelength used was 277 nm, the sensitivity was 0.005 absorption units and the chart speed 10 mm/min. The temperature of separation was ambient (20–22°C).

Standards were prepared in 0.066 mol/L phosphate buffer (pH 6.8) at concentrations of 1.42 mmol/L guanosine, 0.95 mmol/L xanthosine, 0.82 mmol/L guanine, 0.59 mmol/L uric acid and 0.95 mmol/L xanthine. Each standard (0.15 mL) was mixed with 0.1 mL phosphate buffer and treated as serum/substrate mixture.

Serum (0.1 mL) or diluted tissue extract was incubated with 0.15 mL buffered guanosine for 4 h at 37°C. Then, 200 µL cold (4°C) 20 % (w/v) trichloracetic acid was added to the reaction mixture and mixed well. The tubes were then centrifuged at 3000 rpm for 10 min and 200 µL supernatant was added to 200 µL of acetate buffer pH 5.0 (200 mM) and 100 µL allopurinol solution (1.92 mM) were added as an internal standard. The mixture (10 µL) was injected into the

HPLC column. The ratios of peak height against allopurinol were quantified by comparing with the standard solutions.

Ammonium sulphate precipitation

As the presence of guanosine deaminase may be masked by the presence of PNP, a separation of the proteins by ammonium sulphate precipitation was performed. The method of separating guanosine deaminase, guanine deaminase and PNP was that used by Ishida *et al*.[13] For *Pseudamonus complexa.* A fresh liver extract was centrifuged at 20,000 RCF for 20 min in a refrigerated centrifuge. The supernatant was taken for further treatment.

Extract (2 mL) was added to 0.5 mL saturated ammonium sulphate solution to form a 20% saturated solution. This was immediately mixed well to obviate any local precipitation and after 10 min centrifuged for a further 10 min at 5500 RCF. The supernatant was decanted and the precipitate dissolved in 1 mL 0.066 mol/L phosphate buffer (pH 6.8). The supernatant (2 mL) was mixed with 0.8 mL ammonium sulphate to form a 43% saturated solution. The precipitate was collected and redissolved as described above and the supernatant was also reserved.

The dissolved precipitates and the supernatants were incubated with guanosine substrates as described above. The guanosine degradation products present in the two precipitates (dissolved in 1.0 mL 0.066 mol/L phosphate buffer [pH 6.8]) and the final supernatant were examined by HPLC as described for the liver extract. PNP activity was calculated from the total guanine and xanthine present, while guanine deaminase was calculated from the amount of xanthine formed.

Effect of inhibitors

If the apparent deamination of guanosine by human tissue extracts is as a result of PNP and guanine deaminase then it would be expected that any substance that inhibits PNP or guanine deaminase activity (or both) would also inhibit the apparent guanosine deamination. Conversely, any substance that inhibits apparent guanosine deamination should also inhibit either the PNP or the guanine deamination.

If, however, guanosine deamination is due to a discrete enzyme then it would be expected that some inhibitors of the deamination would not inhibit guanine deaminase or PNP. Furthermore, inhibitors of guanine deaminase and PNP would not always inhibit direct guanosine deamination.

The activity of guanosine deaminase has been reported to be inhibited by mersalyl acid.[13] PNP is inhibited by 5-aminoimidazole-4-carboxamide and p-chloromercuribenzoic acid. Guanine deaminase is also inhibited by 5-aminoimidazole-4-carboxamide and p-chloromercuribenzoic. Allantoin and sodium fluoride have been described as inhibitors of guanine deaminase but not PNP.[21]

In this experiment both the colorimetric and HPLC methods described above for guanosine deaminase were utilised. To measure guanine deaminase activity the colorimetric method of Caraway[22] was used. Purine nucleoside phosphorylase activity was measured using the colorimetric assay developed by Chu, Cashion and Jiang.[23]

The phosphate buffer used to prepare substrates for guanosine deaminase, guanine deaminase and PNP was spiked with the inhibitors of these enzymes – mersalyl acid, 5-aminoimidazole-4-carboxamide, p-chloromercuribenzoic acid, allantoin and sodium fluoride. The inhibitors were added to a final concentration in the reaction mixture of 100 µmol/L. Liver extract (containing approximately 250 mg protein/100 mL) was incubated with each buffered substrate spiked with inhibitor, as well as unspiked substrate. Suitable reagent and serum blanks were also prepared. Enzymes activities were compared between inhibitor-spiked buffered substrate and non-spiked buffered substrate.

