PART III

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PURIFICATION OF GLUTAMATE DEHYDROGENASE FROM RAT BRAIN AND LIVER

PURIFICATION OF GLUTAMATE DEHYDROGENASE FROM RAT BRAIN AND LIVER

The most interesting observation that emerged from the studies described earlier was that brain glutamate dehydrogenase and glutamate decarboxylase were affected by a dietary deficiency of protein or vitamin A.

The finding on brain glutamate dehydrogenase was of particular interest as liver glutamate dehydrogenase did not show a similar decrease. It also appeared on the basis of some studies that brain glutamate dehydrogenase had a greater sensitivity to nutritional deficiency than glutamate decarboxylase. Further it has been suggested that the characteristics of glutamate dehydrogenase vary in different tissues. Attempts were therefore made to partially purify this enzyme from the liver and brain and compare their characteristics. Studies were also made of the effects of selected tranquilisers on enzyme activity as tranquilisers are believed to affect the CNS and as such their action in the liver and brain enzyme may not be identical.

PROCEDURE

The basis of the method used for the purification of glutamate dehydrogenase from brain and liver was essentially the same as that employed by Parekh and Sakariah (1971) for the purification of glutamate dehydrogenase from Citrus acida.

The method involves the fractionation with ammonium sulphate, adsorption and elution on calcium phosphate gel and DEAE cellulose.

The protein of the different fractions was estimated by the method of Lowry, Rosebrough, Farr and Randall (1951).

Purification from brain: All the operations described below were carried at 0-1°. A 10% homogenate was prepared in 0.05M potassium phosphate buffer, pH 7.5, by homogenizing in a Potter-Elvehjem homogenizer for 60 seconds at 0° at 4000 rpm. Triton X-100 was added at a level of 2.5 mg/ml to the homogenate, allowed to remain for 30 minutes and centrifuged at 8000 x g for one hour. The supernatant obtained (A) was used for further purification.

Fifty ml of the fraction A were brought to 30% saturation with solid ammonium sulfate (21 g/100 ml) and allowed to stand for 10 minutes with gentle stirring. The precipitate formed was removed by centrifugation at 8000 x g for 10 minutes and the supernatant brought to 50% saturation by adding further 14 g of ammonium sulphate per 100 ml of solution. The resulting precipitate was removed by centrifugation at 8000 x g at 0° for 10 minutes and dissolved in 0.02M potassium phosphate buffer, pH 7.0, to a final volume of 50 ml. This solution was dialysed for 6 hours against 4 litres of 0.02M potassium phosphate buffer, pH 7.0 (Fraction B).

Fifty ml of fraction B were added to the residue obtained after the centrifugation of 25 ml calcium phosphate gel (dry weight, 11 mg per ml) at 6000 x g for 10 minutes. The mixture was allowed to stand for 10 minutes with gentle stirring and the resulting solution centrifuged at 6000 x g for 15 minutes and the supernatant removed. The residue was eluted with 50 ml of 0.2M potassium phosphate buffer, pH 7.0. The eluate was dialysed against 6 litres of 0.005M potassium phosphate buffer, pH 7.0 for 6 hours (Fraction C).

Fifteen ml of fraction C were further fractionated using DEAE cellulose column which was activated as follows. Fifteen g of DEAE-cellulose were shaken gently for five minutes with 200 ml of 1N HCl, allowed to settle down and the supernatant decanted. This was repeated twice. The acid washed resin was then activated with successive additions of 500 ml of 1N NaOH and shaking each time for 5 minutes. A total of 2 litres of 1N NaOH was used for the activation of 15 g of the resin. The activated resin was washed free of excess alkali with distilled water. To the activated DEAE-cellulose a sufficient quantity of 0.02M potassium phosphate buffer, pH 7.0 was added so as to form a thin slurry. The slurry was then added to a 50 ml burette of 0.8 cm diameter in which the bottom was plugged with glass wool so as to form a column of 25 cm in length when settled. The column was equilibrated with 50 ml of 0.02M potassium phosphate buffer, pH 7.0. Fifteen ml of fraction C were introduced and allowed to pass through the column. The rate of flow of the solution was adjusted to 1 ml per minute. After all the enzyme had passed through, the column was washed

successively with 100 ml each of 0.02M, 0.05M and 0.1M potassium phosphate buffer, pH 7.0 and the eluates collected separately each time. Fractions of 15 ml each were collected manually. The different fractions were estimated for glutamate dehydrogenase activity and the second fraction obtained with 0.1M potassium phosphate buffer was found to show enzyme activity (Fraction D).

Purification from liver: The general procedure for the purification of liver glutamate dehydrogenase was the same as that for brain glutamate dehydrogenase except that equal volume of calcium phosphate gel was used and 0.1M potassium phosphate buffer was used for elution of the enzyme from the gel and 10 ml DEAE fractions were collected. The third and fourth fractions obtained with 0.1M potassium phosphate buffer were found to show enzyme activity.

Details of Enzyme assay: Except for the variations specified in the appropriate context, the assay system used in the studies on the purified enzymes are given below:

	brain GDH	liver GDH
buffer	Tris-HCl pH 8.0,20 µmoles	potassium phosphate pH 7.5, 50 µmoles
substrate	0.2 µmoles	0.2 µmoles
$(NH_4)_2SO_4$	100.0 µmoles	150.0 µmoles
$NADH_2$	0.1 µmole	0.1 µmole
enzyme	0.3 ml (10.5 mcg protein)	0.3 ml (9.78 mcg protein)
period of incubation	90 seconds	90 seconds
initiation of reaction	2-oxoglutarate added	2-oxoglutarate added
treatment of blank	2-oxoglutarate omitted	2-oxoglutarate omitted
parameter measured	oxidation of $NADH_2$ measured by reduction in optical density at 340 mµ at 30 seconds interval	oxidation of $NADH_2$ measured by reduction in optical density at 340 mµ at 30 seconds interval

RESULTS

The yield of glutamate dehydrogenase and degree of purification achieved in the different fractions are

