# Chapter VI

## Studies on glutamine synthetase, glutamyltransferase, glutamic-pyruvic transaminase and phosphoglucose isomerase in rat liver

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Many of the enzymes investigated in this study have not been characterised in the present source, viz., rat liver. Attempts were made to achieve the partial purification and characterization of some of these, viz., glutamine synthetase, glutamyltransferase, glutamic-pyruvic transaminase, and phosphoglucose isomerase. The results of these studies are recorded in this chapter.

## Experimental

### Chemicals

Adenosine diphosphate was obtained from British Drug Houses Ltd., England, and p-chloromercury benzoate from L.Light and Co., Bucks, England. The sources of the other chemicals used were as mentioned in the previous chapter.

### Methods

Calcium phosphate gel was prepared according to the method of Keilin and Hartree (201) and had been aged for about a year when used.

The protein content of the different fractions was

determined after suitable dilution by the method of Warburg and Christian (173).

The procedures for the preparation of tissue homogenates and the determination of enzyme activity were the same as described in the preceding chapter.

In the case of glutamine synthetase, additional confirmation about the product formed was sought by chromatographic determination of glutamine content. Save in the following respects, the reaction mixture and procedure used for this purpose were the same as those described previously:

1. Ammonium chloride was substituted for hydroxylamine in the assay system.
2. The reaction was stopped by adding an equal volume of ethanol, the precipitated protein removed by centrifugation at 1500 x g for 15 minutes and the supernatant used for the qualitative detection of glutamine formed, using the circular paper chromatographic method previously described.

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Abbreviations used:

PCMB, p-chloromercury benzoate; CSH, Cysteine; ATP, adenosine triphosphate; ADP, adenosine diphosphate; PGI, phosphoglucose isomerase; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; GHA, glutamyl-hydroxymic acid; EDTA, ethylenediaminetetracetic acid.

(A)

## Glutamine synthetase

### Partial purification

All the operations described below were carried out at 0-5°.

The original tissue extract (6%) was recovered by centrifugation at 18400 x g for 30 minutes and brought to 25% saturation by adding 17.5 gm of solid ammonium sulphate per 100 ml. of the solution and the mixture allowed to stand for 10 minutes. The precipitate was removed by centrifugation at 4600 x g for 30 minutes and the supernatant brought to 35% saturation by adding a further 7.0 gm ammonium sulphate per 100 ml. of the solution. The resulting precipitate was removed by centrifugation at 12800 x g for 10 minutes, dissolved in 0.05 M bicarbonate, and made upto original volume. This procedure resulted in a three-fold purification, attempts at further purification being unsuccessful. 0.1 ml. of this extract was used without dialysis for the subsequent studies unless otherwise specified.

### Results

It can be seen from Tables 8-10 and Figures 5 and 6 that the amount of GHA formed is proportional to enzyme and

Table 8

Effect of enzyme concentration

| Enzyme added (ml) | GHA Formed (micromoles) |
|---|---|
| 0.025 | 0.20 |
| 0.050 | 0.41 |
| 0.100 | 0.82 |
| 0.150 | 0.85 |

Table 9

Effect of glutamate concentration

| Glutamate added (micromoles) | GHA formed (micromoles) |
|---|---|
| 6.25 | 0.20 |
| 12.50 | 0.41 |
| 25.00 | 0.82 |
| 50.00 | 1.00 |

Table 10

Effect of period of incubation

| Period of incubation (minutes) | GHA formed (micromoles) |
|---|---|
| 5.0 | 0.27 |
| 10.0 | 0.54 |
| 15.0 | 0.82 |
| 30.0 | 0.92 |

Table 11

Effect of hydroxylamine concentration

| $NH_2OH$ added (micromoles) | GHA formed (micromoles) |
|---|---|
| 5.0 | 0.20 |
| 10.0 | 0.39 |
| 20.0 | 0.56 |
| 50.0 | 0.82 |
| 60.0 | 0.87 |

Table 12

Effect of $MgSO_4$ concentration

| $MgSO_4$ added (micromoles) | GHA formed (micromoles) |
|---|---|
| 5.0 | 0.39 |
| 10.0 | 0.52 |
| 20.0 | 0.80 |
| 30.0 | 0.65 |

Table 13

Effect of cysteine concentration

| Cysteine added (micromoles) | GHA formed (micromoles) |
|---|---|
| 5.0 | 0.40 |
| 10.0 | 0.56 |
| 20.0 | 0.84 |
| 30.0 | 0.92 |

Table 14

Effect of ATP concentration

| ATP added (micromoles) | GHA formed (micromoles) |
|---|---|
| 0.4 | 0.23 |
| 0.8 | 0.39 |
| 1.6 | 0.64 |
| 2.0 | 0.80 |
| 4.0 | 0.69 |

