C H A P T E R III

Results and Discussion

CHAPTER III

Results and Discussion

Experiment I

Table 15 shows the data on weight gain and cerebral enzymes in rats fed the different diets in experiments Ia and Ib. In the first experiment, glutamic acid supplementation was found to result in decreased weight gains during the early stages, and increased weight gain during the later stages. This is believed to be due to the poor acceptability of this diet and reduced food intake till the animals got used to the taste of glutamic acid. The effect of omission of niacin on weight gain was also more evident in the later stages. In other words, supplementations with glutamic acid was found to have beneficial effect on weight gain after the animals got adapted to the diet, whereas omission of nicotinic acid was found to have a somewhat adverse effect with the progress of treatment. Supplementation with either nicotinic acid or pyridoxine was not found to have a significant effect. As might be expected, the high protein animals showed significantly greater weight gains in both experiments.

In both experiments the low protein animals were found to have lower levels of L-glutamate-NAD-oxidoreductase and L-glutamate-1-carboxy-lyase thus confirming previous

Table 15

Weight gain and the activities of certain cerebral enzymes* in rats fed high and low protein diets

Group	Weight gain(g) per week Expt.Ia (fed ad libitum for 25 weeks) first 6 weeks	Weight gain(g) per week Expt.Ia (fed ad libitum for 25 weeks) total period	Weight gain(g) per week Expt.Ib (pair fed for 6 weeks) total period	L-glutamate:NAD-oxidoreductase Expt. Ia	L-glutamate:NAD-oxidoreductase Expt. Ib	L-glutamate:1-carboxy-lyase Expt. Ia	L-glutamate:1-carboxy-lyase Expt. Ib	4-aminobutyrate:2-oxoglutarate aminotransferase Expt. Ia	4-aminobutyrate:2-oxoglutarate aminotransferase Expt. Ib
I 5% Casein	2.6 ±0.4	1.6 ±0.2	4.5 ±0.2	88 ±1.1	84 ±7.5	14 ±1.1	12 ±0.7	28 ±0.3	32 ±2.8
II 5% Casein (niacin omitted from vit.mixt.)	2.3 ±0.8	1.2 ±0.8	5.0 ±0.2	91 ±3.1	80 ±8.7	16 ±1.4	11 ±0.9	26 ±2.5	30 ±1.2
III 5% Casein + niacin	2.6 ±1.0	2.0 ±0.8	4.9 ±0.1	88 ±5.4	93 ±9.8	17 ±0.7	12 ±0.8	29 ±1.4	32 ±2.0
IV 5% Casein + pyridoxine	3.2 ±0.8	2.0 ±0.8	4.7 ±0.1	87 ±7.3	92 ±7.2	17 ±2.7	14 ±0.1	29 ±1.0	29 ±1.7
V 5% Casein + glutamic acid	2.3 ±1.0	2.5 ±0.2	3.2 ±0.2	109 ±6.5	101 ±5.9	17 ±1.5	19 ±1.9	31 ±1.2	31 ±1.9
VI 20% Casein	5.5 ±0.5	2.8 ±0.8	8.2 ±0.2	112 ±3.2	110 ±8.3	19 ±1.0	18 ±1.6	32 ±0.1	32 ±1.2

*Values are mean enzyme units ± SE's
Six animals in each group were used in expt. Ia and five animals in expt. Ib.

observations in this laboratory (Rajalakshmi,Govindarajan and Ramakrishnan, 1965). The differences in the range of values with regard to the former must be presumed to be due to differences in the method used. The difference with regard to L-glutamate-1-carboxy-lyase was of a smaller order in the present study than in the previous one. This could have been due to the longer period of treatment used in that study or other variables.

No differences were found between low protein and high protein animals with regard to 4-aminobutyrate-2-oxoglutarate aminotransferase, an observation consistent with that in the previous study.

Supplementation with glutamic acid was found to have a beneficial effect on L-glutamate-NAD-oxidoreductase in both experiments. It is possible that it exerts a stimulatory effect in some unidentified way. Although this amino acid is not known to cross the blood brain barrier, normally, it has been known to do so under certain conditions in which the blood brain barrier is broken by means of some physical or chemical methods such as local freezing or by ethyl chloride treatment (Purpura, Girado, Smith and Gomez, 1958; Berl, Purpura, Monteagude and Waelsch, 1960).

