APPENDIX

PUBLICATIONS

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EFFECTS OF PRE-WEANING AND POST-WEANING UNDERNUTRITION ON ACETYLCHOLINE LEVELS IN RAT BRAIN

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Abstract—Studies were made of the effects of undernutrition during the neonatal period and also protein deficiency and undernutrition during the post-weaning period on brain acetylcholine. Rats undernourished from birth to 4 weeks so as to result in a body wt deficit of 43 per cent had an associated deficit in brain wt of 14 per cent, but the concentration of acetylcholine in the brain was not affected. In the case of post-weaning undernutrition, acetylcholine concn was found to be affected in protein deficiency as well as in severe calorie restriction.

ACETYLCHOLINE plays a key role in the transmission of nerve impulses (NACHMANSOHN, 1961). It has been suggested that the concentration of ACh in the brain varies inversely with the functional activity of the brain, as it has been found to be increased in anaesthesia and sleep (RICHTER & CROSSLAND, 1949; CROSSLAND & MERRICK, 1954); in deep narcosis (ELLIOT *et al.*, 1950) and following the administration of CNS depressants (NAIK *et al.*, 1970). The concentration of ACh in the brain was found to be decreased during convulsions, electrical stimulation of brain and emotional excitement (RICHTER & CROSSLAND, 1949). The concentration of ACh in the brain of the rat increases with age up to 100 days or more (CROSSLAND, 1951; NAIK *et al.*, 1970). It is generally accepted that during the neonatal period when the brain is rapidly developing, undernutrition may interfere with normal maturation as judged by brain wt, DNA and lipids (DOBBING, 1968; WINICK & NOBLE, 1966). Since an increase in the concentration of ACh is associated with such maturation, and continues beyond the weaning age, it was considered of interest to study the effects on brain ACh of undernutrition during the neonatal as well as the post-weaning periods.

In previous studies in this laboratory, protein deficiency during the post-weaning period was found to affect the activities of brain glutamate dehydrogenase and glutamate decarboxylase whereas undernutrition did not have a similar effect suggesting that the effects of undernutrition are different from those of protein deficiency (RAJALAKSHMI & RAMAKRISHNAN, 1972). This raises the question of whether protein deficiency has similar effects on other brain constituents such as ACh and whether these effects differ from those of undernutrition. The present studies were therefore concerned with the effects on brain ACh of neonatal undernutrition and of both undernutrition and protein deficiency during the post-weaning period.

METHODS AND MATERIALS

Animals. Albino rats were used in these studies. Neonatal undernutrition was induced by increasing litter size. Pups born on the same day were reared by foster mothers in litters of 8 or 16 up to 7, 14, 21 or 28 days of age. The mothers were given food and water *ad lib.* For studies on the effects of undernutrition and protein deficiency during the post-weaning period five groups of weanling rats, matched for sex and body weight were used, littermates being assigned to the different groups as far as possible. They were fed for 5 weeks on a diet containing 5, 8 or 20% protein *ad lib.* or one containing 20% protein in restricted amounts. In the case of the restricted groups the amounts were adjusted at 50 and 25 per cent of the *ad lib.* food intake of animals fed the 5% protein diet.

Assay of ACh. The whole brain including the olfactory lobes was used for the assay The animals were killed by decapitation and the brains were removed immediately, weighed and ground with pestle and mortar in 3 ml of ice-cold 10% TCA. Further extraction was carried out by the method of TORU & APRISON (1966) ACh was assayed on eserinized frog rectus abdominus muscle. The sensitivity of the muscle was ascertained by repeated applications of standard ACh chloride. The procedure for bioassay was essentially similar to that described in *Pharmacological Experiments of Isolated Preparations* (1968). The only variation was that instead of matching the standard response to the sample, a known amount of standard was used each time and the sample response compared with it. The validity of

Table 1 Effects of neonatal undernutrition on brain acetylcholine at different ages

Litter size (age in days)	Body wt (g) (mean ± S.E.M.) 8	16	Brain wt (g) (mean ± S.E.M.) 8	16	Acetylcholine[1] (mean ± S.E.M.) μg/brain 8	16	μg/g brain 8	16
birth	5		0·21 ±0·01		0·14 (4) ±0·04		0·63 ±0·02	
7	11	9[2]	0·68 ±0·01	0·62 ±0·05	1·17 (2) ±0·15	1·00 (4) ±0·04	1·72 ±0·20	1·63 ±0·02
14	16	11[2]	0·95 ±0·03	0·84[2] ±0·02	1·32 (4) ±0·05	1·07[2] (6) ±0·05	1·39 ±0·06	1·24 ±0·05
21	28	18[2]	1·25 ±0·03	1·13 ±0·02	1·55 (6) ±0·14	1·35 (7) ±0·10	1·23 ±0·11	1·19 ±0·10
28	60	34[2]	1·41 ±0·07	1·22[2] ±0·07	2·57 (6) ±0·17	2·22 (7) ±0·18	1·80 ±0·10	1·80 ±0·14

Number of estimations shown in brackets.
[1] As acetylcholine chloride
[2] Values significantly different from control values ($P < 0·001$).

this procedure was ascertained by separate experiments in which the assays by the two procedures gave almost identical results. All the chemicals used were of research grade purity and were obtained either from B.D.H. or E. Merck.