Table 15

Effect of temperature

| Temperature (°C) | GHA formed (micromoles) |
|---|---|
| 30 | 0.25 |
| 40 | 0.48 |
| 45 | 0.73 |
| 50 | 0.80 |
| 55 | 0.42 |
| 60 | 0.11 |

Table 16

Effect of pH

| pH | GHA formed (micromoles) |
|---|---|
| 6.0 | 0.60 |
| 7.0 | 0.77 |
| 7.2 | 0.80 |
| 7.4 | 0.82 |
| 7.6 | 0.80 |
| 7.8 | 0.67 |
| 8.0 | 0.50 |
| 9.0 | 0.42 |
| 10.0 | 0.25 |

Table 17

Effect of aging

| Period of storage (days) | GHA formed (micromoles) |
|---|---|
| 0 | 0.82 |
| 3 | 0.75 |
| 10 | 0.48 |

Table 18

Effect of metal ions and inhibitors on GHA formation

| Component added | Concentration (micromoles) | Inhibition (%) |
|---|---|---|
| None | - | - |
| Manganese chloride | 10.0 | 94 |
| | 50.0 | 100 |
| Manganese chloride with $MgSO_4$ omitted | 10.0 | 94 |
| Aluminium sulphate | 2.0 | 84 |
| | 4.0 | 92 |
| | 10.0 | 94 |
| Calcium chloride | 10.0 | 100 |
| Ammonium sulphate | 10.0 | 84 |
| | 40.0 | 94 |
| Sodium arsenate | 5.0 | 50 |
| | 10.0 | 70 |
| | 20.0 | 82 |
| Sodium fluoride | 5.0 | 100 |
| Adenosine diphosphate | 1.0 | 84 |

Table 19

Dependence of GHA formation on individual components of the system

| Component omitted | GHA formed (micromoles) |
|---|---|
| Nil | 0.82 |
| Buffer | 0.79 |
| Glutamate | 0.06 |
| $NH_2OH$ | 0.02 |
| $MgSO_4$ | 0.06 |
| Cysteine | 0.14 |
| ATP | 0.06 |
| Enzyme | 0.08 |

STUDIES ON GLUTAMINE SYNTHETASE

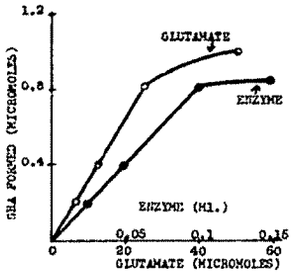


Fig. 5. Effect of enzyme and glutamate concentrations.

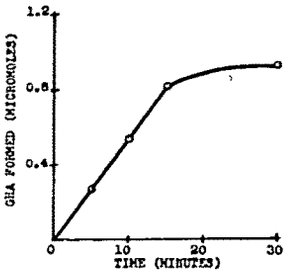


Fig. 6. Effect of period of incubation.

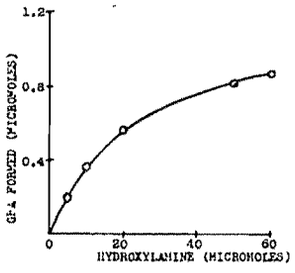


Fig. 7. Effect of hydroxylamine concentration.

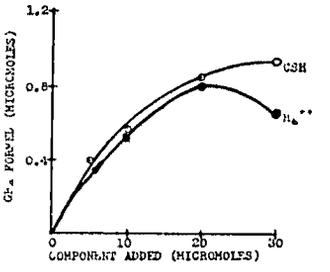


Fig. 8. Effect of magnesium sulphate and cysteine concentrations.

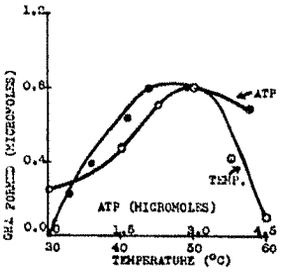


Fig. 9. Effect of ATP concentration and temperature.

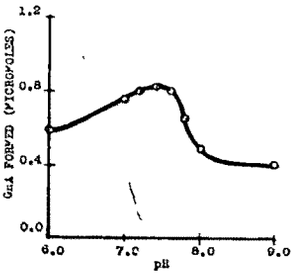


Fig. 10. Effect of pH.

glutamate concentrations and period of incubation.

Tables 11-13 and Figures 7 and 8 show the effect of varying the concentrations of hydroxylamine, $Mg^{++}$ and CSH on GHA formation. It can be seen that the enzyme is slightly inhibited by $Mg^{++}$ beyond a concentration of 20 micromoles.

Table 14 and Figure 9 show the effect of varying the concentration of ATP which is seen to have a slight inhibitory effect at higher concentrations. This may be due to the presence of trace amount of ADP in the preparation.

Table 15 and Figure 9 show the effect of varying the temperature on GHA formation. It is interesting to note that the formation of GHA increases with temperature upto $50^{o}$ and shows sharp decreases thereafter.

The optimum pH of the enzyme as seen from the Table 16 and Figure 10 is found to be between 7.2 and 7.6.