It is possible that a low protein diet may bring about changes in transport of substances across the blood brain barrier. This seems plausible in view of unpublished

observations in this laboratory that, whereas glucose is more rapidly oxidized by brain tissue slices of high protein animals, glutamate is more rapidly oxidized by these of low protein animals, suggesting differences in permeability between the two groups (Thrivikraman, and his associates, unpublished observation). Whatever the explanation, the effect of glutamic acid is certainly intriguing.

Glutamic acid is not found to have a consistent effect on L-glutamate-1-carboxy-lyase although the data of the second experiment suggest a beneficial effect.

Neither omission of niacin nor supplementation with either niacin or pyridoxine are found to have a significant effect. There is some trend in the data to suggest adaptation to a diet free from nicotinic acid, as the activities of L-glutamate-NAD-oxidoreductase and 1-glutamate-1-carboxy-lyase are found to be somewhat more with a longer period of treatment.

Experiment II

The effects of adding different supplements on the nutritive value of kodri are shown in Table 16 from which the supplementations are seen to improve the nutritive value of kodri as might be expected from the improvement brought about in lysine content.

Table 16

Biological data of rats fed kodri(Paspalum scorbiculatum L) with and without different supplements

Parameter	IIa				IIb				
	SUPPLEMENTS USED								
	none	skim milk powder	moth bean	peas	none	skim milk powder	moth bean	peas	lysine
Weight gain (g)	65 ±2.2	125 ±19.7	116 ±14.2	133 ±7.2	7 ±1.0	24 ±2.3	23 ±1.7	26 ±2.5	18 ±2.0
% nitrogen* retained	69 ±6.2	72 ±0.2	72 ±3.9	62 ±3.9	62 ±3.8	73 ±2.4	74 ±2.1	76 ±1.3	78 ±1.1
Liver protein (g/100g.dry wt.)	NE	NE	NE	NE	37.5 ±1.1	42.5 ±0.7	45.7 ±0.9	48.6 ±1.9	41.6 ±0.8
Haemoglogin (g/100 ml. blood) at sacrifice	12.1 ±0.55	13.1 ±0.49	12.6 ±0.29	13.0 ±0.33	10.2 ±0.2	12.7 ±0.6	12.1 ±0.5	11.8 ±0.5	11.1 ±0.4

* nitrogen retention was done four weeks after the dietry treatment. six animals were used in each group.

Data on the performance of different groups on a water maze in experiment IIa are shown in Table 17. Although no significant differences were found between the different groups with regard to errors in either the forward trials or the reversal trials, the relative difficulty of reversal learning as compared to original learning (or reversal index) was found to be less in the supplemented groups. From evidence reviewed by Rajalakshmi and Jeeves (1965) this would appear to be a valid measure of learning performance. The combined scores of the three supplemented groups were found to be significantly less than those of the basal diet group.

The enzyme data are presented in Table 18 from which the improvement in the nutritive value of kodri brought about by the supplementations is found to result in a significant increase in the activities of L-glutamate-NAD-oxidoreductase and L-glutamate-1-carboxy-lyase. The increase in L-glutamate-1-carboxy-lyase is of a smaller order but is quite consistent in the two experiments. The differences are found to be statistically significant in the second experiment. No difference is found in the activity of 4-amino-butyrate-2-oxoglutarate aminotransferase. The results are consistent with these found with differences in protein content.

Table 17

Error scores on a water-maze of rats fed kodri(Paspalum scorbiculatum L) with and without different supplement

Groups	Supplement added	Mean No. of errors		Reversal index*
		foreward trials	reversal trial	
I	none	13 ± 1.5	28 ± 3.1	2.1 ± 0.25
II	skim milk powder	16 ±1.0	19 ± 2.4	1.3 ± 0.39
III	moth bean	15 ± 1.3	25 ± 4.0	1.6 ± 0.74
IV	peas	15 ± 1.4	20 ± 4.3	1.4 ± 0.58
II+III+IV		15 ± 0.7	21 ± 0.6	1.4 ± 0.12

Six animals were used in each group

* Rajalakshmi ans Jeeves 1965.