RESULTS AND DISCUSSION

The results of the experiment on neonatal undernutrition are presented in Table 1. The body and brain wt deficits were as expected on the basis of other studies (DOBBING, 1968). Although the ACh content of the brain was slightly lower, the decrease was statistically significant only at 2 weeks and was in proportion to the decrease in brain wt for the concentration of ACh was not affected. This contrasts with the lower concentration of brain lipids, with a similar degree of neonatal undernutrition (DOBBING, 1968; GUTHRIE & BROWN, 1968). Further studies are needed to ascertain if a decrease in maternal stimulation because of in-

TABLE 2. EFFECTS OF POST-WEANING DEFICIENCIES OF PROTEIN AND CALORIES ON BRAIN ACETYLCHOLINE

Group	Diet	No of animals	Food intake (g/day)	Body wt (g)	Brain wt (g) (mean ± S.E.M.)	Acetylcholine[1] (mean ± S.E.M.) μg/brain	μg/g brain
1	5% protein	6	6	66	1·32[2] ±0·01	2·21[2] ±0·19	1·69[2] ±0·13
2	8% protein	5	7·6	92	1·37[2] ±0·01	2·66[2] ±0·14	1·90 ±0·15
3	20% protein (controls fed *ad lib*)	6	9	136	1·50 ±0·01	3·55 ±0·14	2·36 ±0·13
4	20% protein restricted[3]	4	3	59	1·28[2] ±0·02	2·77 ±0·11	2·15 ±0·08
5	20% protein severely restricted[4]	6	1·5	39	1·26[2] ±0·01	1·62[2] ±0·13	1·30[2] ±0·10

The period of treatment was 5 weeks
[1] As acetylcholine chloride
[2] Values significantly different from control values ($P < 0·01$)
[3] 50% of the *ad lib.* food intake of animals fed 5% protein diet
[4] 25% of the *ad lib.* food intake of animals fed 5% protein diet

crease in litter size counteracts the effects of undernutrition

The results of the experiment during the post-weaning period are presented in Table 2. Both protein deficiency and very severe calorie restriction (group 5) resulted in significantly lower amounts and concentrations of brain ACh. With less severe degrees of food restriction only the total amount in the brain and not the concentration was affected. This was also true of the 8% protein diet. This contrasts with the differential effects of calorie and protein deficiencies during the post-weaning period on brain enzymes (RAJALAKSHMI & RAMAKRISHNAN, 1972). In these previous studies, protein deficiency but not severe undernutrition resulted in lower activities of brain glutamate dehydrogenase and glutamate decarboxylase although body wt in the latter were much less than in the former. It is, however, interesting to note that although the retardation in body wt was comparable in the protein deficient and severely undernourished animals (groups 1 and 4) the amount and concentration of brain ACh were significantly more in the latter case.

The finding of a lower brain ACh with severe undernutrition contrasts with the increase reported in starvation (NAIK *et al.*, 1970). This may be because the effects of chronic undernutrition are different from those of complete starvation. In this connection, in other studies in this laboratory, a low protein diet was found to produce effects different from those of a protein free diet (RAJALAKSHMI & RAMAKRISHNAN, 1972). In conclusion, the concentration of brain ACh in rats was unaffected by undernutrition during the neonatal period but was found to be decreased by both severe undernutrition and protein deficiency during the post-weaning period

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INDIVIDUAL VALUES

INDIVIDUAL VALUES

In the subsequent pages individual values for main biochemical parameters, studied in detailed experiments, are listed. The numbers heading the respective columns denote the parameter as given below :

Column No.	Parameter
1	Number of the animals
2	Body weight (g)
3	Brain weight (g)
4	Brain protein (mg/g brain)
5	Brain DNA (mg/g brain)
6	AChE (µmoles of acetylthiocholine iodide hydrolyzed per minute per gram brain)
7	ChAc (µmoles of radioactive ACh formed per hour per gram brain)
8	ACh (µg per gram brain)

EXPT : Effects of maternal protein deficiency during gestation on prenatal development at birth :

1	2	3	4	5	6	7
GROUP - G^+ (At birth) :						
1.	6.5	0.234				
2.	6.1	0.277	55.8	1.85	1.93	0.36
3.	6.3	0.274				
4.	5.5	0.265				
5.	6.0	0.259	55.5	1.80	2.48	0.32
6.	6.4	0.272				
7.	6.1	0.272				
8.	5.6	0.264	56.0	1.90	2.20	0.40
9.	5.7	0.265				
10.	7.1	0.277				
11.	6.4	0.287	58.0	2.05	2.2	0.35
12.	6.3	0.289				
13.	5.1	0.257				
GROUP - G^- (At birth) :						
1.	4.7	0.192				
2.	4.5	0.191	57.50	1.90	1.93	0.30
3.	4.5	0.190				
4.	4.3	0.191				
5.	4.8	0.185				
6.	4.0	0.220	56.00	1.85	1.93	0.36
7.	4.5	0.212				
8.	4.5	0.190				

Contd....

1	2	3	4	5	6	7
9.	4.5	0.213				
10.	4.6	0.234	58.00	1.90	1.93	0.28
11.	3.5	0.205				
12.	4.3	0.204				
13.	4.0	0.218				
14.	5.0	0.217	56.00	2.05	1.93	0.34
15.	4.5	0.187				
16.	4.5	0.228				
17.	4.6	0.225				
18.	4.7	0.216				
19.	4.2	0.202	57.0	1.95	1.65	0.34
20.	4.3	0.190				
21.	4.3	0.182				
22.	4.5	0.162				
23.	4.2	0.198				
24.	4.3	0.199	51.0	1.80	1.93	0.35
25.	4.5	0.196				
26	4.5	0.179				

EXPT : ACh levels in the brain of pups (G^+) at birth.

1	2	3	8
1.	4.5	0.198	
2.	4.7	0.205	0.860
3.	5.3	0.215	
4.	5.3	0.220	
5.	5.2	0.215	
6.	5.0	0.215	0.693
7.	4.4	0.205	
8.	4.8	0.230	
9.	5.1	0.230	
10.	5.6	0.225	0.532
11.	4.8	0.205	
12.	4.5	0.190	
13.	5.2	0.220	
14.	4.7	0.201	0.454
15.	5.4	0.215	
16.	5.7	0.245	

EXPT : ACh leve in the brain of neonatally undernourished pups.