Table 1. Guanosine deaminase activity in tissue extracts estimated by measuring the amount of ammonia produced by colorimetric means.

Tissue	Guanosine deaminase U/100mg protein	Standard deviation
Liver	2.08	0.16
Kidney	1.53	0.14
Heart	0.14	0.014
Gall bladder	0.09	0.012
Intestine	0.077	0.01
Bladder	None detected	
Bone	None detected	

Results

Colorimetric estimation of ammonia

For easier comparison with published methods, the ammonia measured was expressed in terms of enzyme (guanosine deaminase) activity. The mean (guanosine deaminase) activity of the normal group (n=50) was 0.63 U/L (standard deviation [SD] 0.25 U/L). Mean intra-batch (n=30) enzyme (guanosine deaminase) activity was 0.54 U/L (SD 0.015 U/L [CV 2.7%]). The mean inter-batch (n=30) activity was 5.3 U/L (SD 0.027 U/L [CV 5.1%]). By using sera from both normal and diseased patients a spread of guanosine deaminase results between 0.1 U/L and 25 U/L were obtained.

In addition five separate extracts of each of liver, kidney, heart, gall bladder, bladder, intestine and bone were analysed after estimating their protein content. Extracts were diluted in distilled water to produce enzyme activity below the highest ammonia standard (5.4 U/L)

The results of the guanosine deaminase activities of the tissues are listed in Table 1. The means and the standard deviation of five samples of each, taken from five separate patients were calculated for each organ.

Guanosine deaminase activities estimated by HPLC

When the sera and tissue extracts were reanalysed by HPLC after incubation with guanosine, and the peaks obtained compared to the standard chromatogram (Fig. 2), it was found that only peaks corresponding to guanosine, xanthine and guanine were detected in all sera and tissue extracts. The product of guanosine deamination, xanthosine, was not present.

A typical HPLC trace of the enzymatic breakdown products of guanosine by serum is illustrated in Figure 3. A similar pattern was also found in the tissue extracts (no activity was detected in bone and bladder extracts).

The amount of ammonia produced in 0.25 mL of reaction

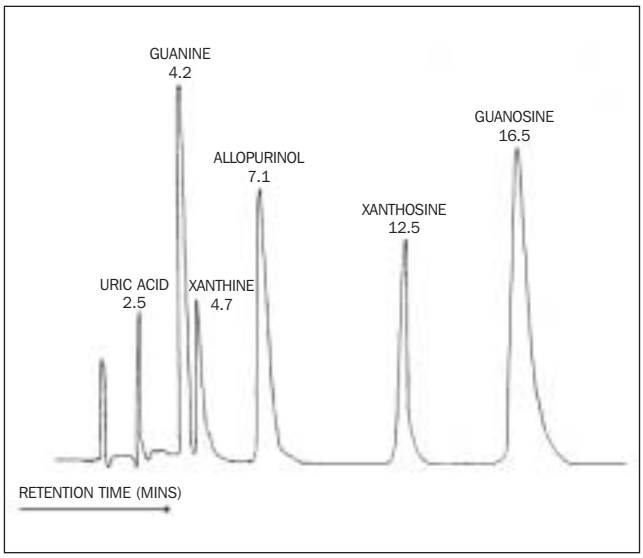

Fig. 2. Illustration of the high-performance liquid chromatogram of the standard solutions of guanosine and related substances. Retention times (min) are the mean of quintuplet analysis and are printed below the title of each peak.

