of liquid paraffin which prevents the rapid spread of infective foci that results in the formation of plaques.

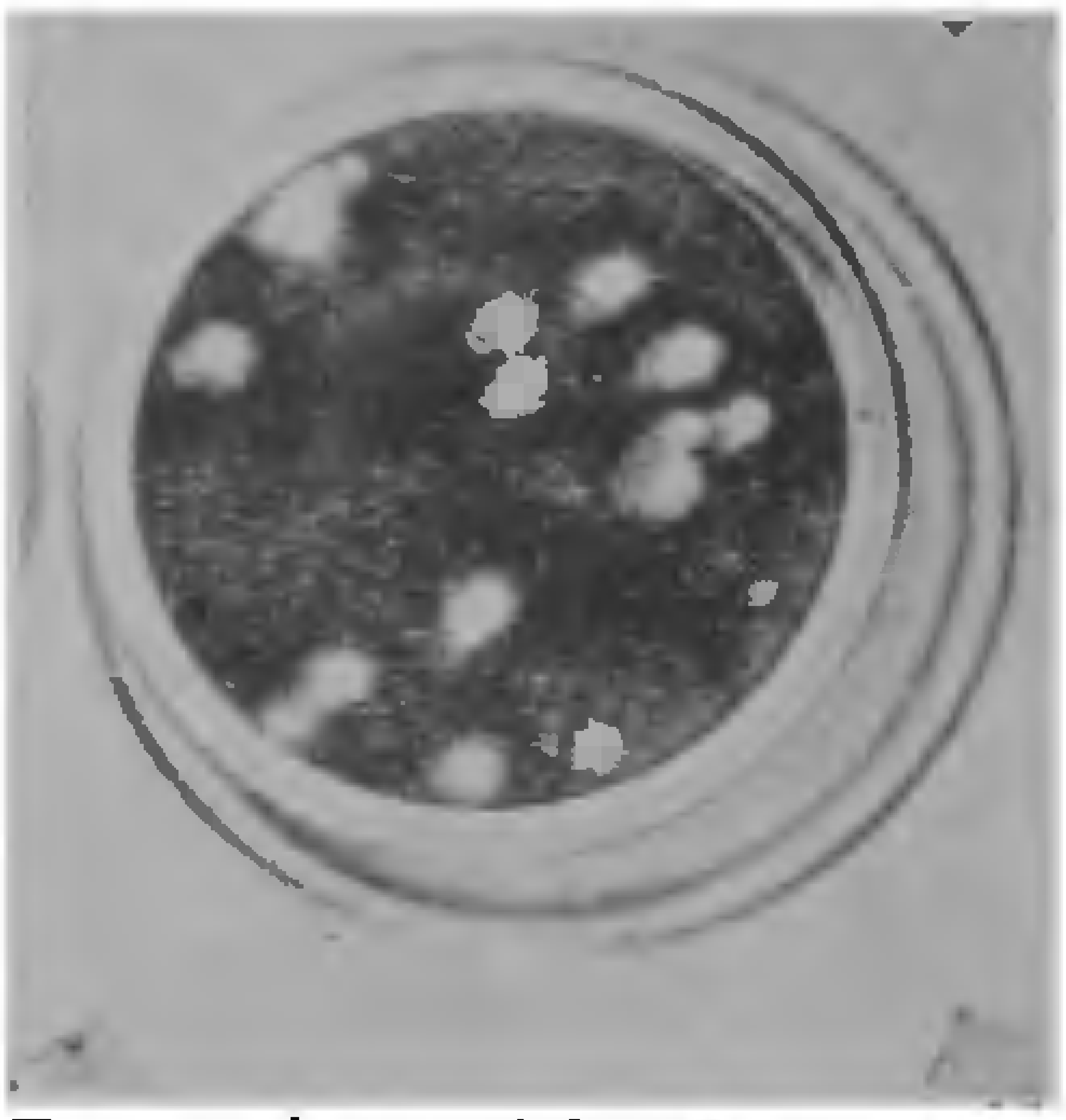

FIG. 2. Plaques of Chandipura Virus.