1	2	3	8
GROUP : 7 days (SL) :			
1.	9.5	0.665	
2.	11.0	0.688	1.92
3.	11.5	0.691	
4.	11.0	0.694	
5.	9.0	0.655	
6.	10.0	0.670	1.50
7.	11.0	0.675	
8.	11.0	0.691	
GROUP : 7 days (LL) :			
1.	9.5	0.604	
2.	8.5	0.660	1.63
3.	8.0	0.655	
4.	9.5	0.640	
5.	8.5	0.615	
6.	8.0	0.565	1.58
7.	8.0	0.590	
8.	8.5	0.620	
9.	8.0	0.575	
10.	8.5	0.660	1.69
11.	8.0	0.510	
12.	8.5	0.660	

Contd...

1	2	3	8
GROUP : 14 days (SL) :			
1.	16.5	0.96	1.549
2.	17.0	0.97	
3.	19.5	1.050	1.255
4.	19.0	1.005	
5.	18.5	1.050	1.388
6.	12.0	0.750	
7.	12.5	0.870	1.393
8.	14.5	0.940	
GROUP : 14 days (LL) :			
1.	10.5	0.950	1.26
2.	12.5	0.790	
3.	10.5	0.850	1.27
4.	9.0	0.910	
5.	9.5	0.810	1.39
6.	11.0	0.870	
7.	9.0	0.820	1.35
8.	10.0	0.815	
9.	11.0	0.775	1.13
10.	11.5	0.950	
11.	9.5	0.905	
12.	10.0	0.905	1.07
13.	11.5	0.745	

Contd...

1	2	3	8
GROUP : 21 days (SL) :			
1.	30	1.290	1.35
2.	32	1.280	
3.	29	1.255	1.02
4.	33	1.290	
5.	35	1.265	1.32
6.	27	1.245	
GROUP : 21 days (LL) :			
1.	22	1.150	1.37
2.	14	1.005	
3.	13	0.995	0.96
4.	17	1.120	
5.	25	1.210	1.11
6.	22	1.155	
7.	20	1.135	1.28
8.	24	1.290	
9.	19	1.115	
10.	17	1.030	
11.	16	1.030	1.21
12.	27	1.240	
13.	23	1.115	0.98
14.	22	1.113	
15.	24	1.210	1.43

Contd...

1	2	3	8
GROUP : 28 days (SL) :			
1.	71	1.44	1.51
2.	70	1.36	1.84
3.	62	1.37	1.88
4.	55	1.35	1.85
GROUP : 28 days (LL) :			
1.	26	1.105	1.86
2.	47	1.104	1.70
3.	41	1.256	1.77
4.	33	1.260	2.02

EXPT : Effects of neonatal undernutrition on cholinergic enzymes :

1	2	3	4	5	6	7
GROUP : 8 Litter size (28 days) :						
1.	59	1.440	80.95	1.99	10.0	3.8
2.	63	1.515	85.70	2.42	10.0	4.0
3.	61	1.425	85.70	2.29	9.5	3.6
4.	60	1.420	92.80	2.06	9.0	3.6
5.	59	1.380	92.80	2.25	9.5	3.8
6.	58	1.315	88.09	1.93	8.5	4.0
7.	52	1.345	85.70	2.25	10.5	3.4
8.	64	1.475	97.62	1.95	9.0	4.0

contd...

1	2	3	4	5	6	7
GROUP : 16 Litter size (28 days) :						
1	39	1.330	92.8	2.20	11.03	3.5
2.	32	1.170	85.7	1.85	10.48	4.0
3.	33	1.180	85.6	1.95	9.00	3.8
4.	35	1.250	88.90	2.00	9.50	3.2
5.	35	1.255	92.8	2.10	9.38	3.2
6.	29	1.255	88.90	1.95	9.38	3.8
7.	30	1.145	85.60	2.00	9.00	3.6
8.	36	1.265	85.60	2.10	11.48	4.0

EXPT : Effects of prenatal and/or postnatal undernutrition on the cholinergic system in the pregeny :

1	2	3	4	5	6	7
GROUP : G^+L^+ (7 days) :						
1.	17	0.720	58.0	2.0	4.41	0.75
2.	20	0.805	60.5	2.2		
3.	15	0.705	55.0	1.7	3.25	0.55
4.	18	0.740	57.5	2.0		
5.	17	0.720	59.0	2.15	3.10	0.80
6.	20	0.785	60.5	2.40		
7.	15	0.685	55.5	1.8	3.50	0.60
8.	17	0.715	58.0	2.0		

Contd...

1	2	3	4	5	6	7
GROUP : G^+L^- (7 days) :						
1.	8	0.620	55.0	1.80	4.41	0.80
2.	12	0.705	58.0	2.20		
3.	10	0.650	60.5	1.85	3.50	0.55
4.	9	0.630	55.0	1.85		
5.	9	0.640	55.0	1.75	3.10	0.60
6.	12	0.710	60.5	1.90		
7.	10	0.655	58.0	2.20	4.41	0.55
8.	8	0.633	58.0	1.70		
GROUP : G^-L^- (7 days) :						
1	10	0.580	60.5	2.0	2.50	0.50
2.	7	0.510	58.0	1.9		
3.	7	0.520	55.5	1.9	3.10	0.45
4.	6	0.480	55.0	1.8		
5.	6	0.490	58.0	2.0	2.25	0.55
6.	8	0.520	59.5	2.1		
7.	8	0.510	60.0	1.8	2.10	0.50
8.	7	0.500	55.5	1.9		
GROUP : G^+L^+ (14 days) :						
1.	26	1.180	75.5	2.18	4.41	1.95
2.	27	1.205	80.5	2.25	5.51	2.00
3.	28	1.116	85.0	2.00	4.41	1.85
4.	27	1.243	65.5	2.45	5.51	1.85
5.	29	1.390	70.5	2.25	4.41	2.00
6.	28	1.152	75.5	2.10	4.96	1.75
7.	27	1.147	80.0	1.95	4.41	1.75

Contd....