Table 23: Purification of glutamate dehydrogenase from the rat liver and brain

fraction assayed	total volume (ml)		total protein (mg)		total activity (units)*		specific activity (units/mg protein)		activity yield (per cent)		protein recovery (per cent)	
	brain	liver	brain	liver	brain	liver	brain	liver	brain	liver	brain	liver
A. crude extract	50	50	380	875	15	53	0.04	0.06	100	100	100	100
B. ammonium sulphate fraction (30-50% saturation)	50	50	85	600	15	45	0.18	0.08	100	85	22	69
C. calcium phosphate gel eluate	50	50	25	200	15	45	0.60	0.23	100	85	7.0	23
D. DEAE-cellulose eluate**	50	50	1.8	8.7	6.0	40	3.4	4.6	40	75	0.5	1.0

*amount of enzyme which catalyses the oxidation of 1 micromole of $NADH_2$ in one minute.
**the values are extrapolated for 50 ml of original solution.

shown in table 23. The specific activity of glutamate dehydrogenase in the DEAE cellulose eluate is seen to increase to 85 and 77 fold over that of the crude extract for brain and liver glutamate dehydrogenase respectively. This increase is accompanied by a loss of 60 per cent and 25 per cent in total activity for brain and liver glutamate dehydrogenase respectively.

The effects of varying enzyme concentration, period of incubation, substrate concentration, pH and buffer ion concentration on the activity of brain and liver glutamate dehydrogenase are shown in tables 24, 25, 26, 27 and 28. Enzyme activity was found to be proportional to enzyme concentration, period of incubation and 2-oxoglutarate concentration within a certain range (Figs. 1, 2 and 3). The pH optimum was found to be 8.0 for brain and 7.5 for liver glutamate dehydrogenase (Fig. 4). The km for 2-oxoglutarate was found to be 7.3×10^{-4}M and 1.3×10^{-3}M for brain and liver glutamate dehydrogenase respectively.

Enzyme activity increased proportionately with $NADH_2$ concentration upto 0.1 µmole in both cases (Fig.5, Table 29). Higher concentrations of $NADH_2$ were found to be inhibitory in both the cases. The km for $NADH_2$ was found to be 3.7×10^{-3}M and 4.7×10^{-3}M for brain and liver glutamate dehydrogenase respectively. The

Table 24: Effects of variations in enzyme concentration on glutamate dehydrogenase activity in the rat brain and liver

brain GDH		liver GDH	
enzyme added (mcg of protein)	$NADH_2$ oxidised (micromole)	enzyme added (mcg of protein)	$NADH_2$ oxidised (micromole)
0	0	0	0
1.75	0.009	1.63	0.011
3.50	0.018	3.26	0.023
5.25	0.028	6.52	0.045
7.00	0.036	9.78	0.068
10.50	0.054	13.04	0.072
14.00	0.060	-	-

Effects of variations in enzyme concentration on glutamate dehydrogenase activity in the rat brain and liver

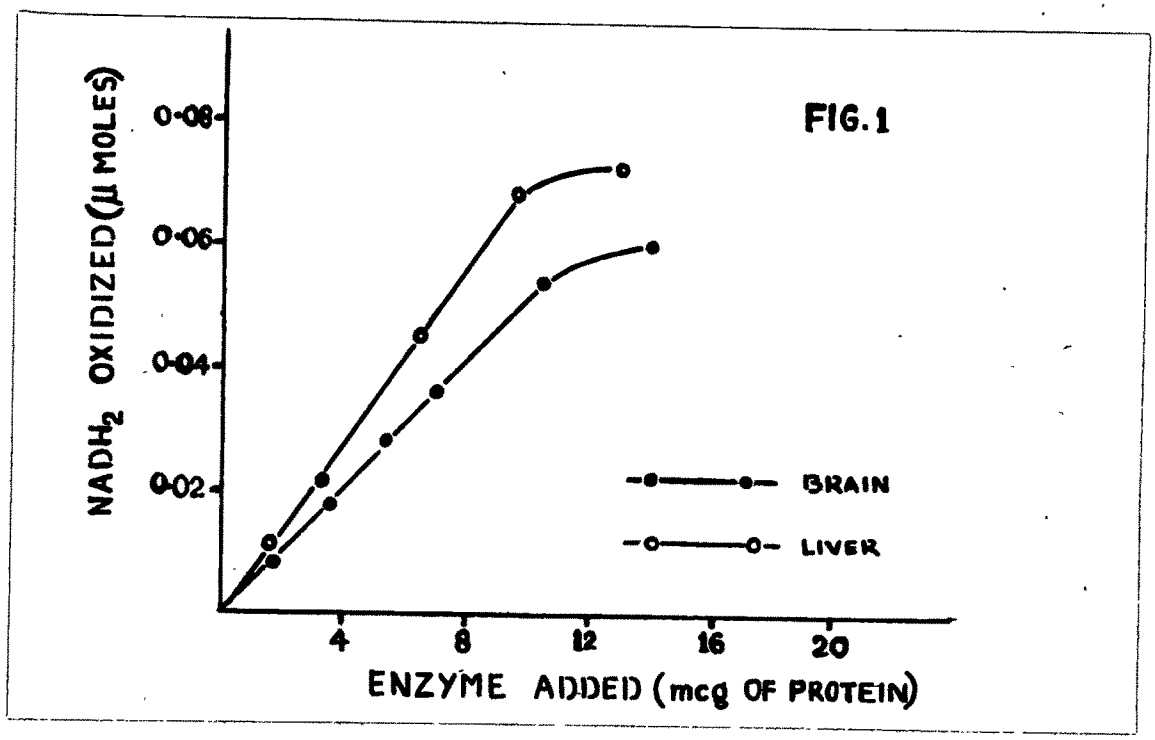


Table 25: Effect of variation in period of incubation on glutamate dehydrogenase activity in the rat brain and liver

period of incubation (minutes)	$NADH_2$ oxidised (μmoles)	
	brain GDH	liver GDH
0	0	0
0.5	0.020	0.023
1.0	0.038	0.045
1.5	0.054	0.068
2.0	0.061	0.076
2.5	0.066	0.082
3.0	0.070	0.085