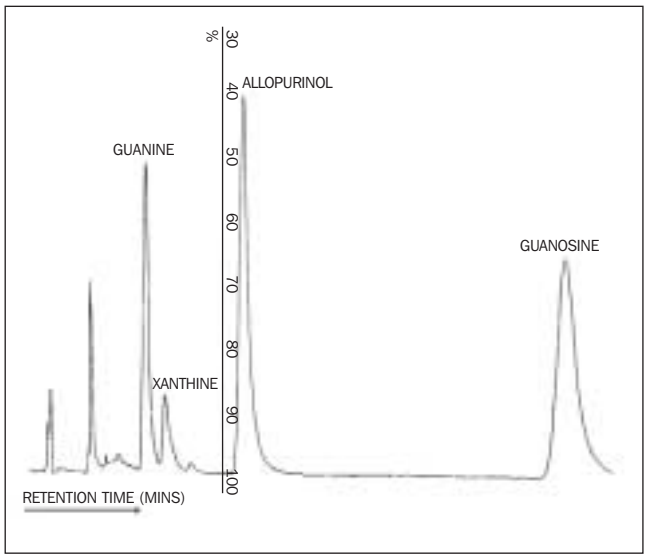

Fig. 3. Typical high-performance liquid chromatogram resulting from a separation of the products of the enzymatic breakdown of buffered guanosine.

mixture when sera were incubated for 4 h at 37°C with buffered guanosine was correlated with the amount of xanthine formed in the same reaction mixtures. The results are shown in Figure 4.

It was possible that other enzymes present in liver extract may degrade the xanthosine formed from the deamination of guanosine. However, when 0.15 mL 1.42 mmol/L xanthosine (pH 6.8) was incubated with 0.1 mL liver extract for 4 h at 37°C and the breakdown products measured by HPLC the reaction mixture still contained the same quantity of xanthosine as at the start of the reaction.

Change of enzyme activity

In order to examine the total products (and to ensure that no undetected product was formed) over a spread of enzyme activity levels, a series of liver extract dilutions containing from 38–228 mg protein/L were incubated with buffered guanosine. The ammonia produced was measured by colorimetry and the other products were measured by HPLC.

No xanthosine was detected in the reaction mixture. The amounts of guanine, xanthine and ammonia formed, as well as the total guanosine remaining at each concentration level, are listed in Table 2.

To ensure that no products were formed transiently, the reaction was examined over timed intervals. Diluted liver extract (0.5 mL, containing approximately 250 mg protein/100 mL) was incubated at 37°C with 0.75 mL 1.42 mmol/L buffered guanosine substrate. Samples (0.25 mL) were taken for HPLC analysis after 5, 30, 60 and 120 min. No xanthosine was detected in the reaction mixture during any of the time periods. A summation of all the products formed is provided in Figure 5.

Ammonium sulphate precipitation of liver extract protein

After incubation with guanosine, the fractions were measured by HPLC as described above and found to contain only guanine, xanthine and unconverted guanosine. The amount of PNP was calculated from the sum of guanine and xanthine produced while guanine deaminase was calculated

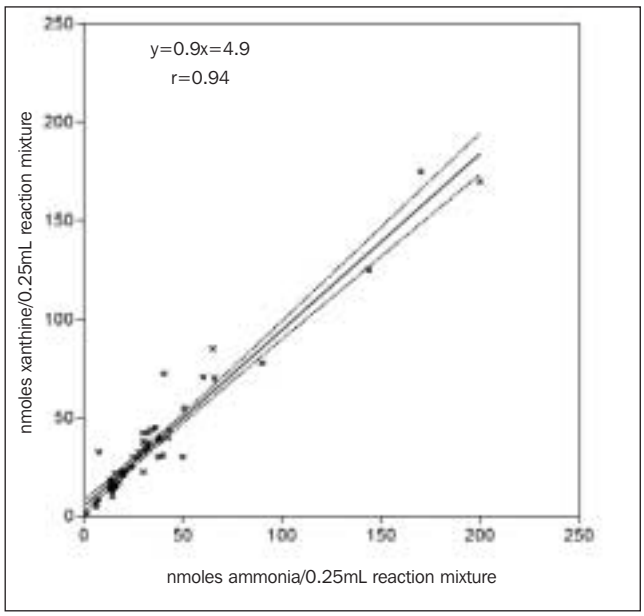

Fig. 4. Scattergraph to compare the xanthine formed measured by high performance liquid chromatography with the ammonia produced measured by colorimetry as a result of the incubation of both normal and abnormal sera with buffered guanosine.

from the amount of xanthine produced. The ammonium sulphate fractions were found to contain the enzyme activities shown in Table 3.