1	2	3	4	5	6	7
GROUP : G^+L^- (14 days) :						
1.	8	0.689	65.5	1.90	3.82	1.20
2.	9	0.746	70.5	1.95	5.51	1.30
3.	10	0.855	70.0	2.00	3.90	1.60
4.	9	0.767	80.5	2.00	4.56	1.65
5.	13	0.980	80.5	2.25	4.24	1.80
6.	13	1.020	75.5	2.25	3.90	1.80
7.	12	0.995	80.5	2.10	3.90	1.65
8.	14	1.115	75.5	2.10	5.51	1.80
GROUP : G^-L^- (14 days) :						
1.	9	0.757	70.5	1.85	3.86	1.40
2.	8	0.718	70.0	1.90	3.64	1.30
3.	6	0.680	65.5	2.00	3.53	1.05
4.	8	0.713	75.5	2.25	3.64	1.45
5.	9	0.750	75.5	2.15	3.64	1.60
6.	9	0.745	80.5	2.10	3.86	1.45
7.	7	0.560	70.5	2.10	3.53	1.30
8.	8	0.710	80.5	2.00	3.64	1.50
GROUP : G^+L^+ (21 days) :						
1.	41	1.413	90.45	2.12	8.27	3.40
2.	41	1.347	95.80	2.34	8.77	3.65
3.	43	1.400	109.48	2.25	8.82	4.00
4.	47	1.413	98.50	2.81	9.92	4.50
5.	45	1.323	95.80	2.12	8.82	4.35
6.	44	1.395	90.45	2.03	10.47	3.90

Contd...

1	2	3	4	5	6	7
GROUP : $G^{+}L^{-}$ (21 days) :						
1.	11	1.039	90.45	2.12	11.03	3.4
2.	12	1.192	95.80	2.25	10.48	4.0
3.	14	1.223	95.80	2.03	9.93	4.0
4.	13	1.035	90.45	2.12	11.03	3.4
5.	13	1.170	98.50	2.03	9.38	3.0
6.	15	1.012	98.50	2.34	11.03	3.4
7.	13	1.110	95.80	2.03	10.48	3.0
GROUP : $G^{-}L^{-}$ (21 days) :						
1.	15	1.041	95.80	2.12	8.07	3.50
2.	12	1.081	95.80	2.25	8.82	3.35
3.	13	1.118	90.45	2.05	7.72	3.25
4.	13	1.022	90.45	2.12	7.72	3.50
5.	13	1.173	98.5	2.12	8.27	3.50
6.	10	0.930	95.80	2.03	8.27	3.00
7.	9	0.920	95.8	2.03	7.82	2.80

EXPT : Effects of dietary rehabilitation on brain ACh, AChE and ChAc activity of rats undernourished in early life.

1	2	3	6	7
GROUP : $G^+L^+R^+$:				
1	135	1.570	8.61	4.5
2.	140	1.585	8.51	4.3
3.	145	1.605	10.54	4.8
4.	130	1.520	8.51	4.0
5.	128	1.380	9.45	3.8
6.	126	1.380	9.00	4.3
GROUP : $G^+L^-R^+$:				
1.	80	1.385	10.54	4.20
2.	75	1.370	10.54	3.90
3.	72	1.425	9.93	3.70
4.	70	1.280	9.93	3.50
5.	74	1.330	12.05	3.85
6.	75	1.300	9.93	4.00
GROUP : $G^-L^-R^+$:				
1.	70	1.350	12.05	4.00
2.	65	1.300	10.54	3.75
3.	62	1.270	8.85	3.00
4.	66	1.285	9.93	3.50
5.	60	1.260	8.85	3.50
6.	67	1.295	10.54	4.50

EXPT : Effects of maternal protein deficiency during gestation and/or lactation on brain ACh levels of the progeny at 21 days.

	1	2	3	8
GROUP : G^+L^+ :				
	1.	40	1.360	1.45
	2.	42	1.385	1.50
	3.	46	1.400	1.65
	4.	47	1.410	1.75
	5.	45	1.380	1.55
	6.	48	1.415	1.70
GROUP : G^+L^- :				
	1.	10	1.020	1.10
	2.	14	1.223	1.40
	3.	12	1.190	1.20
	4.	14	1.220	1.55
	5.	15	1.250	1.60
	6.	13	1.100	1.40
GROUP : G^-L^- :				
	1.	9	0.925	0.90
	2.	10	0.940	1.05
	3.	14	1.050	1.55
	4.	14	1.100	1.40
	5.	12	1.085	1.25
	6.	13	1.100	1.20

EXPT : Effects of post-weaning protein deficiency on cholinergic system :

1	2	3	8
GROUP : 5 % Protein :			
1.	74	1.34	1.86
2.	59	1.28	1.79
3.	56	1.32	1.83
4.	73	1.35	1.15
5.	63	1.33	2.00
6.	75	1.31	1.42
GROUP : 8 % Protein :			
1.	83	1.34	1.94
2.	71	1.34	2.38
3.	86	1.41	1.70
4.	106	1.36	1.89
5.	102	1.35	2.05
6.	98	1.43	1.22
GROUP : 20 % Protein ad lib. :			
1.	139	1.52	2.40
2.	120	1.51	2.91
3.	140	1.54	2.12
4.	145	1.58	2.03
5.	130	1.44	2.37
6.	144	1.45	2.50

Contd....