Table 18

Effect of various supplements to kodri(Paspalum scorbiculatum L) on certain cerebral enzymes* in rats

SUPPLEMENTS	Expt. IIa (38 weeks of treatment)					Expt. IIb (6 weeks of treatment)				
	none 1	skim milk powder 2	moth bean 3	peas 4	2+3+4	none 1	skim milk powder 2	moth bean 3	peas 4	lysine 5	2+3+4+5
Weight of cerebrum(g)	0.98 ±0.04	1.06 ±0.03	1.10 ±0.026	1.04 ±0.04	1.06 ±0.02	0.93 ±0.03	0.93 ±0.03	0.94 ±0.04	0.91 ±0.06	0.92 ±0.05	0.93 ±0.02
L-glutamate:NAD oxidoreductase	83 ±3.6	111 ±6.6	103 ±3.9	104 ±10.3	106 ±4.6	84 ±4.6	100 ±4.2	100 ±4.6	106 ±7.6	98 ±5.8	101 ±3.8
L-glutamate:1-carboxy-lyase	14 ±1.4	17 ±2.5	17 ±1.15	17 ±1.7	17 ±0.94	13 ±0.67	16 ±1.38	16 ±1.02	17 ±1.17	15 ±0.6	16 ±0.61
4-aminobutyrate 2-oxoglutarate aminotransferase	25 ±0.5	25 ±0.55	27 ±0.55	29 ±1.35	27 ±0.65	23 ±1.8	25 ±1.3	28 ±1.51	28 ±1.6	25 ±2.0	27 ±1.0

* Values are mean enzyme units ± SE's

six animals were used in each group.

Experiment III

The results of experiment III are presented in Table 19 from which the diet formulated and fed at the centre is found to have a superior nutritive value as compared to that fed at home. Group II fed the diet based on the food intake of children given breakfast and lunch at the centre and taking dinner and tea at home compares with group III fed only the diet provided at the centre. This is perhaps not surprising as the diet provided at the centre was designed so as to correct the basic deficiencies in the diet.

The differences in the nutritive value of the diets as judged by weight gain and liver xanthine oxidase are found to be associated with differences in L-glutamate-NAD-oxidoreductase and L-glutamate-1-carboxy-lyase as in the case of diets differing in protein quality and content. A difference is also found in the case of 4-aminobutyrate: 2-oxoglutarate aminotransferase which may be due to differences other than in protein content.

The data on psychological performance on the Hebb-Williams maze are in accord with previous studies suggesting an association between the activities of certain cerebral enzymes and psychological performance.

The results are of great significance from the standpoint of practical nutrition and are consistent with the

Table 19

Biological data, cerebral enzymes and psychological performance of rats fed home diet, home formulated diet and formulated diet.

	Home diet I	Diet at centre + diet at home II	Diet at the centre III
Body composition			
Weight grain (g)	70 ± 6.5	85 ± 4.8	90 ± 4.9
Blood hemoglobin(g/100ml)	13.5±0.14	13.5±0.14	14.0±0.12
Liver xanthine oxidase* (units per g. wet tissue)	6.2±0.10	7.6±0.13	7.6±0.06
Cerebral enzymes			
L-glutamate:NAD-oxidoreductase	61 ± 0.83	65 ± 0.71	68 ± 0.80
L-glutamate:1-carboxy-lyase	16 ± 0.91	19 ± 0.82	23 ± 0.61
4-aminobutyrate:2-oxoglutarate aminotransferase	22 ± 0.54	24 ± 0.63	28 ± 0.61
Psychological performance			
Error scores in Hebb-Williams maze	241 ± 9.2	184 ± 13.4	171 ± 11.7

* Taken from the data of coinvestigators (Ramachandran and associates, Unpublished data).

results of several human studies cited earlier (Chapter I pp. 10 to 13) suggesting a relation between malnutrition and psychological performance.

It must be pointed out that the diets in this experiment were fed ad libitum and the calorie intake of group I was 85% of that in groups II and III, whereas in the field studies the calorie intake of the controls was only 65% of that of the experimentals. Had the amount of food fed to the animals been so controlled as to resemble this situation the differences might perhaps have been greater. However, it is difficult to state this catagorically without actual experimentation as mere calorie restriction is found to be without effect on either cerebral enzymes or psychological performance (Rajalakshmi, Ali and Ramakrishnan, 1967).

Experiment IV:

The weight changes of the groups are shown graphically in figure 1 which gives an idea of the early growth arrest in the experimental animals. However, this was not found to affect either nitrogen retention after rehabilitation or status at death with regard to liver protein and blood hemoglobin (Table 20). Their weight gains after rehabilitation were comparable to those in stock animals.