Inhibition studies

The results of inhibition, by the compounds studied, on the enzymatic deamination of guanosine (measured by colorimetry and HPLC), guanine deaminase and PNP are listed in Table 4. Mersalyl acid inhibited guanosine deamination when either the ammonia or xanthine was measured. HPLC showed that guanosine was broken down to guanine but that guanine was not further degraded to xanthine.

Table 2. Fate of the guanosine after differing concentrations of liver extract were incubated with buffered guanosine. Amounts of guanosine remaining and the total of products and substrates are also shown.

					Total guanine
Dilution of liver	Ammonia formed	Guanine formed	Xanthine formed	Guanosine remaining	xanthine and guanosine
extract	nmoles/0.25mL	nmoles/0.25mL	nmoles/0.25mL	nmoles/0.25mL	nmoles/0.25mL
1 in 10 (38mg protein/L)	11.5(1.25	42.5(1.5)	19.9(0.62)	160(5.00)	222(7.12)
2 in 10	23(1.75)	65.5(3.75)	26.3(1.25)	137(5.00)	229(10.0)
(78mg protein/L)					
4 in 10	46.9(2.5)	72.5(4.75)	52.5(2.00)	95.5(4.50)	221(11.3)
(158mg protein/L)					
6 in 10 (228mg protein/L)	76.2(3.25)	67.8(5.00)	81(3.00)	73.0(2.50)	222(10.5)
8 in 10	98.8(0.45)	55.3(4.5)	105(3.25)	59.5(2.25)	220(10.0)
(316mg protein/L)					
NEAT	121(5.50)	38.2(1.5)	125.3(4.00)	43.2(3.00)	207(8.50)
(380mg protein/L)					
(SD of quintiplet estimations)					

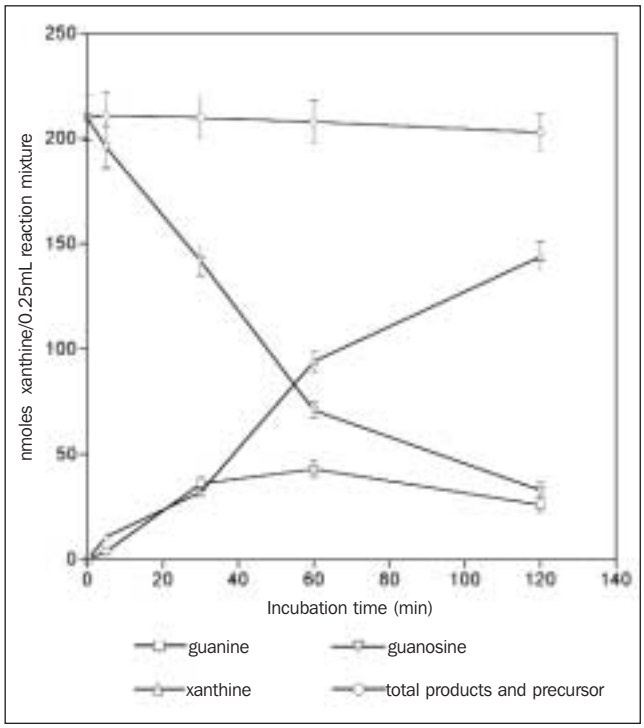

Fig. 5. Illustration of the breakdown products of buffered guanosine by human liver extract measured at time intervals.

In the examination of individual activities, PNP was not inhibited but guanine deaminase was inhibited by mersalyl acid. HPLC showed that p-chloromercuricbenzoic acid inhibited the breakdown of guanosine and thus no guanine was available for deamination to ammonia. Furthermore, no ammonia was detected by colorimetry. The p-chloromercuricbenzoic acid was also found to inhibit guanine deaminase and PNP when estimated individually. 5-aminoimidazole acted similarly to p-chloromercuricbenzoic acid. Allantoin did not inhibit any of the enzymes at the concentrations used.

Table 3. Distribution of enzyme activity in the ammonium sulphate fractions prepared by precipitation of liver extract.

Ammonium sulphate fraction %	Protein content %	Guanine deaminase %	PNP deaminase %	Guanosine %
Crude extract	100	100	100	0
0 - 20	14	1	2	0
20 - 43	32	14	12	0
43	42	75	72	0
Recovery	88	90	86	-

HPLC showed that sodium fluoride did not inhibit the breakdown to guanine but did inhibit deamination of guanine to xanthine. This was confirmed colorimetricaly by the absence of ammonia formation. Sodium fluoride also inhibited guanine deaminase but not PNP when estimated separately.