The effect of aging on glutamine synthetase activity is shown in Table 17 from which it is evident that storage at $-10^{o}$ for about 10 days results in a loss of about 42% of the original activity.

The enzyme activity is found to be inhibited by calcium and aluminium ions, ammonium sulphate, adenosine diphosphate and arsenate as shown in Table 18. It can also be seen from the table that $Mn^{++}$ cannot replace $Mg^{++}$ and that the addition of the former to the complete system results in a total suppression of GHA formation.

As is evident from Table 19, deletion studies made on individual components of the assay system showed that whereas the omission of the buffer merely resulted in a slight decrease in GHA formation, the omission of any other component resulted in a complete absence of the same.

Glutamine was confirmed to be the end product formed by paper chromatographic determination using $NH_4Cl$ in place of hydroxylamine in the assay system.

## Discussion

The enzyme glutamine synthetase has been isolated from a number of animal and plant sources (199,202-207). A comparison of the characteristics of the enzyme isolated from rat liver with those of the enzyme isolated from other sources shows several points of similarity.

The enzyme isolated from rat liver shows an optimal

activity between pH 7.2 and 7.6 and is, in this respect, similar to that isolated from green peas (204), hen heart (199) and guinea pig kidney (207). However, the optimum temperature for the activity of the enzyme in the present case is seen to be $50^{\circ}$, and in this respect it resembles that isolated from hen heart (199).

In its inhibition by calcium and activation by magnesium, this enzyme seems to be similar to that isolated from brain tissue. However, the equivalence of $Mn^{++}$ and $Mg^{++}$ for the activation of this enzyme which has been reported by some investigators (208,209) has not been verified in the present case. The former, on the contrary, is found to inhibit the enzyme.

(B)

## Glutamyltransferase

Partial purification

The method of preparation of cell-free extract was the same as that for synthetase except that 10% extracts were prepared in this case instead of 6%.

The cell-free extract (A) was brought to pH 5.0 by the addition of cold 0.2M HCl, care being taken to ensure that the above as well as the subsequent operations were carried out in the cold room maintained at $0^{\circ}$. The resulting precipitate was removed by centrifugation for 15 minutes at 4600 x g, dissolved in 0.05M bicarbonate and stood for two hours (B).

Fraction 'B' was then frozen overnight in a deep-freeze at $-20^{\circ}$C. The frozen mass was thawed and the resulting solution allowed to remain in cold room for about an hour. The denatured protein was removed by centrifugation and discarded. The supernatant was saved (C).

The supernatant fraction 'C' was mixed with half the volume of calcium phosphate gel (10 mg. dry material per ml.) and kept for 10 minutes with occasional stirring. Because of its unstability the supernatant recovered by centrifugation was saved and tested without dialysis (D).

Table 20

Summary of purification procedure

| | Step | Total Volume (ml) | Total protein mg. | Total Units | Units/mg Protein | Yield % |
|---|---|---|---|---|---|---|
| A | Cell-free extract | 64* | 1920 | 640 | 0.33 | 100 |
| B | pH 5.0 fraction | 64 | 832 | 588 | 0.7 | 46 |
| C | pH 5.0 supernatant | 62 | 150 | 213 | 1.4 | 34 |
| D | Calcium phosphate gel supernatant | 60 | 84 | 172 | 2.0 | 27 |

* From 7.5 g. wet weight of liver tissue.

Table 21

Effect of enzyme concentration

| Enzyme added (ml) | GHA formed (micromoles) |
|---|---|
| 0.025 | 0.20 |
| 0.05 | 0.46 |
| 0.1 | 0.90 |
| 0.2 | 1.63 |

Table 22

Effect of glutamine concentration

| Glutamine added (micromoles) | GHA formed (micromoles) |
|---|---|
| 10.0 | 0.21 |
| 20.0 | 0.43 |
| 30.0 | 0.66 |
| 40.0 | 0.90 |
| 60.0 | 1.13 |

Table 23

Effect of period of incubation

| Period of incubation (minutes) | GHA formed (micromoles) |
|---|---|
| 7.5 | 0.23 |
| 15.0 | 0.48 |
| 30.0 | 0.92 |
| 45.0 | 1.12 |

Table 24

Effect of hydroxylamine concentration

| Hydroxylamine added (micromoles) | GHA formed (micromoles) |
|---|---|
| 2.5 | 0.28 |
| 5.0 | 0.57 |
| 10.0 | 0.92 |
| 20.0 | 0.60 |
| 40.0 | 0.37 |
| 60.0 | 0.23 |

Table 25

Effect of ATP concentration

| ATP added (micromoles) | GHA formed (micromoles) |
|---|---|
| 0.1 | 0.60 |
| 0.2 | 0.71 |
| 0.8 | 0.86 |
| 1.0 | 0.94 |
| 2.0 | 0.62 |
| 4.0 | 0.56 |
| 6.0 | 0.16 |