Effects of variations in period of incubation on glutamate dehydrogenase activity in the rat brain and liver

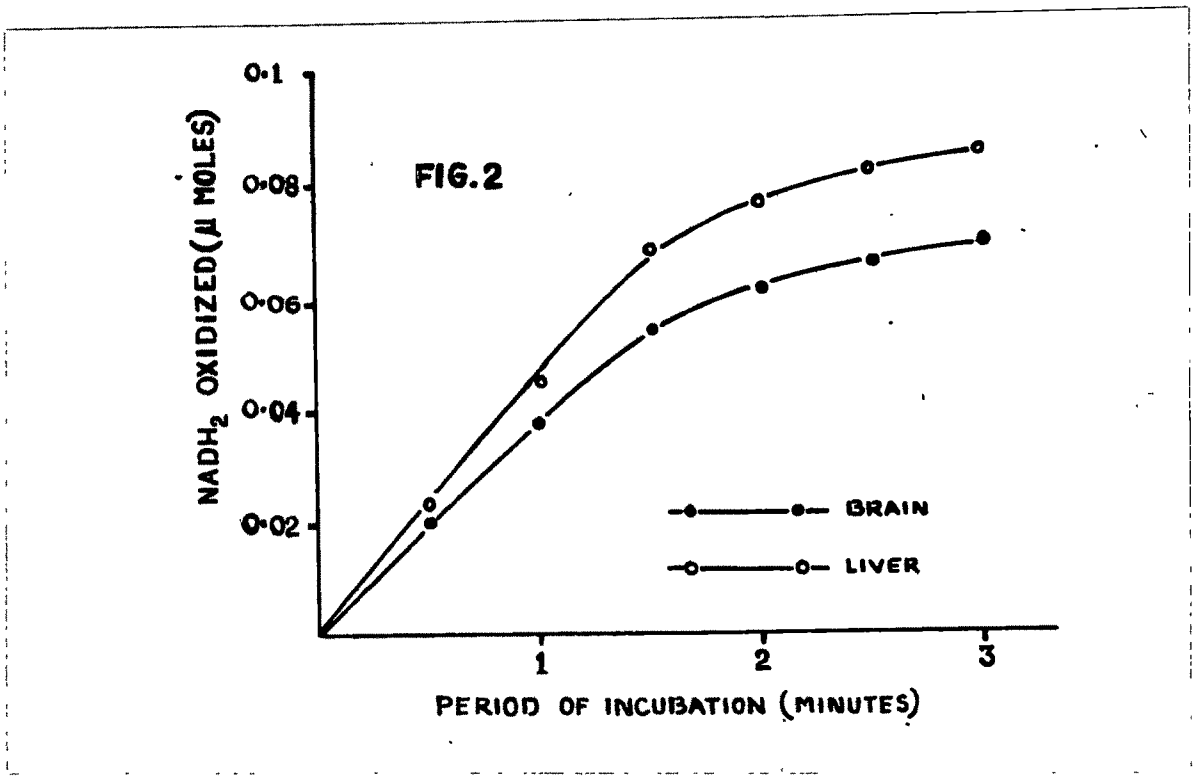


Table 26: Effects of variations in 2-oxoglutarate concentration on glutamate dehydrogenase activity in the rat brain and liver

2-oxoglutarate added (μmoles)	$NADH_2$ oxidised (μmoles)	
	brain GDH	liver GDH
0	0	0
0.025	-	0.009
0.05	0.014	0.018
0.10	0.028	0.036
0.15	0.044	0.052
0.20	0.056	0.068
0.30	0.058	0.073
0.40	0.060	0.075

Effects of variations in 2-oxoglutarate concentration on glutamate dehydrogenase activity in the rat brain and liver