1	2	3	8
GROUP : 20% Protein Pair-fed to 5% protein :			
1.	93	1.32	2.40
2.	95	1.39	2.50
3.	88	1.43	1.70
4.	89	1.31	1.98
5.	110	1.31	1.73
6.	95	1.36	1.85
GROUP : 20% Protein fed at 50% intake of 5% Protein Group :			
1.	63	1.28	2.40
2.	63	1.39	2.06
3.	56	1.24	2.06
4.	55	1.30	2.11
GROUP : 20 % Protein fed at 25% intake of 5% Protein Group :			
1.	39	1.28	1.48
2.	37	1.23	1.21
3.	38	1.25	1.30
4.	36	1.23	1.60
5.	45	1.31	0.92
6.	39	1.26	1.29

EXPT : Effects of postweaning deficiencies of protein and calories on cholinergic system.

1	2	3	4	5	6	7
GROUP : 5 % Protein ad lib.						
1.	60	1.545	134.6	1.26	9.93	4.8
2.	48	1.447	112.2	1.12	7.62	3.8
3.	60	1.493	97.9	1.58	8.62	4.3
4.	67	1.501	130.6	1.58	10.54	5.5
5.	80	1.495	112.2	1.57	8.51	4.8
GROUP : 8 % Protein ad lib.						
1.	108	1.480	115.0	1.38	10.47	5.2
2.	109	1.490	124.9	1.58	8.82	3.8
3.	90	1.460	124.9	1.50	9.93	3.8
4.	92	1.405	107.1	1.45	9.37	5.0
5.	130	1.405	83.6	1.29	11.03	4.5
GROUP : 20 % Protein ad lib.						
1.	93	1.496	115.0	1.32	10.54	3.8
2.	133	1.560	120.2	1.38	7.28	4.8
3.	123	1.590	120.2	-	8.61	4.3
4.	123	1.526	120.2	1.45	8.51	4.5
5.	140	1.695	96.1	1.32	8.51	5.2
6.	117	1.528	124.9	1.25	7.62	4.0
7.	205	1.811	102.0	-	12.8	5.5

Contd....

1	2	3	4	5	6	7
GROUP : 20% Protein pair-fed to 5% Protein Group :						
1.	90	1.525	115.0	1.45	8.61	3.8
2.	106	1.540	115.0	1.51	9.93	5.0
3.	96	1.620	124.9	1.45	7.62	3.8
4.	95	1.495	134.6	1.45	7.62	4.5
5.	108	1.465	120.2	1.45	9.93	4.5
6.	130	1.580	107.1	1.64	10.54	5.0
7.	118	1.295	102.0	1.51	10.54	5.15
GROUP : 20% Protein fed at 50% intake of 5% protein Group :						
1.	68	1.425	129.8	1.64	9.93	4.80
2.	57	1.385	134.6	1.57	7.62	3.75
3.	45	1.380	124.9	1.51	7.62	3.45
4.	60	1.420	105.7	1.57	9.93	4.65
5.	42	1.330	124.9	1.45	7.62	3.85
6.	87	1.555	117.3	1.54	10.54	4.85
7.	91	1.565	97.0	1.41	9.93	4.65
GROUP : 20% Protein fed at 25% intake of 5% protein Group :						
1.	42	1.290	124.9	1.45	9.93	4.80
2.	35	1.315	134.6	1.58	7.62	3.45
3.	37	1.220	115.9	1.64	7.62	4.0
4.	47	1.440	110.5	1.32	9.93	4.80
5.	50	1.300	124.9	1.38	10.54	3.75
6.	50	1.436	89.8	1.51	8.61	3.45
7.	50	1.435	112.2	1.58	9.93	4.00
8.	52	1.423	93.9	1.51	10.54	4.80

EXPT : Post-weaning protein deficiency for a period of 65 weeks.

	1	2	3	4	5	6	7
GROUP : 5 % Protein diet ad lib.							
	1.	123	1.590	129.8	1.92	6.16	4.0
	2.	175	1.532	124.9	1.99	8.81	4.65
	3.	118	1.412	129.8	2.08	6.61	4.85
	4.	170	1.550	120.2	1.49	7.71	5.00
GROUP : 20% Protein diet ad lib.							
	1.	216	1.712	120.18	1.92	7.71	4.50
	2.	328	1.855	129.80	1.67	6.61	5.06
	3.	183	1.602	124.90	1.83	6.61	4.26
	4.	260	1.775	124.90	2.08	7.71	4.10

EXPT : Effects of pre- and post-natal thiamine deficiency on brain ACh levels :

1	Pair-fed Control			Thiamine deficient		
	2	3	8	2	3	8
GROUP : 7 days :						
1	10	0.628	1.55	7	0.485	1.35
2.	8	0.520		7	0.500	
3.	8	0.515	1.42	8	0.510	1.45
4.	7	0.505		9	0.525	
5.	7	0.500	1.40	6	0.465	1.25
6.	9	0.525		6	0.470	
7.	8	0.520	1.35	9	0.520	1.60
8.	7	0.485		8	0.510	
GROUP : 14 days :						
1.	16	0.885	1.65	15	0.980	1.70
2.	10	0.780		8	0.640	
3.	12	0.850	1.40	7	0.625	1.30
4.	14	0.880		10	0.740	
5.	10	0.665	1.20	12	0.860	1.35
6.	15	0.890		8	0.710	
7.	12	0.855	1.70	7	0.630	1.05
8.	14	0.855		10	0.770	
GROUP : 21 days :						
1.	25	1.285	1.80	18	1.185	1.45
2.	20	1.184		14	1.050	
3.	16	1.050	1.25	13	1.030	1.30
4.	19	1.175		20	1.205	
5.	24	1.305	1.40	12	0.985	0.85
6.	16	1.060		16	1.105	
7.	15	1.045	1.60	17	1.105	0.95
8.	26	1.300		16	1.085	
GROUP : 28 days :						
1.	32	1.350	1.85	24	1.305	1.24
2.	24	1.260	1.60	22	1.205	1.05
3.	20	1.145	1.36	16	1.020	0.85
4.	30	1.346	1.90	20	1.080	0.96

EXPT : Effects of pre- and post-natal thiamine deficiency on cholinergic system of the progeny.