Table 26

Effect of $MnCl_2$ concentration

| $MnCl_2$ added (micromoles) | GHA formed (micromoles) |
|---|---|
| 2.0 | 0.36 |
| 5.0 | 0.89 |
| 6.0 | 0.80 |
| 8.0 | 0.69 |
| 10.0 | 0.60 |
| 15.0 | 0.48 |

Table 27

Effect of pH

| pH | GHA formed (micromoles) |
|---|---|
| 4.2 | 0.64 |
| 4.4 | 0.72 |
| 4.6 | 0.80 |
| 5.0 | 0.92 |
| 5.2 | 0.72 |
| 5.4 | 0.52 |

Table 28

Effect of temperature

| Temperature (°C) | GHA formed (micromoles) |
|---|---|
| 30 | 0.70 |
| 35 | 0.80 |
| 37 | 0.92 |
| 40 | 0.90 |
| 45 | 0.74 |
| 50 | 0.51 |
| 60 | 0.12 |

Table 29

Comparative effect of manganese and magnesium ions on the formation of GHA

| $MgSo_4$ added (micromoles) | $MnCl_2$ added (micromoles) | GHA formed (micromoles) |
|---|---|---|
| - | 5.0 | 0.92 |
| 5.0 | 5.0 | 0.42 |
| 1.0 | - | 0.03 |
| 5.0 | - | 0.03 |

Table 30

Dependence of GHA formation on individual components of the system

| Component omitted | GHA formed (micromoles) |
|---|---|
| Nil | 0.92 |
| Glutamine | 0.04 |
| $NH_2OH$ | 0.09 |
| $MnCl_2$ | 0.05 |
| ATP | 0.05 |
| Enzyme | 0.0 |

Table 31

Effect of metal ions, inhibitors and activators

| Addition | Concentration (micromoles) | GHA formed (micromoles) | Inhibition (%) |
|---|---|---|---|
| None | - | 0.92 | 0 |
| Sodium fluoride | 1.0 | 0.60 | 35 |
| | 5.0 | 0.46 | 50 |
| Phosphate (as phosphate buffer) | 4.0 | 0.97 | 0 |
| Sodium pyrophosphate | 1.0 | 0.20 | 78 |
| | 5.0 | 0.04 | 96 |
| Calcium chloride | 10.0 | 0.13 | 86 |
| | 20.0 | 0.02 | 98 |
| Aluminium sulphate | 1.0 | 0.23 | 75 |
| | 5.0 | 0.06 | 93 |
| Ammonium sulphate | 10.0 | 0.42 | 54 |
| | 50.0 | 0.09 | 90 |
| Potassium cyanide | 5.0 | 0.74 | 20 |
| PCMB | 0.05 | 0.13 | 86 |
| | 0.25 | 0.09 | 90 |
| PCMB + cysteine | 0.05 + 10 | 0.90 | 0 |
| Adenosine diphosphate | 0.5 | 1.09 | 0 |
| | 1.0 | 0.97 | 0 |
| | 2.0 | 0.74 | 20 |
| Sodium arsenate | 0.1 | 1.09 | 0 |