The data on psychological performance are shown in Table 21. Although the stock animals appear to have learned both the discrimination and the reversal in fewer

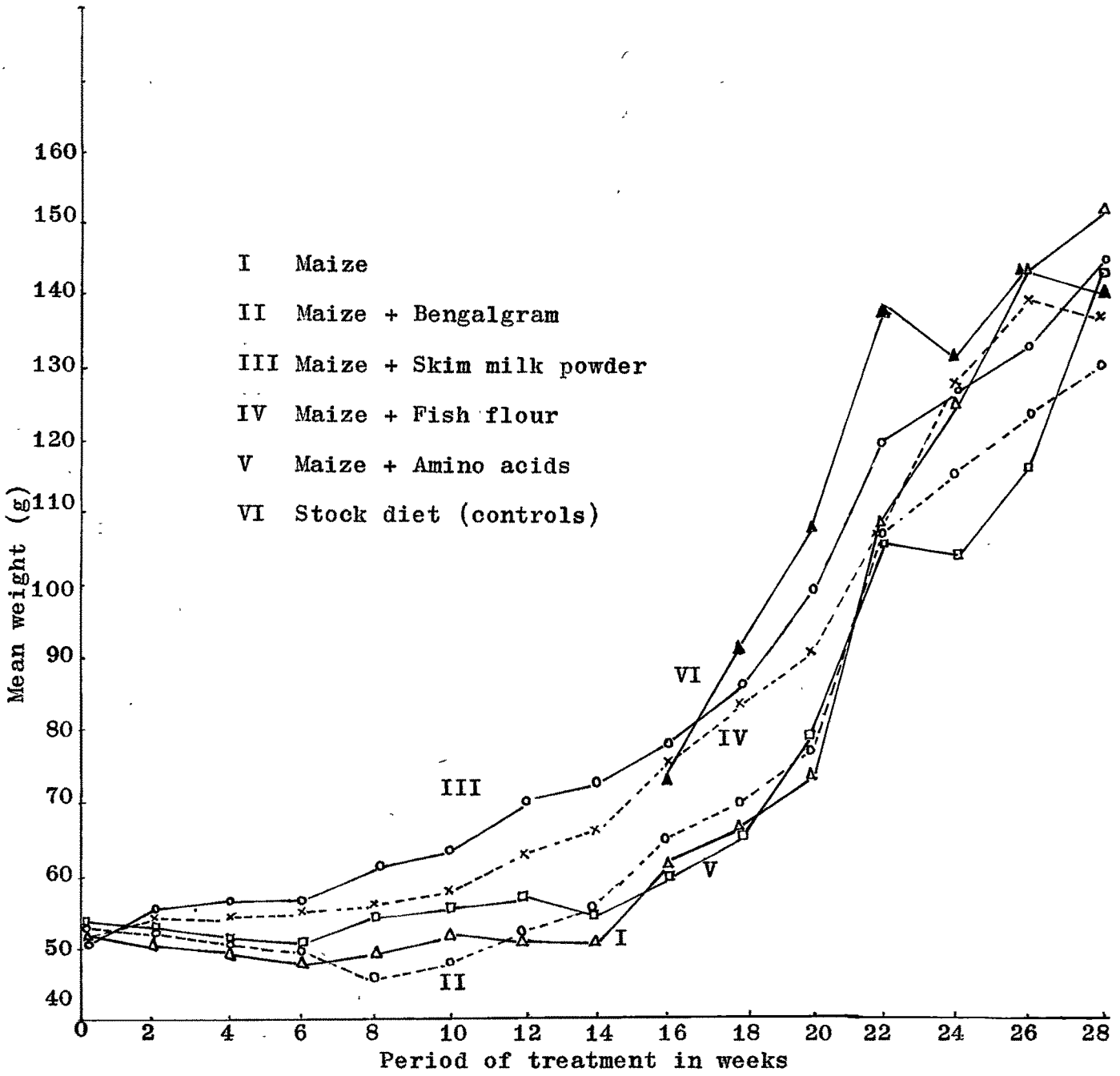
I Maize
II Maize + Bengalgram
III Maize + Skim milk powder
IV Maize + Fish flour
V Maize + Amino acids
VI Stock diet (controls)
Mean weight (g)
Period of treatment in weeks
160
150
140
130
120
110
100
90
80
70
60
50
40
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
I
II
III
IV
V
VI

Fig. 1.