1	2	3	4	5	6	7
GROUP : Pair-fed Control (7 days) :						
1.	8	0.510	60.9	2.20	2.75	0.50
2.	8	0.515				
3.	7	0.515	73.4	2.30	3.31	0.55
4.	7	0.505				
5.	7	0.500	55.5	1.85	3.39	0.60
6.	9	0.520				
7.	8	0.510	55.4	1.90	4.41	0.75
8.	10	0.630				
GROUP : Thiamine Deficient (7 days) :						
1.	6	0.480	55.4	1.80	3.08	0.55
2.	8	0.500				
3.	8	0.505	52.8	1.85	3.31	0.55
4.	7	0.500				
5.	7	0.490	65.7	2.10	3.52	0.65
6.	9	0.510				
7.	8	0.500	68.2	2.00	4.41	0.75
8.	9	0.515				
GROUP : Pair-fed Controls (14 days) :						
1.	18	0.910	85.0	2.50	6.5	2.0
2.	16	0.885	80.5	2.00	6.0	1.80
3.	10	0.780	65.0	1.90	3.75	1.45
4.	12	0.850	68.0	2.10	5.00	1.65
5.	14	0.855	75.0	2.30	-	-
6.	15	0.890	75.5	2.25	-	-
7.	12	0.850	68.0	2.00	-	-
8.	18	0.900	84.0	2.45	-	-

Contd....

	1	2	3	4	5	6	7
GROUP : Thiamine Deficient (14 days) :							
	1.	14	0.984	80.0	2.5	6.00	1.80
	2.	8	0.640	68.0	1.9	4.75	1.65
	3.	8	0.735	66.5	1.9	4.00	1.30
	4.	9	0.745	74.00	2.1	4.75	1.40
	5.	10	0.770	72.5	2.1	-	-
	6.	8	0.720	65.5	1.7	-	-
	7.	9	0.780	76.5	1.85	-	-
	8.	8	0.780	76.0	1.75	-	-
GROUP : Pair-fed Controls (21 days) :							
	1.	20	1.180	88.00	2.00	6.62	3.45
	2.	16	1.100	76.50	1.85	6.62	3.30
	3.	24	1.305	85.68	2.60	7.72	3.65
	4.	19	1.180	78.00	2.00	6.06	3.40
	5.	25	1.290	88.00	2.60	-	-
	6.	16	1.060	75.50	1.70	-	-
	7.	15	1.050	72.00	1.80	-	-
	8.	28	1.350	86.00	2.60	-	-
GROUP : Thiamine Deficient (21 days) :							
	1.	20	1.184	86.5	2.40	6.62	3.45
	2.	18	1.174	85.0	2.35	6.06	3.30
	3.	14	1.055	75.0	2.10	5.75	3.00
	4.	13	1.025	70.5	2.00	5.75	2.85
	5.	12	0.980	80.5	1.85	-	-
	6.	16	1.105	75.5	1.95	-	-
	7.	14	1.052	68.00	1.75	-	-
	8.	15	1.062	74.00	1.75	-	-
GROUP : Pair-fed Controls (28 days) :							
	1.	20	1.150	84.5	2.0	8.15	3.20
	2.	24	1.275	86.0	2.2	8.40	3.40
	3.	32	1.350	88.5	2.5	8.85	4.40
	4.	26	1.305	85.5	2.1	8.20	3.45
GROUP : Thiamine Deficient (28 days) :							
	1.	16	1.025	79.5	1.80	7.45	3.0
	2.	18	1.105	82.0	1.90	7.90	3.4
	3.	24	1.300	86.5	2.20	8.60	4.0
	4.	20	1.150	85.5	2.00	7.90	3.2

EXPT : Effects of pre- and post-natal thiamine deficiency on weights of brain regions at 21 days.

Sample No.	1	2	Cortex (g)	Cerebellum (g)	M & P (g)
GROUP : G^+L^+ - Pair-fed Control :					
1	1.	22	0.923	0.166	0.095
	2.	26	0.962	0.165	0.088
	3.	22	0.918	0.132	0.127
	4.	24	0.983	0.158	0.108
2	5.	24	0.950	0.150	0.130
	6.	23	0.915	0.145	0.115
	7.	25	0.979	0.140	0.105
	8.	26	0.970	0.140	0.105
3	9.	19	0.908	0.125	0.130
	10.	26	1.020	0.145	0.135
	11.	21	0.902	0.148	0.115
	12.	21	0.920	0.135	0.105
4	13.	21	0.980	0.158	0.122
	14.	22	0.915	0.153	0.117
	15.	23	0.980	0.152	0.120
	16.	24	0.984	0.160	0.125
GROUP : G^-L^- - Thiamine deficient :					
1	1.	16	0.798	0.127	0.075
	2.	21	0.845	0.147	0.128
	3.	18	0.835	0.139	0.110
	4.	16	0.798	0.135	0.086
2	5.	16	0.878	0.112	0.098
	6.	19	0.820	0.140	0.130
	7.	19	0.828	0.120	0.104
	8.	17	0.822	0.116	0.085
3	9.	18	0.950	0.148	0.122
	10.	19	0.946	0.148	0.124
	11.	17	0.976	0.147	0.098
	12.	19	0.945	0.151	0.115
4	13.	18	0.853	0.138	0.097
	14.	19	0.860	0.120	0.136
	15.	17	0.905	0.122	0.116
	16.	20	0.880	0.145	0.110

EXPT : Effects of pre- and post-natal thiamine deficiency on AChE activity in the brain regions of rats at 21 days.