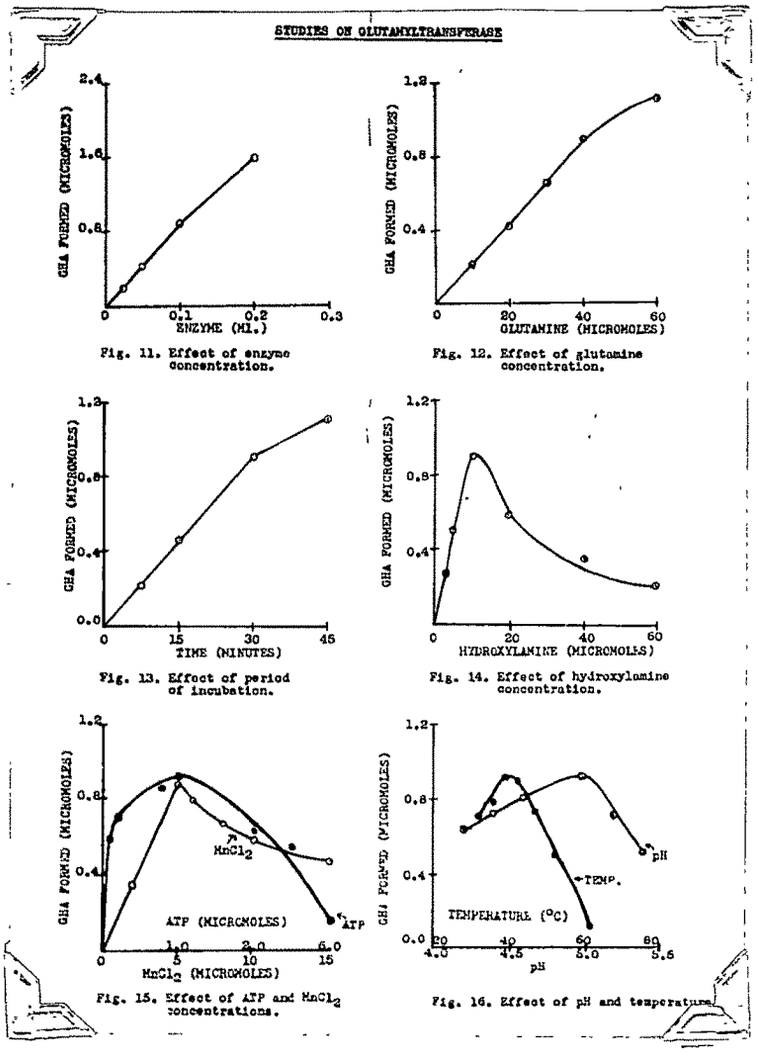


Fig. 11. Effect of enzyme concentration.

Fig. 12. Effect of glutamine concentration.

Fig. 13. Effect of period of incubation.

Fig. 14. Effect of hydroxylamine concentration.

Fig. 15. Effect of ATP and $MnCl_2$ concentrations.

Fig. 16. Effect of pH and temperature.

The effect of varying the temperature on GHA formation is shown in Table 28 and Figure 16 from which it can be seen that the formation of GHA increases with temperature upto $37^{o}$ and decreases at higher temperatures.

The effect of addition of $Mg^{++}$ on the formation of GHA is shown in Table 29. It is found that the substitution of $Mg^{++}$ for $Mn^{++}$ not only fails to activate the enzyme but also inhibits it.

Table 30 shows the effect of omission of individual components from the assay system on GHA formation. It can be seen that the deletion of any component results in the formation of negligible amounts of GHA.

The effect of addition of various inhibitors and activators on GHA formation is shown in Table 31. Pyrophosphate, $Ca^{++}$, $Al^{+++}$, ammonium sulphate and PCMB are found to have a marked inhibitory effect, and fluoride and cyanide, to a lesser extent. However, the inhibition caused by PCMB is completely reversed by the addition of cysteine. Adenosine diphosphate and arsenate are found to have a stimulatory effect.

## Discussion

The enzyme glutamyltransferase has also been isolated from other animal and plant sources (204,210-213). A comparison of the characteristics of the enzyme isolated from rat liver tissue with those of the enzyme isolated from other sources shows several points of similarity.

In its pH requirement of about 5.0, the enzyme is, similar to that isolated from brain with an optimal pH of about 5.5 (212). In its metal ion requirement, the enzyme is different from those isolated from microorganisms in that the latter do not require metallic ion. In its requirement of $Mn^{++}$ and inhibition by $Mg^{++}$ and in regard to its optimum temperature the enzyme contrasts sharply with glutamine synthetase. It is pertinent to point out in this context that although the same enzyme has been hypothesized to catalyse both the synthesis and transfer reactions, recent findings indicate that glutamyltransferase in Mycobacterium pheli is not endowed with glutamine synthetase activity (214).

In its inhibition by hydroxylamine, this enzyme seems to be similar to that isolated from brain. Inhibition by PCMB and reversal of the same by cysteine suggest it to be a sulphhydryl enzyme.

(C)

## Glutamic-pyruvic transaminase

Partial purification

All operations described below were carried out at 0-5°. The method of preparation of cell-free extract was the same as that for glutamyltransferase.

The cell-free extract (A) was brought to 30% saturation with solid ammonium sulphate (21.0 gm per 100 ml.) and allowed to stand for 15 minutes. After removal of the precipitate by centrifugation at 12800 x g for 15 minutes, the supernatant was brought to 40% saturation by adding a further 7.0 gm of ammonium sulphate per 100 ml. of the original solution. The resulting precipitate removed by centrifugation at 4500 x g for 15 minutes was dissolved in 0.05M bicarbonate and made upto the original volume (B).

The above fraction 'B' was then mixed with equal volume of calcium phosphate gel (10 mg. dry material per ml.) and allowed to stand for 15 minutes with occasional stirring. The supernatant recovered by centrifugation was saved and tested (C). 0.2 ml. of this fraction was used for the subsequent studies unless otherwise stated.

## Results

The purification of glutamic-pyruvic transaminase is given in Table 32 from which it can be seen that the purification procedure resulted in nearly a 12-fold increase in specific activity.

It can be seen from Tables 33 and 34 and Figures 17 and 18 that the amount of alanine formed is proportional to enzyme concentration and period of incubation.

Table 35 and Figure 19 show the effect of varying the concentration of glutamate on alanine formation. The optimum concentration of glutamate is seen to be 20 micromoles.

Table 36 and Figure 20 show the effect of varying the temperature on alanine formation. The optimum temperature is seen to be around $60^{\circ}$.