Table 20

Biological data on rats subjected to growth arrest and subsequent growth

Initial diet	weight gain after rehabilitation (g)	% nitrogen retained after rehabilation	liver protein(g/100g. wet tissue	hemoglobin g/100 ml.blood
Maize	101	80	17.0	12.8
Maize + bengal gram	90	77	18.2	NE
Maize + skim milk powder	72	85	23.7	14.4
Maize + fish flour	65	68	19.5	14.6
Maize + aminoacid	78	86	19.2	13.2
CONTROLS (from stock colony)	66	81	19.4	15.5

NE. not estimated.

Table 21

Discrimination and reversal learning of rats subjected to growth arrest

Group	Initial diet	Period of early inanition(weeks)	Number of trials required for Discrimination learning			Reversal learning		
			Mean	Median	Mode	Mean	Median	Mode
I	Maize	17	90±7.4	60	60	130±6.8	150	190
II	Maize + bengal gram	15	93±7.4	90	90	170±2.3	175	180
III	Maize + skim milk powder	7	92±3.7	95	95	161±9.0	165	165
IV	Maize ± fish flour	13	123±6.0	120	120	161±7.4	170	190
V	Maize ± amino acids	15	130±3.2	130	130	148±11.9	140	140
VI	Controls from stock colony	0	77±4.4	60	60	117±11.1	100	100

Five animals were used in each group.

trials there is no statistically significant difference between this group and group I subjected to maximum inanition. The performance of the various groups does not seem to be related to the period of inanition. The results are difficult to interpret because of the complex factors involved. The data on cerebral enzymes of these animals are shown in Table 22. Again no consistent differences are observed between the period of inanition and the activities of the cerebral enzymes studied.

Thus contrary to expectation severe growth arrest resulting from accidental causes were not found to affect significantly either growth, psychological performance or cerebral enzymes after rehabilitation. However, this experiment suffered from a number of drawbacks. The age as well as the initial diet of the experimental groups and control animals were different. Further the experimental animals suffered varying degrees of growth retardation only after weaning. The results might have been different if such retardation had occured prior to weaning. The results of this experiment, although not admitting of definite interpretation, paved the way for a carefully controlled experiment subsequently carried out (Rajalakshmi, Ali and Ramakrishnan, 1967). The present results are in accord with the results obtained in that study.

Table 22

Activities of certain cerebral enzymes*in rats subjected to growth arrest

Group	Initial diet	Weight of the cerebrum(g)	L-glutamate: NAD-oxidoreductase	L-glutamate:1 -carboxy-lyase	4-aminobutyrate:2-oxoglutarate aminotransferase.
I	Maize	0.93±0.055	96±9.57	13 ± 2.80	25 ±3.34
II	Maize+ bengal gram	0.99±0.057	84±3.87	14 ± 2.20	29 ±2.12
III	Maize+ skim milk powder	0.93±0.042	103±9.48	15 ± 1.73	24 ±3.19
IV	Maize+ fish flour	0.95±0.047	105±5.3	13 ±1.73	26 ±2.00
V	Maize+ amino acid	0.95±0.030	105±14.3	14 ±0.71	26 ±2.00
Vi	Controls from stock colony	1.01±0.020	110±9.57	14 ±1.91	24 ±3.50
I+II+III+IV+V		0.95±0.021	99±4.0	14 ±0.5	26 ±0.84

* Values are mean enzyme units ± SE'S

5 animals were used in each group.

Experiment V

The data on shock avoidance of animals subjected to different treatments are presented in Table 23. Unfortunately the data are difficult to interpret as even animals not subjected to electroconvulsive shock (Group IIId) fail to show a clear cut shock avoidance. In general, the stock animals were found to show a greater degree of shock avoidance as compared to low protein and high protein animals, as judged by the time taken for stepping off the platform. This might have been due to the greater extent to which the low and high protein animals, which were weighed once a week during dietry treatment, were handled as compared to stock animals which were not handled prior to testing. In this connection handling has been reported to affect response to electric shock (Rajalakshmi, personal communication).