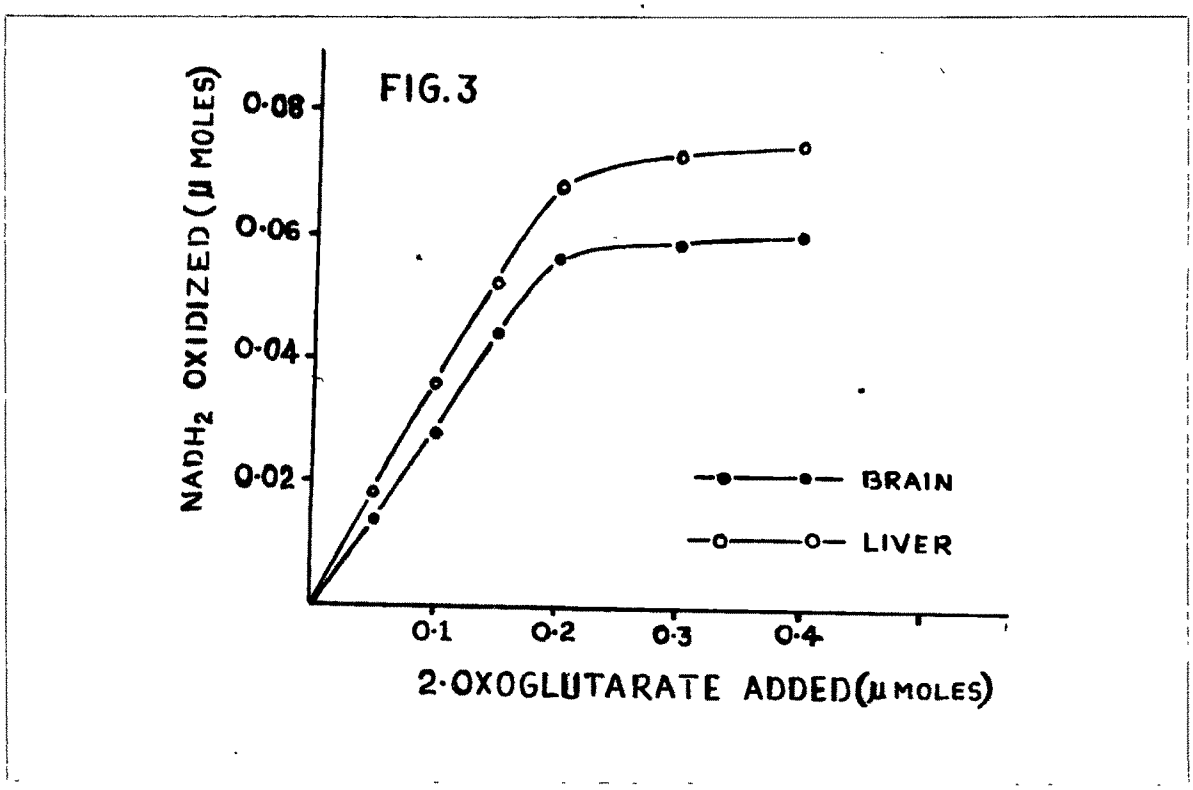


Table 27: Effects of variations in pH on glutamate dehydrogenase activity in the rat brain and liver

pH*	$NADH_2$ oxidised (µmoles)	
	brain GDH	liver GDH
6.0	0.014	-
6.5	0.028	0.051
7.0	0.034	0.064
7.5	0.040	0.067
8.0	0.055	0.022
8.5	0.040	0.011
9.0	0.010	0.007

* 6.0 - 6.5 citrate phosphate buffer
  7.0 - 7.5 potassium phosphate buffer
  8.0 - 9.0 Tris-HCl buffer

Effects of variations in pH on glutamate dehydrogenase activity in the rat brain and liver

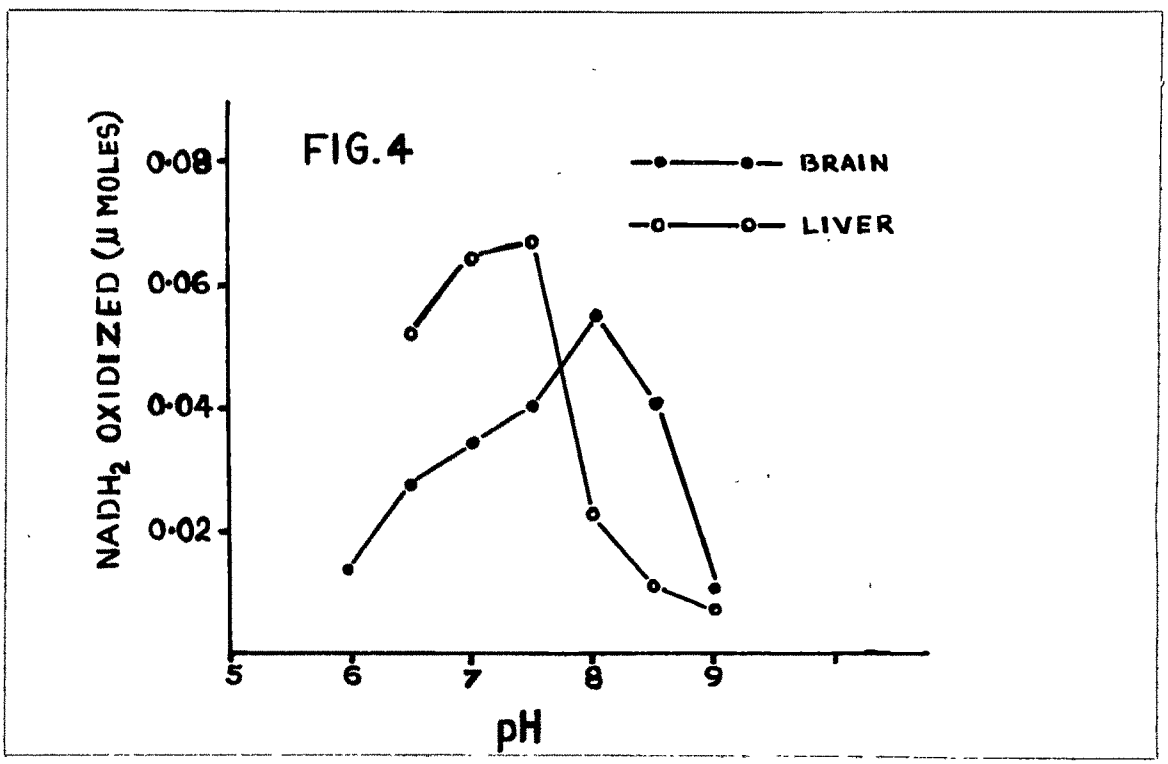


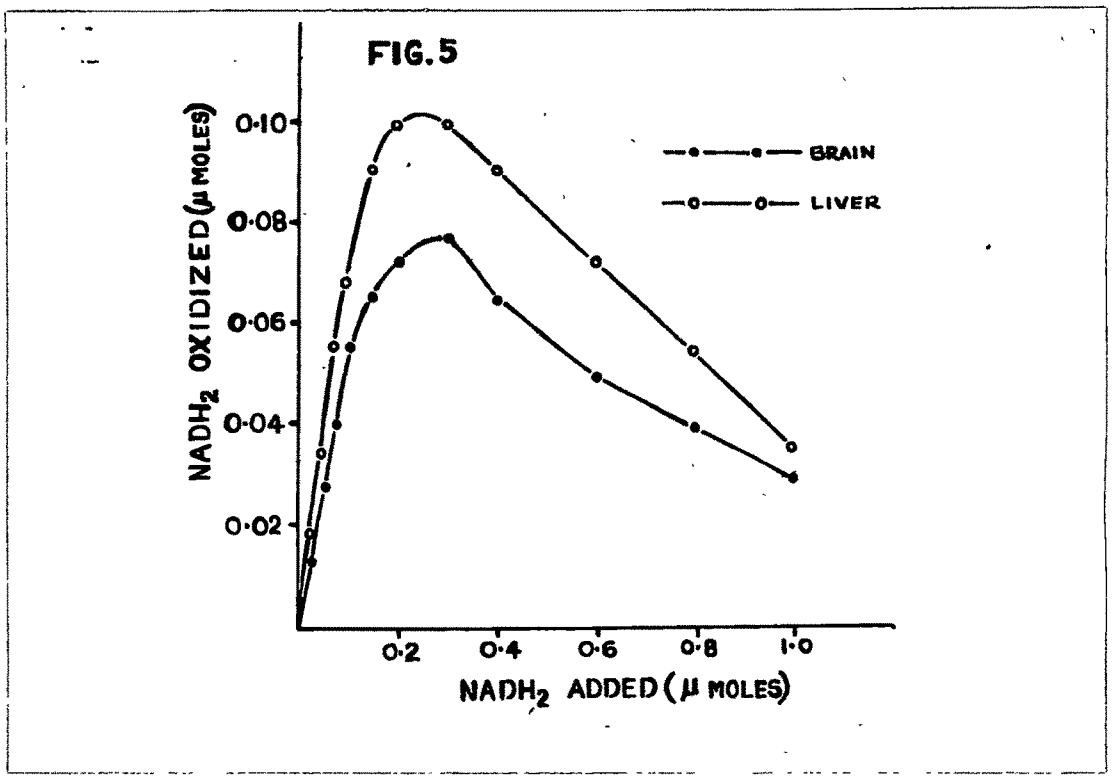
Table 28: Effects of variations in buffer ion concentrations on glutamate dehydrogenase activity in the rat brain and liver