Sample No.	Pair-fed Control			Thiamine Deficient		
	Cortex	Cerebellum	M & P	Cortex	Cerebellum	M & P
			Brain DNA (mg/g)			
1	1.73	5.71	1.96	1.61	6.43	1.49
2	1.61	6.55	1.96	1.61	5.71	1.61
3	1.55	6.07	1.90	1.73	6.78	1.84
4	1.61	6.43	1.90	1.61	6.79	1.85
			Brain protein (mg/g)			
1	80.90	90.50	78.50	90.50	83.50	85.70
2	80.90	88.10	114.30	85.70	90.50	92.80
3	88.10	83.30	102.40	76.20	92.80	85.70
4	71.40	88.10	92.80	90.50	8.90	88.10
			AChE - umoles of subs. hydrolyzed/min./g. brain.			
1.	1.654	1.654	3.860	2.206	2.206	2.757
2	1.654	1.654	3.088	2.206	1.544	1.875
3	2.426	1.094	4.412	3.309	1.324	2.016
4	2.206	2.206	3.300	2.206	1.875	3.419

EXPT : Effects of postnatal thiamine deficiency on brain ACh levels.

1	Pair-fed Control			Thiamine Deficient		
	2	3	8	2	3	8
GROUP : 7 days :						
1	8	0.540	1.45	8	0.530	1.45
2	10	0.610		7	0.520	
3	10	0.608	1.50	9	0.530	1.60
4	8	0.510		10	0.605	
5	7	0,525	1.40	8	0.535	1.45
6	9	0.528		9	0.525	
7.	10	0.580	1.65	10	0.610	1.40
8.	8	0.520		8	0.510	
GROUP : 14 days :						
1	15	0.855	1.44	12	0.855	1.30
2	10	0.780		16	0.890	
3	18	0.980	1.85	10	0.860	1.65
4	17	0.960		9	0.780	
5	12	0.850	1.45	14	0.860	1.59
6	14	0.860		13	0.855	
7	16	0.865	1.58	9	0.780	1.60
8	15	0.870		11	0.828	
GROUP : 21 days :						
1	35	1.275	1.44	25	1.225	1.55
2	40	1.310	1.62	20	1.125	1.30
3.	36	1.280	1.75	18	1.110	1.20
4.	44	1.380	1.75	28	1.320	1.92
5	45	1.380	1.80	30	1.350	1.80
6	38	1.280	1.67	26	1.340	1.65
7	40	1.305	1.78	22	1.140	1.25
8	36	1.275	1.80	28	1.320	1.90
GROUP : 28 days :						
1	60	1.460	2.25	40	1.385	1.50
2	65	1.504	1.96	46	1.400	1.60
3	58	1.420	1.75	38	1.365	1.44
4.	60	1.465	1.78	39	1.370	1.48
5	56	1.410	1.70	36	1.340	1.44
6	55	1.380	2.45	34	1.340	1.40
7	54	1.360	1.95	34	1.330	1.38
8	60	1.455	1.76	40	1.385	1.55

EXPT : Effects of post-natal thiamine deficiency on cholinergic system of the progeny.

1	2	3	4	5	6	7
GROUP : Pair-fed Control (7 days) :						
1.	7	0.510	53.6	1.94	2.75	0.55
2.	9	0.575	75.5	2.66	3.31	0.50
3.	8	0.550	60.9	2.26	3.31	0.60
4.	8	0.535	73.1	2.26	3.97	0.75
5.	9	0.595	68.2	2.02	3.31	0.65
6.	7	0.495	63.3	2.10	4.41	0.85
GROUP : Thiamine Deficient (7 days) :						
1.	8	0.530	65.7	1.94	3.75	0.60
2.	8	0.540	65.7	2.50	3.08	0.50
3.	8	0.585	60.9	2.18	3.31	0.50
4.	9.	0.535	60.9	2.18	4.41	0.65
5.	7	0.425	80.3	2.81	5.29	0.80
6.	5	0.565	43.8	1.77	3.52	0.60
7.	7	0.585	53.6	2.20	3.31	0.55
8.	8	0.570	68.2	2.34	4.41	0.80
GROUP : Pair-fed Control (14 days) :						
1.	14	0.860	75.5	2.18	4.00	1.50
2.	10	0.780	65.5	1.95	3.75	1.45
3.	16	0.865	80.5	2.25	6.00	1.80
4.	18	0.980	85.0	2.50	6.50	2.00
5.	12	0.850	70.5	2.00	4.50	1.50
6.	14	0.860	75.5	2.45	5.00	1.65
7.	15	0.870	78.0	2.25	5.75	1.80
8.	15	0.870	80.0	2.10	6.00	2.00
GROUP : Thiamine Deficient (14 days) :						
1.	11	0.828	70.5	2.10	5.00	1.60
2.	16	0.890	80.5	2.60	6.00	1.80
3.	10	0.860	65.5	2.20	4.75	1.65
4.	9	0.780	64.0	1.95	4.00	1.20
5.	12	0.870	75.0	2.40	5.50	1.30
6.	14	0.860	80.0	2.50	5.50	1.65
7.	9	0.780	70.0	1.90	4.00	1.60
8.	10	0.820	78.5	2.30	4.75	1.65

Contd.....