The data on cerebral enzymes are presented in Table 24. The differences between animals fed low protein and high protein diets with respect to L-glutamate:NAD-oxidoreductase and L-glutamate:1-carboxy-lyase are consistent with the results of experiment I. It is not possible to conclude, in the absence of proper controls, whether the shock differentially affects the two groups. However, from the data on stock animals, it would appear that animals given both electroconvulsive shock and foot shock do not differ from those given

Table 23

Effects of electroconvulsive shock on avoidance of foot shock in animals fed different diets

Interval between foot shock and ECS.	Low protein (LP) I		High protein (HP) II		Stock diet III							
					Matched for weight with LP group a		Matched for age with LP & HP groups					
							Given FS and ECS b		not given FS c		not given ECS d	
	Median time taken by rats (minutes) to step down from the platform											
	1	2	1	2	1	2	1	2	1	2	1	2
0 Sec.	0.10	0.16	0.03	0.42	0.05	0.23	0.09	0.42	0.09	0.10	0.08	0.75
10 Sec.	0.17	1.17*	0.06	0.25	0.09	0.20	0.23	0.24	0.20	0.34≠	0.09	0.60*
5 Min.	0.08	0.29	0.09	0.33	0.10	3.00	0.30	3.00**	0.14	0.04	0.09	2.94
All intervals combined	0.13	0.64	0.08	0.42	0.10	0.48	0.15	2.3	0.24	0.15	0.08	0.75

* One of the rats failed to step down from the platform.
** Two rats failed to step down from the platform.
≠ One of the rats got paralysed.

Three animals were used in each group.

ECS, electrocunvulsive shock.
FS , foot shock.
1 - before foot shock.
2 - after foot shock.

Table 24

Effects of electroconvulsive shock on certain cerebral enzymes in rats fed different diets

Diet	L-glutamate:NAD-oxidoreductase				L-glutamate:1-carboxy-lyase				4-aminobutyrate:2-oxoglutarate aminotransferase			
	Interval between foot shock and electroconvulsive shock											
	0 sec. 1	10 sec. 2	5 min. 3	1+2+3	0 Sec. 1	10 sec. 2	5 min. 3	1+2+3	0 Sec. 1	10 sec. 2	5 min. 3	1+2+3
I Low protein	80±3.0	71±2.1	72±2.7	74±2.5	13±1.5	12±1.2	13±1.2	13±0.7	27±1.7	26±4.1	28±1.8	27±1.4
II High protein	97±12.1	98±6.8	97±4.4	97±4.6	18±1.8	16±1.5	18±2.6	17±1.1	30±4.7	28±3.2	29±0.7	29±1.7
III Stock diet: (a) matched for wt. with low protein	97±7.2	89±3.9	93±2.1	93±2.7	18±1.6	17±2.7	17±1.5	17±0.82	29±2.8	29±2.2	27±2.6	28±1.4
(b) matched for age with groups I & II	101±17.2	90±3.2	108±9.6	100±7.1	18±1.5	18±1.4	17±0.7	18±0.8	26±2.1	27±1.5	25±0.7	26±0.6
(c) matched for age as in (b) and given only ECS.	98±12.6	99±1.7	108±10.8	102±4.8	18±2.1	18±1.6	17±1.[illegible]	18±0.95	26±1.5	30±1.2	28±2.7	28±1.1
(d) matched for age as in (b) and given only foot shock.	98±7.[illegible]	93±4.6	109±10.[illegible]	102±5.3	20±4.0	17±1.2	17±1.[illegible]	18±1.4	19±1.0	21±2.7	21±1.2	20±0.[illegible]

Values are mean enzyme units ± SE's. Three animals were used in each group.

either alone with regard to these two enzymes. These results need confirmation by further experimentation. 4-aminobutyrate:2-oxoglutarate aminotransferase, on the other hand, appears to show increased activity with the administration of electroconvulsive shock (Group IIIc and IIId). It is unlikely that this increased activity is due to the synthesis of the enzyme in view of the short interval of fifteen minutes between the administration of the electroconvulsive shock and sacrifice. The apparent increase in activity must therefore be presumed to be due to factors such as destruction of some inhibitor or release of some activators. Sulphahydril groups are known to get released during electroconvulsive treatment (Ellman and Sullivan, 1965). Serum transaminases levels are also considerably increased during electroconvulsive therapy (Poloni, 1956).

Further investigations are necessary to identify the mechanism involved and to find out to what extent foot shock alone affects the parameters measured.