buffer added (µmoles)	$NADH_2$ oxidised (µmoles)	
	brain GDH	liver GDH
0	0.012	0.045
5	0.014	0.045
10	0.028	0.050
20	0.055	0.054
40	0.055	0.063
50	0.056	0.068
100	0.056	0.068

Table 29: Effects of variations in $NADH_2$ concentration on glutamate dehydrogenase activity in rat brain and liver

$NADH_2$ added (µmoles)	$NADH_2$ oxidised (µmoles)	
	brain GDH	liver GDH
0.025	0.013	0.018
0.05	0.027	0.034
0.075	0.040	0.055
0.10	0.055	0.068
0.15	0.065	0.091
0.20	0.072	0.100
0.30	0.077	0.100
0.40	0.065	0.091
0.60	0.050	0.073
0.80	0.040	0.055
1.00	0.030	0.036

Effects of variations in $NADH_2$ concentration on glutamate dehydrogenase activity in the rat brain and liver



concentration of ammonium sulphate required for optimum activity was found to be 100 and 150 μmoles for brain and liver glutamate dehydrogenase respectively (Table 30, Fig. 6). Studies were made of the stability of the enzyme at different temperatures (Table 31). Both the enzymes were stable upto 45°. Complete inactivation occured when the enzyme was kept at 55° for 7 minutes.

The effect of period of storage on the enzyme activity of both the crude extract and the purified enzyme from the brain and liver were studied by storing them in test tubes at -10° (Tables 32 and 33). Different batches were removed at regular intervals and assayed for enzyme activity. In both cases retention of full activity was found in the crude extract upto four weeks after which there was a progressive decline, complete loss of activity occuring in 7 weeks in the case of the liver enzyme and 10 weeks in that of the brain enzyme. The partially purified enzymes from both tissues were stable upto second week but by 5th week complete activity was lost in both cases.

Studies were made of the effects of several metal ions on enzyme activity. Copper, mercury, zinc and silver were found to inhibit the enzyme from both sources (Table 34). Iron was not found to inhibit the brain

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Table 30: Effects of variations in $(NH_4)_2SO_4$ concentration on glutamate dehydrogenase activity in the rat brain and liver

$(NH_4)_2SO_4$ added (µmoles)	$NADH_2$ oxidised (µmoles)	
	brain GDH	liver GDH
0	0	0
25	0.014	0.013
50	0.028	0.023
75	0.044	0.036
100	0.056	0.051
150	0.056	0.070
200	0.056	0.070

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Effects of variations in $(NH_4)_2SO_4$ concentration on glutamate dehydrogenase activity in the rat brain and liver