Discussion

When the colorimetric method of estimating guanosine deaminase was used, the activities found in normal human sera and post-mortem tissues agreed with previous reports.[17,18] This proved that the technique used and the assay conditions were in agreement with those reported earlier.

When the products of guanosine breakdown were examined by HPLC, no xanthosine was detected as a product of either serum or tissue degradation. In all cases (other than bone and bladder extracts, where no activity was detected) only guanine and xanthine were detected. The amount of ammonia present as a result of deamination correlated well with the amount of xanthine formed (r=0.94), implying that they were both products of the same reaction.

Table 4. Inhibition of guanosine deaminase, guanine deaminase and purine nucleoside phosphorylase. Guanosine deaminase inhibition was examined by measuring the ammonia produced using colorimetry and also the xanthine and guanine formed using HPLC The product measured are indicated in brackets.

Inhibitor	Guanosine deaminase (ammonia formed) colorimetric	Guanosine deaminase (xanthine formed) HPLC	Guanosine deaminase" (guanine formed) HPLC	Guanine deaminase activity colorimetric	Purine nucleoside phosphorylase activity colorimetric
mersalyl acid,	inhibited	inhibited	not inhibited	inhibited	not inhibited
5-aminoimidazole	inhibited	inhibited	inhibited	inhibited	inhibited
p-chloromercuric-	inhibited	inhibited	inhibited	inhibited	inhibited
benzoic acid					
allantoin	not inhibited	not inhibited	not inhibited	not inhibited	not inhibited
sodium fluoride	inhibited	inhibited	not inhibited	inhibited	not inhibited

When differing concentrations of liver extract were incubated with guanosine, it was found that the sum of guanine and xanthine detected equalled the amount of guanosine broken down at all concentrations. The breakdown of all the guanosine present in the substrate was accounted for and thus no other reactions took place. In addition, no enzyme was present in human liver extract that was able to break down xanthosine to xanthine under the conditions described. Thus, any xanthosine formed as a result of the presence of guanosine deaminase should be present in the reaction mixture.

When the reaction sequence was followed over a timed period, it was found that the linear breakdown of guanosine over the first 60 min was not reflected in the linearity of guanine and xanthine formation. This is likely to be a consequence of a precursor-product relationship, as a result of consecutive lag phases prior to the ultimate attainment of a steady state. More importantly, at no time was any xanthosine detected.

Thus, the ammonia measured by the colorimetric method purporting to be as a result of direct enzymatic breakdown of guanosine was likely to have been due to the deamination of the guanine, which was formed from guanosine.

The ammonium sulphate fractions were found to contain guanine deaminase and PNP, present mainly in the 43% supernatant. Lesser quantities of these enzymes were also found in the 20–43% fraction. These results are in agreement with those found in *P. convexa* by Isheda *et al*., except that no xanthosine was found in the products produced by the 20–43% fraction. Thus, this experiment did not detect the presence of guanosine deaminase in human liver extract as found in *P. convexa*

Inhibition studies show that, with the exception of allantoin, all the substances studied were found to inhibit guanosine deaminase, both when the reaction was studied by estimating the ammonia formed or by the formation of xanthine by HPLC. The inhibition correlated with that found for the individual enzymes, guanine deaminase and PNP.

In conclusion, degradation of guanosine was due to two enzymes – PNP (EC 2.4.15) and guanine deaminase (EC 3.5.4.3). The former degraded guanosine to guanine and the latter deaminated guanine to xanthine and ammonia. This reaction sequence agrees with the results of the study by Kalckar[10] in 1948 and those quoted in modern texts.[11]

This reaction sequence is in line with the guanosinuria and the hypouricaemia found in patients with severe PNP immunodeficiency,[24] and implies that guanosine cannot be broken down to uric acid via xanthine when PNP is absent.