The advantage of plaquing under this overlay is that it permits the virus to form plaques in liquid tissue culture medium itself and the effect of LPFN is only indirect on the plaques. The easy availability of LPFN makes it an useful adjunct to plaque study.

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PHYSIOLOGICAL STUDIES ON THE EFFECTS OF NUTRITIONAL IMBALANCE ON THE CENTRAL NERVOUS SYSTEM: EFFECTS OF THIAMINE DEFICIENCY ON THE REGIONAL PROTEIN METABOLISM IN THE BRAIN OF CHICKEN, *GALLUS DOMESTICUS*

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ABSTRACT

The levels of RNA, protein and the activity levels of Aspartate amino-transferase (AAT) and Alanine aminotransferase (AlAT) in the cerebrum, cerebellum and medulla oblongata of thiamine-deficient chicken increased indicating an augmentation in the activity of the protein synthetic machinery in B_1-hypovitaminous birds.

THE proteins in the brain of vertebrates are known to exist in a dynamic state[1]. The synthesis and catabolism of proteins in the mammalian brain have been extensively studied[2-4]. It has been demonstrated that the levels of protein, total nitrogen and DNA remain practically unchanged, even in states of severe protein deficiency or starvation[6-8].

However, information about the regional distribution and metabolism of protein in the vertebrate brain during thiamine deficiency is lacking. Since marked changes in the enzymes connected to carbohydrate metabolism occur in animals with thiamine deficiency[5,9], it might be expected that the brain would also show alterations connected to protein metabolism during thiamine deficiency. Little work has been done to test this possibility. Hence, the present study was undertaken.

MATERIAL AND METHODS

Two days old, male white leghorn chicken, *Gallus domesticus* (10–12 g) were reared in the laboratory at $36 \pm 2°$ C. The controls were fed on standard chicken feed. The experimental animals were fed on polished rice to induce thiamine deficiency[5]. Water was available *ad libitum* to both the groups.

The normal and experimental birds were decapitated after 28 days. The brain was dissected and kept in Ringer at 0° C. The different regions of the brain (cerebrum, cerebellum and medulla oblongata) were separated with sterilized instruments, weighed in ice-cold Ringer and immediately used for analysis.

Proteins from the samples were precipitated by trichloro acetic acid (BDH) and estimated by the micro-biuret method[10].

RNA was extracted by the method of Schmidt-Thanhausser-Schneider[11] and estimated by orcinol colour reaction following the colorimetric procedure described by Glick[12].

The Aspartate aminotransferase (AAT, E.C. 2·6·1·1) and alanine amino transferase (AlAT, E.C. 2·6·1·2) activities were determined following the colorimetric procedure of Reitman and Frankel as described by Bergmeyer[13].

Results and Discussion

Results are summarized in Tables I–III.

Brain weight and body weight decreased considerably in the thiamine-deficient state (Table I).

Table I

Body and Brain weight of normal (control) and thiamine-deficient chicken[1]

Body weight in g Control	Body weight in g Test	Brain weight in g Control	Brain weight in g Test
82·7	31 3	1·97	1·37
±	±	±	±
11·0	5·5	0·2	0·2
75·8–98 8*	29–40·8*	1·8–2·2*	1·1–1·5*

[1] Values are mean ± S.D. of 28 observations.
* Represents the weight range.
Test = Thiamine-deficient chicken.

Such a differential response of the different regions of the brain may be related to the differences in the functional status of the region concerned. However, the general increase in RNA and protein levels observed in the present investigation points to an augmentation in the activity of the protein synthetic machinery in B_1-hypovitaminous birds.

The activity levels of AAT and AlAT, in general, showed an increase in all the compartments of the brain studied, *viz.*, cerebrum, cerebellum and medulla oblongata during thiamine deficiency (Table III). It is also seen (Table III) that the increase in the levels of the aminotransferase activities showed variation in relation to the region of the brain. Further, the brain stem showed marked response for the changes in the activity levels of AAT and the cerebrum demonstrated the maximum response for changes in the activity levels of AlAT (Table III). It is also noteworthy that the activity level of AAT was more than that of AlAT in the CNS of the thiamine-deficient birds. The general increase in the activity levels of both the aminotransferases during thiamine deficiency may be either due to the activation of the enzyme synthesis by the altered cellular environment prevailing in the brain, or due to an increase in the rate of enzyme synthesis. Supporting this suggestion, an increase in the levels of RNA and proteins have been detected (Table II).