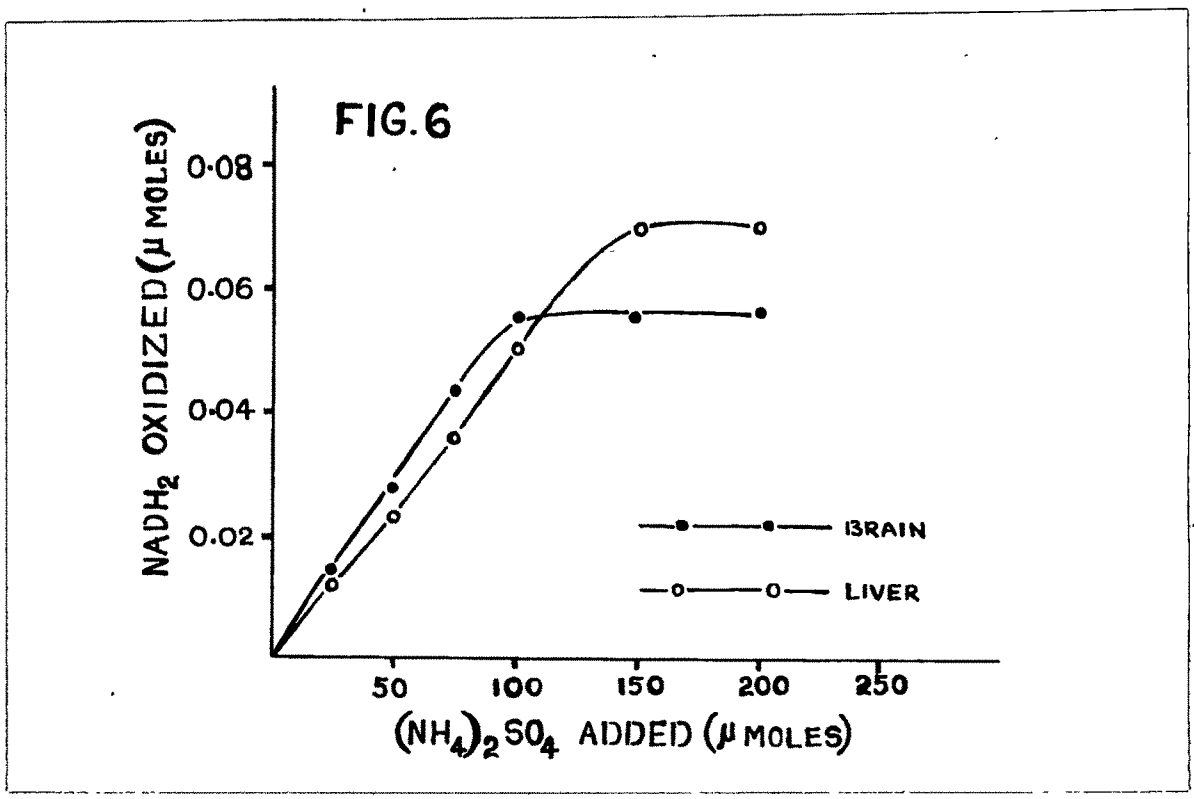


Table 31: Effects of variations in temperature on glutamate dehydrogenase* activity in the rat brain and liver

temperature	$NADH_2$ oxidised (µmoles)		% activity	
	brain GDH	liver GDH	brain GDH	liver GDH
0	0.055	0.067	100	100
37	0.056	0.067	100	100
40	0.056	0.068	100	100
45	0.055	0.068	100	100
50	0.044	0.054	80	80
55	0	0	0	0

*the enzyme was kept on a water bath maintained at the specified temperature for 7 minutes, cooled to 0° and then assayed.

Table 32: Effects of storage* on glutamate dehydrogenase activity in the rat brain

	period of storage (weeks)										
	0	1	2	3	4	5	6	7	8	9	10
	% of original activity										
crude extract	100	100	100	100	100	90	90	80	60	40	00
purified enzyme	100	100	90	70	20	00	00	00	00	00	00

*stored at -10°

Table 33: Effects of storage* on glutamate dehydrogenase activity in the rat liver.

	period of storage (weeks)							
	0	1	2	3	4	5	6	7
	% of original activity							
crude extract	100	100	100	100	100	80	40	00
purified enzyme	100	100	100	50	20	00	00	00

*stored at -10°.

Table 34: Effects of metal ions on glutamate dehydrogenase activity in the rat brain and liver

addition	amount added (μmoles)	$NADH_2$ oxidised (μmoles)		% inhibition	
		brain GDH	liver GDH	brain GDH	liver GDH
none	-	0.055	0.068	-	-
$FeCl_3$	0.01	0.055	0.068	0	0
	0.10	0.055	0.025	0	63
	1.00	0.055	0.0	0	100
$CuSO_4.5H_2O$	0.1	0.055	0.068	0	0
	1.0	0.023	0.022	58	68
	2.0	0.0	0.0	100	100
$Hg(C_2H_3O_2)_2$	0.01	0.055	0.068	0	0
	0.10	0.015	0.034	73	50
	1.0	0.0	0.0	100	100
$ZnSO_4.7H_2O$	0.01	0.054	0.068	0	0
	0.02	0.024	0.068	56	0
	0.05	0.0	0.030	100	66
	0.10	0.0	0.016	100	76
	0.20	0.0	0.0	100	100
$AgNO_3$	0.00001	0.054	0.068	0	0
	0.0005	0.027	0.068	50	0
	0.001	0.0	0.068	100	0
	0.002	0.0	0.034	100	50
	0.004	0.0	0.012	100	82
	0.005	0.0	0.0	100	100

enzyme though it inhibits the liver enzyme. The brain enzyme also seemed to be more sensitive to silver as compared to the liver enzyme. Addition of 0.001 µmole of silver was found to inhibit completely the brain enzyme whereas only 50% activity was lost even with the addition of 0.002 µmole in the case of the liver enzyme.

The effects of AMP, ADP and ATP on glutamate dehydrogenase activity are shown in Table 35. AMP was found to activate both the enzymes but the pattern and the extent of activation differed in the two tissues. In the case of the brain enzyme maximum activation (136%) was found with very low concentration of AMP (0.0004 µmole), no greater activation being found with further increases in the concentration. In the case of the liver enzyme a higher concentration of 0.05 µmole was needed to achieve an increase of 100% in activity but raising the concentration to 0.5 µmole resulted in a further five fold increase in activity.

Activation by ADP was found to show a more or less similar pattern in both the cases. 79% activation is obtained with 0.2 µmole of ADP in the case of brain enzyme. In the case of liver enzyme 89% activation was obtained with 0.1 µmole of ADP. No greater increase was found with further increase in the concentration of ADP.

Table 35: Effects of AMP, ADP and ATP on glutamate dehydrogenase activity in the rat brain and liver*

addition	amount added (μmoles)	$NADH_2$ oxidised (μmoles)		% inhibition or activation	
		brain GDH	liver GDH	brain GDH	liver GDH
none	-	0.028	0.010	0	0
AMP	0.0001	0.028	0.010	0	0
	0.0002	0.040	0.010	+43	0
	0.0004	0.066	0.010	+136	0
	0.01	0.066	0.010	+136	0
	0.05	0.066	0.020	+136	+100
	0.10	0.066	0.040	+136	+300
	0.5	0.066	0.060	+136	+500
	1.0	0.066	0.060	+136	+500
none	-	0.028	0.035	0	0
ADP	0.025	0.028	0.035	0	0
	0.050	0.028	0.050	0	+43
	0.075	0.032	0.062	+14	+77
	0.10	0.044	0.066	+57	+89
	0.20	0.050	0.066	+79	+89
	1.0	0.050	0.066	+79	+89
	5.0	0.050	0.066	+79	+89
none	-	0.028	0.035	0	0
ATP	0.5	0.028	0.035	0	0
	1.25	0.035	0.046	+25	+31
	2.5	0.042	0.060	+50	+72
	5.0	0.048	0.066	+71	+89
	7.5	0.042	0.068	+50	+94
	10.0	0.032	0.070	+14	+100
	20.0	0.020	0.070	-29	+100

*for the estimation of liver GDH with AMP, the enzyme was diluted 7 times since the activation obtained was beyond the measurable range. In other cases it was diluted two times.