Table 37 and Figure 21 show the effect of varying the pH on alanine formation. The optimum pH is found to be near 7.8.

The effect of various inhibitors, activators, and metal ions is shown in Table 38, from which it can be seen that the enzyme is not activated by metallic ions and is

Table 32

Summary of purification procedure

| Purification step | Total volume (ml) | Total protein (mg) | Total units | Specific activity (units/ mg. protein) | Yield % |
|---|---|---|---|---|---|
| (A) Cell-free extract | 58.0* | 2030 | 2436 | 1.2 | 100 |
| (B) $(NH_4)_2SO_4$ fraction 30-40% | 58.0 | 295 | 1113 | 3.7 | 46 |
| (C) Calcium phosphate gel supernatant | 56.8 | 85 | 1226 | 14.4 | 50 |

* From 7.0 g. wet weight of liver tissue.

Table 33

Effect of enzyme concentration

| Enzyme added (ml) | Alanine formed (micromoles) |
|---|---|
| 0.025 | 0.48 |
| 0.050 | 1.08 |
| 0.100 | 2.28 |
| 0.200 | 4.32 |
| 0.300 | 5.16 |

Table 34

Effect of period of incubation

| Period of incubation (minutes) | Alanine formed (micromoles) |
|---|---|
| 7.5 | 0.96 |
| 15 | 2.04 |
| 30 | 4.32 |
| 45 | 4.80 |
| 60 | 5.40 |

Table 35

Effect of glutamic acid concentration

| Glutamate added (micromoles) | Alanine formed (micromoles) |
|---|---|
| 2.5 | 0.60 |
| 5.0 | 1.20 |
| 10.0 | 2.16 |
| 20.0 | 4.44 |
| 30.0 | 5.04 |

Table 36

Effect of temperature

| Temperature (°C) | Alanine formed (micromoles) |
|---|---|
| 30 | 4.32 |
| 35 | 4.80 |
| 40 | 5.64 |
| 45 | 6.00 |
| 50 | 6.60 |
| 60 | 7.80 |
| 70 | 1.80 |

Table 37

Effect of pH

| pH | Alanine formed (micromoles) |
|---|---|
| 6.4 | 1.80 |
| 6.8 | 2.90 |
| 7.0 | 4.32 |
| 7.4 | 4.80 |
| 7.6 | 5.20 |
| 7.8 | 5.76 |
| 8.0 | 5.20 |
| 9.0 | 4.60 |
| 10.0 | 1.60 |

Table 38

Effect of metal ions, pyridoxal phosphate, dicarboxylic acids and other inhibitors

| Addition | Concentration (micromoles) | Alanine formed (micromoles) | Percentage of the original activity |
|---|---|---|---|
| Control | - | 2.79 | 100.0 |
| PCMB | 0.1 | 0.12 | 4.2 |
| PCMB + Cysteine | 0.1 + 10.0 | 2.20 | 89.7 |
| Cysteine | 20.0 | 1.09 | 39.0 |
| KCN | 10.0 | 2.25 | 80.6 |
| Pyridoxal phosphate | 0.1 | 2.09 | 110.7 |
| $MgSO_4$ | 10.0 | 2.79 | 100.0 |
| $MnCl_2$ | 10.0 | 2.60 | 93.1 |
| $NH_2OH$ | 2.0<br>5.0 | 1.68<br>1.38 | 60.2<br>49.4 |
| Succinate | 30.0 | 2.40 | 86.0 |
| Fumarate | 30.0 | 2.40 | 86.0 |
| Malate | 30.0 | 2.40 | 86.0 |

* One day old enzyme fraction C was used.

STUDIES ON GLUTAMIC-PYRUVIC TRANSAMINASE

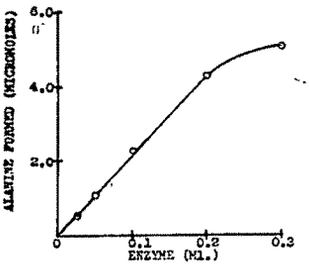


Fig. 17. Effect of enzyme concentration.

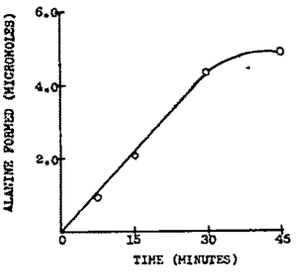


Fig. 18. Effect of period of incubation.

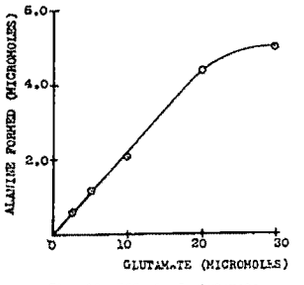


Fig. 19. Effect of glutamate concentration.

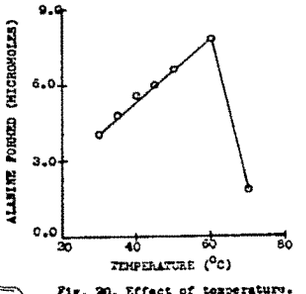


Fig. 20. Effect of temperature.