These findings suggest an absence of guanosine deaminase in human metabolism because this enzyme would offer an alternative pathway for guanosine degradation, which would result instead in an accumulation of xanthosine as well as a hypouricaemia due to an inability to degrade xanthosine to xanthine □

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References

1 Jones DD, Bahijiri S, Roberts EL *et al*. Activity of serum cytidine deaminase during pregnancy. *Br J Obstet Gynaecol* 1982; **89**:314–7.

2 Okamura T, Kigasawa K. Re-evaluation of the colorimetric assay for cytidine deaminase *Prenat Diagn* 1994; **14**: 213–8.

3 Thompson PW, Jones DD, Currey HLF. Cytidine deaminase activity as a measure of acute inflammation in rheumatoid arthritis *Ann Rheum Dis* 1986; **45**:9–14.

4 Goldberg DM, Fletcher MJ, Watts C. Serum adenosine deaminase in hepatic disease *Clin Chim Acta* 1966; **14**: 720–8.

5 Giblett ER, Anderson JE, Cohen F *et al*. Adenosine deaminase in two patients with severely impaired cellular immunity. *Lancet* 1972; **ii**:1067–9.

6 Piras MA, Budroni M, Andreoni G. Adenosine deaminase in pleural effusion: an aid to diagnosis *BMJ* 1978; **2** (17): 51–2.

7 Delia S, Masteroianni CM, Massetti AP *et al*. Adenosine deaminase and acquired immunodifficiency syndrome *Clin Chem* 1987; **33**: 1675.

8 Ito M., Takaoka. T, Mori H, Teruo A. A sensitive new method for measurement of guanase with 8-azaguanine in bicine bis-hydroxy ethyl glycine buffer as substrate. *Clin Chim Acta* 1981; **115**: 135–44.

9 Jones W. On the physiological agents which are concerned in the nuclein fermentation, with special reference to four independant desamidases. *J Biol Chem* 1911; **ix**: 169–80.

10 Kalckar HM. Differential spectrophotometry of purine compounds by means of specific enzymes. *J Biol Chem*1947; **167**: 429–75.

11 Rodwell VW. In: *Harpers's biochemistry* (23rd edn). Connecticut: Appleton and Lange, 1993: 372.

12 Carver JD, Walker A.. The role of nucleotides in human nutrition. *Nutr Biochem* 1995; **6**: 58–72
13 Ishida Y, Shirafuji S, Kida M *et al*. Studies on guanosine degrading system in bacterial cell. Part III. Purification of guanosine daeminase. *Agr Biol Chem* 1969; **33**: 384–90.
14 Kaur S, Raman K, Pandey BR *et al*. Selective inhibition of xanthine oxidase by substituted pyridopyrimidines. *Res Commun Chem Pathol Pharmacol* 1978; **21**: 103–13.
15 Krawczyk B, Pertynska T, Torbus, B *et al*. Protein and lipoprotein fractions, cholesterol levels and guanosine deaminase in serum of women in early normal pregnancy. *Ginekol Pol* 1969; **40**(2):103–13.
16 Giorgelli F, Bottai C, Mascia L *et al*. Recycling of a-D-ribose 1-phosphate for nucleoside interconversion. *Biochem Biophys Acta* 1997; **1335**: 16–22.
17 Jones DD, Roberts EL, Davies AG. The estimation of serum guanosine deaminase in liver disease. *J Clin Chem Clin Biochem*1983; **21**: 835–40.
18 Kalkan A, Bulut V, Erel O *et al*. Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. *Mem Inst Oswaldo Cruz* 1999; **94**:383–6.
19 Sherwood RA. The measurement of nucleoside deaminases by high performance liquid chromatography and their use in clinical chemistry. *Biomed Chromatogr*1991; **5**: 235–9.
20 Varley H. *Practical clinical chemistry* (4th edn). London: Heinemann, 1969: 27.
21 Zillner H. In: *Handbook of enzyme inhibitors*. VCH, 1993: 208–436.
22 Caraway WT. Colorimetric determination of serum guanase activity. *Clin Chem* 1966; **12** (4).
23 Chu SY, Cashion P, Jiang M. A new colorimetric assay for purine nucleoside phosphorylase. *Clin Biochem* 1989; **22**: 357–62
24 Simmonds HA. In: Holton JB, ed. The inherited metabolic diseases (2nd edn). Edinburgh: Churchill Livingstone, 1994: 297–350.