With ATP a maximum activation of 100% was obtained in the case of the liver enzyme whereas only 70% was obtained in the case of the brain enzyme. The concentration needed was 10 µmoles in the former case and 5 µmoles in the latter, higher concentrations having an inhibitory effect in the case of brain enzyme.

In order to investigate the possibility that the drugs such as tranquilisers which act mainly on the CNS may have differential effects on the liver and brain glutamate dehydrogenases, studies were made of the effects of selected tranquilisers on the partially purified glutamate dehydrogenase and the activity of the same in the crude extract of rat brain and liver (Tables 36 and 37). The structures of the tranquilisers used are given on pages 112 and 113 . Fluphenazine dihydrochloride inhibited the purified brain enzyme completely at a concentration of 50 mcg. A much higher concentration of 500 mcg was needed for the complete inhibition in the case of liver glutamate dehydrogenase. The inhibitory effect was reduced to about half with the use of crude homogenates.

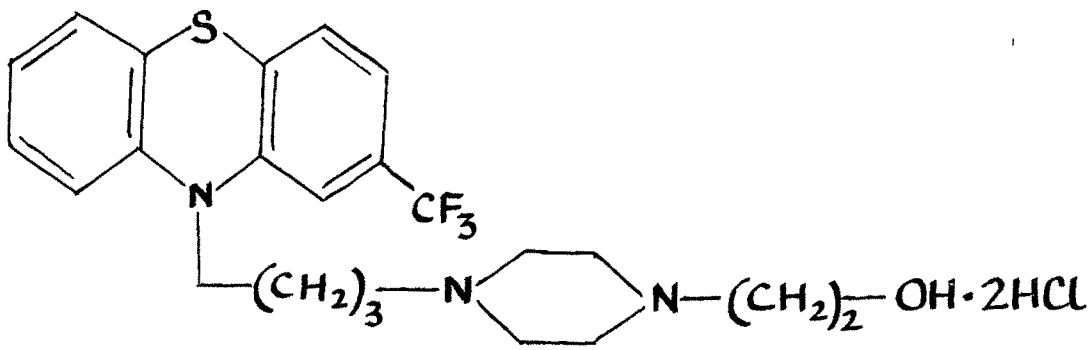
Triflupromazine inhibited the partially purified brain glutamate dehydrogenase at a concentration of

STRUCTURE AND FORMULAE OF THE DRUGS USED

(USDIN AND EFRON, 1972)

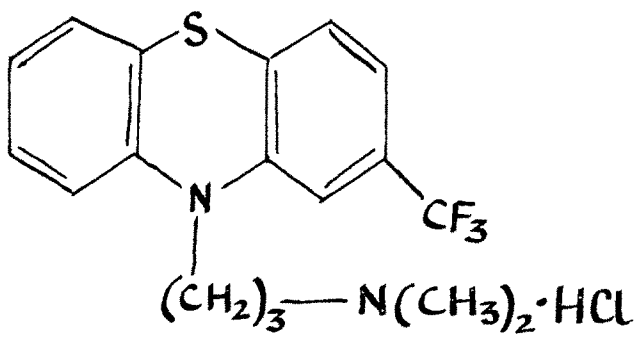
Fluphenazine dihydrochloride (Anatensol)

4-[3-(2-Trifluoromethyl)phenothiazin-10-yl] - prophyl-1-piperazine ethanol dihydrochloride



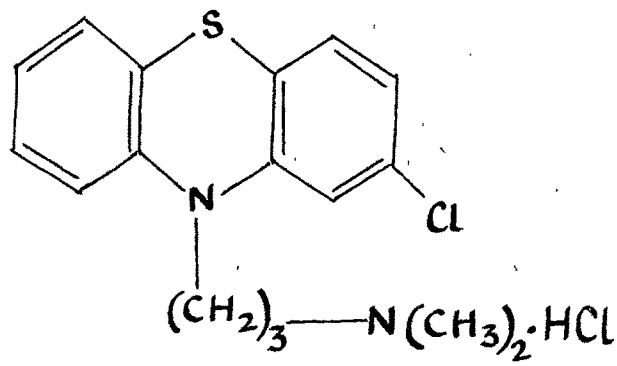
Triflupromazine (Siquil)

10 [3-(Dimethylamino)propyl] -2-trifluoromethyl-phenothiazine hydrochloride



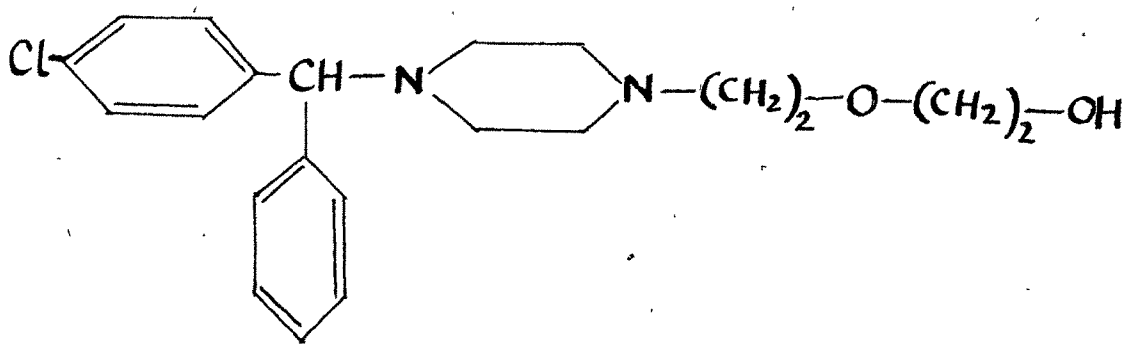
Chlorpromazine (Largactil)

2-chloro-10 [3-(dimethyl amino) propyl] - phenothiazine hydrochloride



Hydroxyzine (Atarax)

2- {2- [4-(4-chlorophenyl phenylmethyl)piperazinyl] - ethoxy} ethanol



Fluphenazine dihydrochloride, triflupromazine and chlorpromazine are soluble in water. Hydroxyzine is partially soluble and the suspension is used.