1	2	3	4	5	6	7
GROUP : Pair-fed Control (21 days) :						
1.	41	1.325	88.06	2.18	7.17	3.50
2.	38	1.385	78.54	2.12	7.72	3.65
3.	34	1.185	90.44	2.34	6.06	3.30
4.	36	1.225	109.48	2.25	6.62	3.40
5.	43	1.345	85.68	2.81	-	-
6.	36	1.310	78.54	2.19	-	-
7.	37	1.260	85.68	1.87	-	-
8.	36	1.315	88.06	2.03	-	-
GROUP : Thiamine Deficient (21 days) :						
1.	20	1.255	88.06	2.62	6.62	3.45
2.	27	1.275	80.92	2.19	6.06	3.40
3.	25	1.220	85.68	2.19	6.06	3.00
4.	21	1.260	95.20	-	6.62	3.50
5.	21	1.245	78.54	-	-	-
6.	22	1.200	80.92	2.12	-	-
7.	25	1.205	78.54	1.87	-	-
8.	22	1.205	80.92	1.94	-	-
GROUP : Pair-fed Control (28 days) :						
1.	60	1.495	90.47	1.99	9.65	4.20
2.	57	1.490	90.47	2.32	8.85	3.80
3.	57	1.415	88.09	2.19	8.60	3.35
4.	60	1.445	85.79	2.06	9.50	3.80
5.	58	1.405	90.47	2.25	-	-
6.	57	1.415	88.57	2.38	-	-
7.	55	1.470	88.09	1.93	-	-
8.	57	1.435	90.47	1.93	-	-
GROUP : Thiamine Deficient (28 days) :						
1.	43	1.395	85.70	1.74	9.40	4.00
2.	35	1.350	97.60	1.74	8.60	3.35
3.	37	1.400	92.80	2.12	8.60	3.60
4.	37	1.440	-	2.18	8.25	3.80
5.	40	1.410	85.70	2.26	-	-
6.	38	1.425	-	2.18	-	-
7.	38	1.370	92.85	2.25	-	-
8.	36	1.420	97.62	2.52	-	-

EXPT : Effects of dietary rehabilitation in Pre- and Post-natally thiamine deficient rats.

1	Control			Rehabilitated		
	2	3	8	2	3	8
1.	125	1.480	2.10	91	1.310	1.65
2.	135	1.560	2.40	110	1.480	1.90
3.	110	1.460	2.00	135	1.540	2.40
4.	105	1.380	1.85	98	1.380	1.80
5.	130	1.540	1.80	125	1.495	2.40
6.	140	1.580	3.20	125	1.485	2.00

EXPT : Effects of post-weaning thiamine deficiency on thiamine content (ug/g) in the tissues.

Group	Sample No.	Cortex	Cerebellum	M & P
I. Ad lib. Control	1	3.20	4.60	4.15
	2	2.82	4.07	7.05
	3.	2.83	2.65	4.38
	4	3.17	2.93	4.95
II. Pair-fed Control	1	3.43	2.54	4.56
	2	2.88	1.82	4.69
	3	2.78	2.38	4.66
	4.	1.96	2.90	2.76
III. Thiamine Deficient	1	0.72	0.50	2.39
	2	0.93	0.48	1.19
	3	0.70	0.69	1.91
	4	1.38	1.16	1.29

EXPT : Effects of post-weaning thiamine deficiency on brain ACh levels.

	1	3	8		1	3	8
Gr.I : 100% Thiamine ad.lib.				Gr.VI : 0 % Thiamine ad lib.			
	1.	1.305	3.60		1.	1.050	0.50
	2.	1.550	2.22		2.	1.150	1.39
	3.	1.450	2.00		3.	1.310	1.00
	4.	1.600	2.10		4.	1.360	1.00
Gr.II : 100% Thiamine P.F.*				Gr.III : 50% Thiamine P.F.*			
	1.	1.265	2.30		1.	1.390	1.84
	2.	1.250	2.33		2.	1.320	1.60
	3.	1.260	-		3.	1.090	1.34
	4.	1.150	1.82		4.	1.415	1.21
	5.	1.360	-		5.	1.405	1.36
	6.	1.410	1.63		6.	1.410	-
	7.	1.360	-		7.	1.260	2.00
	8.	1.350	1.72				
Gr. IV: 25% Thiamine P.F.*				Gr. V : 10% Thiamine P.F.*			
	1.	1.450	1.60		1.	1.425	1.55
	2.	1.310	1.10		2.	1.305	2.30
	3.	1.280	-		3.	1.180	1.54
	4.	1.100	1.41		4.	1.245	1.32
	5.	1.415	-		5	1.430	1.12
	6.	1.260	1.08		6.	1.310	1.02
	7.	1.365	1.21		7.	1.300	1.10
	8.	1.300	1.69				

* P.F = Pair-fed to ad lib. intake of 0% thiamine group (Gr. VI).

EXPT : Effects of post-weaning thiamine deficiency on moisture content (%) of the different tissues :

No.	Liver	Heart	Cortex	Cerebellum	M & P
GROUP : Thiamine Deficient :					
1.	69.13	78.01	78.25	77.03	71.42
2.	70.64	77.14	77.96	76.68	70.96
3.	70.42	74.72	77.61	81.34	71.61
4.	69.63	83.05	77.92	76.88	77.61
5.	69.91	73.11	77.95	75.96	70.94
6.	68.67	78.36	77.75	76.76	70.77
GROUP : Control ad lib.					
1.	69.18	75.78	78.51	77.22	72.51
2.	69.21	76.45	81.19	77.51	71.89
3.	68.88	76.02	77.84	77.36	71.42
4.	69.43	77.68	78.05	76.73	71.74
5.	68.90	76.16	78.35	77.04	72.38
6.	69.36	76.28	78.21	76.75	75.46
GROUP : Pair-fed Control.					
1.	68.84	-	79.19	77.32	71.97
2.	68.25	73.25	77.89	78.65	65.86
3.	64.90	76.21	78.53	73.30	73.96
4.	68.61	76.20	78.39	77.25	66.66
5.	71.16	76.49	78.40	77.01	72.29
6.	68.90	77.02	79.03	78.00	72.78