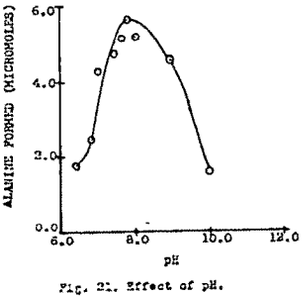


Fig. 21. Effect of pH.

inhibited to a slight extent by $Mn^{++}$. PCMB strongly inhibits the enzyme, this inhibition being reversed by cysteine. The latter, however, shows an inhibitory effect when used singly at higher concentrations. Cyanide is also found to inhibit the enzyme but to a lesser extent. The enzyme shows a marked inhibition by hydroxylamine and to a lesser extent by dicarboxylic acids. On the other hand, pyridoxal phosphate at a very low concentration is found to result in slight activation. The enzyme preparation shows considerable decrease in activity on dialysis.

## Discussion

The enzyme, glutamic-pyruvic transaminase, of high activity has been prepared from pig heart by a number of workers (215, 217).

A comparison of the characteristics of the enzyme isolated from rat liver with transaminases isolated from other sources shows some points of similarity. The enzyme isolated in the present case shows an optimum activity at about 7.8 pH, which lies within the range of 7.5 reported generally for other animal transaminases, and 8.0 reported for bacterial transaminases (218-220).

The optimum temperature of 60° would appear to suggest the stability of this enzyme at relatively high temperatures. However, the enzyme is seen to show a fall in activity at higher temperatures. In this connection, it is interesting to note that the enzyme glutamic-aspartic transaminase isolated from pig heart was found to show no impairment in activity on heating at 60° for as long as one hour (216).

In its inhibition by hydroxylamine, it resembles animal and bacterial transaminases and other $B_6$-catalysed reactions (221,222). A similar inhibition has also been observed with regard to other transaminases (223,224).

The inhibition by PCMB and reversal of the same by cysteine show the enzyme to be sulphhydral. The absence of marked activation by pyridoxal phosphate shows that it is not dissociated during purification and is bound firmly to the apoenzyme. However, the absolute requirement of this vitamin cannot be estimated till the enzyme is resolved.

(D)

## Phosphoglucose isomerase

Partial purification

The method of preparation of cell-free extract was the same as that for synthetase except that 1% extracts were prepared in this case. All operations described below were carried out at 0-5°.

The cell-free extract (A) was brought to 50 per cent saturation with solid ammonium sulphate (35 gm/100 ml.) and allowed to stand for 15 minutes. The precipitate removed by centrifugation was discarded and the supernatant brought to 65% saturation by adding a further 10.5 gm. of ammonium sulphate per 100 ml. The resulting precipitate was removed by centrifugation at 12800 x g for 15 minutes, dissolved in 0.05M bicarbonate and made to original volume (B).

The ammonium sulphate fraction 'B' was mixed with half the volume of calcium phosphate gel (10 mg. dry material per ml.), stood for 15 minutes and centrifuged, the resulting supernatant being discarded. The protein adsorbed on the gel was eluted by an equal volume of 0.05M bicarbonate for 10 minutes and the supernatant recovered

by centrifugation saved and tested without dialysis (C).

0.2 ml. of fraction 'B' was used for the characterization studies unless otherwise specified.

## Results

The purification of phosphoglucose isomerase is given in Table 39, from which it can be seen that the purification procedure resulted in a nearly 20-fold increase in specific activity.

It can be seen from Tables 40-42 and Figures 22-24 that fructose-6-phosphate formed is proportional to enzyme and substrate concentrations and period of incubation.

Table 43 and Figure 25 show the effect of varying the pH on fructose-6-phosphate formation. The enzyme shows a wide range of activity with a broad optimum pH between 7.8 and 8.5.

Table 44 and Figure 26 show the effect of varying the temperature on fructose-6-phosphate formation. The optimum temperature is seen to be 40°.

The effect of various metal ions and inhibitors is shown in Table 45. None of the metal ions used is found to activate the enzyme which is markedly inhibited by $Zn^{++}$ and $Ca^{++}$. The enzyme is also inhibited by cyanide and

Table 39

Summary of purification procedure

| Purification step | Total volume (ml) | Total protein (mg.) | Total units | Specific activity (units per mg. protein) | Yield % |
|---|---|---|---|---|---|
| (A) Cell-free extract | 98* | 294 | 5390 | 18.3 | 100. |
| (B) Ammonium sulphate fraction 50-65% | 98 | 16 | 1960 | 122.5 | 36 |
| (C) Calcium phosphate gel eluate | 96 | 2 | 720 | 365.0 | 13 |

* From 1.0 gm wet weight of liver tissue.