Table 36: Effects of drugs on glutamate dehydrogenase activity in crude extracts of rat brain and liver

addition	amount added (mcg)	$NADH_2$ oxidised (µmoles)		% inhibition	
		brain GDH	liver* GDH	brain GDH	liver GDH
none	-	0.046	0.070	0	0
fluphenazine dihydrochloride	1.0	0.046	0.070	0	0
	50.0	0.018	0.070	61	0
	100.0	0.0	0.070	100	0
	500.0	0.0	0.035	100	50
triflupromazine	50.0	0.046	0.070	0	0
	400.0	0.027	0.070	41	0
	500.0	0.0	0.070	100	0
chlorpromazine	1.0	0.046	0.070	0	0
	50.0	0.023	0.070	50	0
	100.0	0.0	0.070	100	0
	500.0	0.0	0.056	100	20
hydroxyzine	200.0	0.046	0.070	0	0
	400.0	0.027	0.070	41	0

*liver enzyme was diluted 5 times.

Table 37: Effect of drugs on partially purified glutamate dehydrogenase of rat brain and liver

addition	amount added (mcg)	$NADH_2$ oxidised (µmoles)		% inhibition	
		brain GDH	liver GDH	brain GDH	liver GDH
none	-	0.054	0.065	-	-
fluphenazine dihydrochloride	1.0	0.054	0.065	0	0
	20.0	0.022	0.065	60	0
	50.0	0.0	0.065	100	0
	100.0	0.0	0.066	100	0
	200.0	0.0	0.052	100	20
	500.0	0.0	0.0	100	100
trifluopromazine	50.0	0.056	0.065	0	0
	200.0	0.028	0.065	50	0
	400.0	0.0	0.066	100	0
	500.0	0.0	0.065	100	0
chlorpromazine	1.0	0.055	0.065	0	0
	10.0	0.042	0.065	24	0
	50.0	0.0	0.065	100	0
	100.0	0.0	0.065	100	0
	200.0	0.0	0.022	100	66
	500.0	0.0	0.0	100	100
hydroxyzine	200.0	0.055	0.065	0	0
	400.0	0.040	0.065	27	0

400 mcg but had no effect on liver enzyme. The pattern of inhibition remained the same with crude homogenate of the two tissues though the extent of inhibition was decreased in the case of the brain enzyme.

Chloropromazine was also found to have a greater inhibitory effect on the brain enzyme than the liver enzyme. Similarly, hydroxyzine inhibited brain glutamate dehydrogenase but had no effect on the liver enzyme.

DISCUSSION

The characteristics of the enzyme glutamate dehydrogenase isolated from a variety of sources (Frieden, 1965) show that the enzymes in different sources differ with regard to kinetic characteristics, metabolic functions and molecular properties (Table 38). The kinetics of the glutamate dehydrogenases purified from rat brain and liver in the present study show certain similarities such as inhibition at higher concentrations of $NADH_2$, the km for $NADH_2$, stability on storage, and heat lability. Inhibition at high concentration of $NADH_2$ has also been reported in other cases (Goldin and Frieden, 1971) and there is evidence that this inhibition arises from NADH binding to a

Table 38: Comparison of the characteristics of glutamate dehydrogenase obtained from different sources

source	pH	km with 2-oxoglutarate	km with $NADH_2$	inhibition by metal ions	reference
rat liver	7.5	1.3×10^{-3}	4.7×10^{-3}	Fe^{+++}, Cu^{++}, Hg^{++}, Zn^{++}, Ag^{+}	present study
rat brain	8.0	7.3×10^{-4}	3.7×10^{-3}	Cu^{++}, Hg^{++}, Zn^{++}, Ag^{+}	present study
beef liver	7.6	0.7×10^{-3}	9.6×10^{-5}	Ag^{+}, Zn^{++}, Fe^{+++}	Olson and Anfinsen (1953)
frog liver	8.0	5×10^{-3}	-	-	Fahien, Wiggert and Cohen (1965)
ox liver	8.5	1.23×10^{-4}	-	-	Strecker (1953)
Citrus acida	8.0	1.3×10^{-3}	-	Ag^{+}, Zn^{++}, Fe^{+++}	Parekh and Sakariah (1971)
Phaseolus mungo	8.0	-	-	-	Damodaran and Nair (1938)
corn leaves	8.1	1.5×10^{-3}	-	-	Bulen (1956)
N. crassa	8.4	-	-	-	Roberts (1966)

second non-active site. It has also been reported that the reciprocal plots with respect to either NAD (Fahien, Wiggert and Cohen, 1965; Olson and Anfinsen, 1953; Wiggert and Cohen, 1966; Corman and Kaplan, 1967) or NADH (Fahien, Wiggert and Cohen, 1965; Wiggert and Cohen, 1966; Corman and Kaplan, 1967) are strongly nonlinear. However in the present case no non-linearity was observed in the double reciprocal plots.

However, the enzymes from the two sources differ with regard to certain other properties such as the km for 2-oxoglutarate. The two enzymes also differ markedly in their inhibition by iron. The liver enzyme was found to be inhibited by iron while the brain enzyme was not.

The most important difference was noted in the case of the effects of tranquilisers. All the drugs studied showed an inhibitory effect on brain enzyme. The liver enzyme was however not affected by trifluprómazine and hydroxyzine whereas fluphenazine dihydrochloride and chlorpromazine inhibited the enzyme only at a much higher concentration. All the three adenine nucleotides AMP, ADP and ATP were found to activate both brain and liver glutamate dehydrogenase. This is in agreement with other studies (Goldin and Frieden, 1971).