Table II

Changes occurring in the levels of RNA and protein in different regions of the brain of normal (control) and Thiamine-deficient chicken, Gallus domesticus[1]

	Cerebrum Control	Cerebrum	Cerebrum Test	Cerebellum Control	Cerebellum	Cerebellum Test	Medulla Control	Medulla	Medulla Test
RNA mg/g	4.75 ± 1·1	[a] +86·3 $p>$ 0·01	8·85 ± 1·2	4·8 ± 1·3	[a] +233·4 $p>$ 0·01	16·0 ± 4·0	4·3 ± 1·2	[a] +88·2 $p>$ 0·01	8·0 ± 1·9
Protein mg/g	106 ± 4·8	[a] + 0·46 NS	106·5 ± 7·6	105·5 ± 17·9	[a] + 5·6 NS	111·5 ± 13·9	75·0 ± 6·8	[a] + 8·0 NS	81·0 7

[1] Values are mean ± S.D. of 10 observations.
[a] Percentage change, signs (+) indicate an increase over controls.
NS = not significant.
Test = Thiamine-deficient birds.

In general, the protein content increased (negligible in the cerebrum and cerebellum and considerable in the brain stem) on inducing thiamine deficiency (Table II) Paralleling the increase in proteins, RNA levels also increased (significant) in all the regions investigated during B_1-hypovitaminosis. Further, marked differences in the protein and RNA contents of the cerebrum, cerebellum and medulla oblongata were noted (Table II). A remarkable feature was that the cerebrum demonstrated least response for changes in RNA and also protein in the thiamine deficient state. Maximum response for changes in RNA was exhibited by cerebellum and for protein levels was exhibited by the brain stem on inducing thiamine deficiency (Table II).

AAT and AlAT are of great clinical significance[14]. The levels of these aminotransferases in serum have been studied as an index for the diagnosis of disease-conditions like hepatic and cardiac infractions[13]. The liver aminotransferase activities have been known to increase under several pathological conditions[14,15]. In support of this the aminotransferase activities of the brain have shown a significant augmentation during

TABLE III

Aminotransferase activities in different regions of the Brain of normal (controls) and Thiamine-deficient chicken[1] Gallus domesticus

	AAT			AlAT		
	Cerebrum	Cerebellum	Medulla	Cerebrum	Cerebellum	Medulla
Controls	1·15[2]	0·92	1·2	1·02	1·1	1·34
	±	±	±	±	±	±
	0·06	0·06	0·076	0·07	0·06	0·23
Test	8·7	8·7	11·6	2·4	1·95	2·6
	±	±	±	±	±	±
	2·6	1·5	1·5	0·05	0·2	0·08
	[a]+656 5	[a]+846·6	[a]+866·7	[a]+134·7	[b]+ 77·2	[a]+94·0
	$p > 0.01$	$p > 0.01$	$p > 0.01$	$p > 0.05$	$p > 0.01$	$p > 0.05$

[1] Activity expressed as micromoles of keto acid formed /g wet weight of the tissue/hr.

[2] Values are Mean ± S.D. of 12 observations.

[a] Percentage change, sign (+) indicating an increase over controls.

thiamine deficiency in chicken. Since the aminotransferases are known to link amino acid and carbohydrate metabolism[14], an increase in aminotransferase activities during thiamine deficiency indicates that the contribution of glucogenic amino acids to the carbohydrate metabolism is more. It is therefore possible that carbohydrate metabolism is not maintained at a steady level during thiamine deficiency. The lower activity levels of AlAT compared to the activity levels AAT account for the decreased production of from pyruvic acid in the brain of B_1-hypovita- THE birds. This is of significance in the metabolism to as alanine plays a vital role in linking carbo- and ca and protein metabolism[14]. This is in accord- have both the earlier reports of Peters[5] and Nalini strate Nayeemunnisa[9].

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