Table 40

Effect of enzyme concentration

| Enzyme added (ml) | F-6-P Formed (micromoles) |
|---|---|
| 0.05 | 0.083 |
| 0.10 | 0.166 |
| 0.15 | 0.255 |
| 0.20 | 0.356 |
| 0.30 | 0.398 |

Table 41

Effect of glucose-6-phosphate concentration

| Glucose-6-phosphate added (micromoles) | F-6-P Formed (micromoles) |
|---|---|
| 0.5 | 0.085 |
| 1.0 | 0.180 |
| 2.0 | 0.356 |
| 2.5 | 0.485 |

Table 42

Effect of period of incubation

| Period of incubation (minutes) | F-6-P Formed (micromoles) |
|---|---|
| 1.25 | 0.082 |
| 2.50 | 0.174 |
| 5.00 | 0.356 |
| 7.50 | 0.398 |
| 10.00 | 0.420 |
| 15.00 | 0.439 |

Table 43

Effect of pH

| pH | F-6-P Formed (micromoles) |
|---|---|
| 6.0 | 0.300 |
| 6.4 | 0.330 |
| 7.0 | 0.356 |
| 7.4 | 0.446 |
| 7.8 | 0.464 |
| 8.0 | 0.489 |
| 8.5 | 0.498 |
| 9.0 | 0.307 |
| 10.0 | 0.307 |

Table 44

Effect of temperature

| Temperature (°C) | F-6-P Formed (micromoles) |
|---|---|
| 20 | 0.091 |
| 30 | 0.356 |
| 35 | 0.398 |
| 40 | 0.514 |
| 45 | 0.498 |
| 50 | 0.398 |
| 60 | 0.024 |

Table 45

Effect of metal ions and inhibitors

| Addition | Concentration (micromoles) | F-6-P Formed (micromoles) |
|---|---|---|
| Control | - | 0.356 |
| Magnesium sulphate | 10.0 | 0.343 |
| Magnesium chloride | 10.0 | 0.340 |
| | 20.0 | 0.348 |
| Zinc sulphate | 10.0 | 0.249 |
| Potassium chloride | 10.0 | 0.365 |
| Calcium chloride | 10.0 | 0.290 |
| Ethylenediamine-tetracetic acid | 10.0 | 0.348 |
| Potassium ferricyanide | 10.0 | 0.340 |
| Sodium-pyrophosphate | 10.0 | 0.356 |
| Cysteine | 5.0 | 0.323 |
| Potassium cyanide | 10.0 | 0.307 |

STUDIES ON PHOSPHOGLUCOSE ISOMERASE

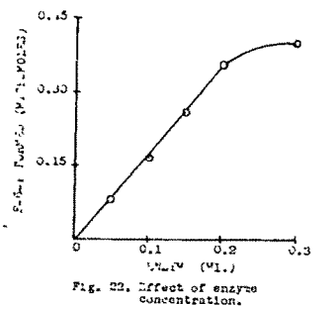


Fig. 22. Effect of enzyme concentration.

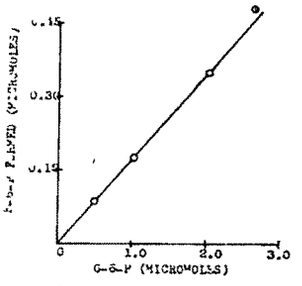


Fig. 23. Effect of glucose-6-phosphate concentration.

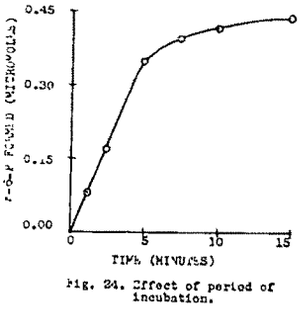


Fig. 24. Effect of period of incubation.

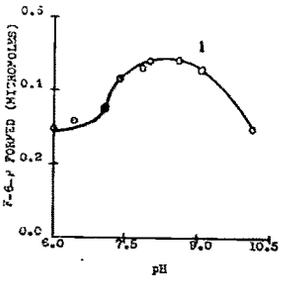


Fig. 25. Effect of pH.

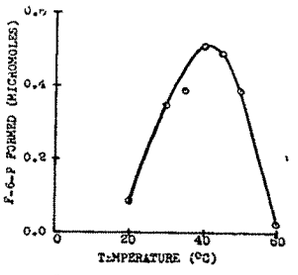


Fig. 26. Effect of temperature.

cysteine but to a lesser extent.

## Discussion

The properties of phosphoglucose isomerase isolated from liver are in general similar to those reported for the enzyme from other sources (177,225,226).

In its non-inhibition by phosphate the enzyme resembles that isolated from A.Niger (227) and differs from muscle isomerase, resembling the latter, however, in its non-activation by metallic ions. The wide pH range (7.8-8.5) in which the enzyme shows optimal activity contrasts with the optimal pH of 9.0 reported generally for the muscle enzyme, although that isolated from Lingcod muscle is found to have a much broader range (228).