Effects of Thiamine Deprivation on Friend Virus Leukemia in Mice (32952)

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As a result of increasing interest in the biological properties of shellfish extracts, particularly those concerned with antiviral and antineoplastic modes of action, there has been a growing number of reports (1–4) in the literature associated with the specific effects of these substances. These reports have been primarily concerned with the therapeutic aspects of various preparations of shellfish extracts against a variety of experimental model systems including transplantable tumors and virus-induced neoplasia. Still to be elucidated, however, is the biologic mechanism of action of these shellfish extracts in an antiviral or antineoplastic capacity. In the light of previously reported (5) biologic enzyme degradation effects of certain fish and shellfish tissues, it was thought that study of these systems might provide some insight into the actual antineoplastic mechanism of action of these substances.

In certain shellfish and fish tissues there is a heat labile enzyme which destroys thiamine. Attention was first drawn to this "thiaminase" by the appearance of Chastek paralysis in foxes fed a diet containing 10% or more of uncooked fish. The disease is characterized by weakness, anorexia, progressive ataxia, and spastic paraplegia. The similarity between the lesions in the fox and the lesions seen in man in Wernicke's syndrome lent support to the same basic etiology of thiamine deficiency.

Kupstas and Hennessy (6) in 1957 showed the isolation, proof of structure, and synthesis of ichthiamin, a product of the action of minced clam tissue on thiamine, and the mechanism of inactivation of this vitamin by the clam.

Because of similarities noted in the preparation of clam extracts used in the ichthiamin experiments and those extracts used later in tumor control experiments, the suggestion of a possible similar mechanism of action was entertained.

Because thiamine is so obviously important in several aspects of metabolism and, in view of the demonstrated destruction of thiamine by extracts also shown to be oncostatic, it was decided to test for a correlation between the two. Since Mercenaria extracts have been used successfully in the Friend virus murine leukemia system (4), it was decided to employ this system in a test of the hypothesis.

Materials and Methods. In each of two separate experiments, 200 DBA/2 female mice were obtained from a breeder [2] at 6 weeks of age and immediately randomly separated into three groups, 100 (Group A), 50 (Group B) and 50 (Group C), for purposes of virus inoculation and dietary control.

Diet. Specific groups were fed either a normal diet of Purina lab chow and water, *ad libitum*, or a special pelleted food product containing all necessary amino acids, minerals, and vitamins with the exception of thiamine, and water *ad libitum* (Table I). This thiamine-free test diet was obtained from a commercial manufacturer.[3]

Procedures and Results. All animals after being randomly grouped were immediately begun on specific diets. The group of 100 (Group A) and one of the 50 (Group C) were placed on the thiamine-free diet while the remaining group of 50 (Group B) was maintained on a normal diet. This was done to metabolically eliminate all endogenous thiamine prior to viral inoculation. Seven days later, all mice, except for the group of 50 put on the thiamine-free diet (Group C), were inoculated with 0.1 ml of a 1:4 ml saline dilution of Friend virus cell-free filtrates, intraperitoneally.

Three weeks after virus inoculation 50 mice were selected randomly from the group of 100

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TABLE I. Comparative Analysis of Diets Used.

Control diet (lab chow)	(%)	Thiamine-free diet	(%)
Crude protein	23	Crude protein (casein hydrolyzate assayed vitamin free)	18
Carbohydrate	67		
Fat	4.5	Carbohydrate	68
Crude fiber	5.5	Hydrogenated vegetable oil	10
		USP no. 14 salt mixture	4

Vitamins	(per 100 gm)	Vitamins	(per 100 gm)
B_{12}	1.25 μg	Alpha tocopherol	165 mg
Calcium pantothenate	1700 μg	Calcium pantothenate	2500 μg
Choline chloride	11 mg	Choline chloride	20 mg
Folic acid	80 μg	Menadione	50 μg
Riboflavin	500 μg	Riboflavin	20 μg
Thiamine	480 μg	Pyridoxine HCl	20 μg
Niacin	4.35 mg	Niacin	4 mg
Vitamin A	400 USP units	Vitamin A conc. (200,000 units/gm)	20,000 IU
D (activated sterol)	438 IU	Vitamin D conc. (400,000 units/gm)	40,000 IU
Vitamin E	350 μg	*i*-inositol	100 mg

TABLE II. Mean Body and Spleen Weights.[a]

Female mice	Treatment	Expt. no.	MIBW (gm)	MTBW (gm)	MSW (mg)
Group A	Thiamine deficient + virus	1	17.0 ± 1.2	10.34 ± 1.8	23.7 ± 2.5
100		2	16.8 ± 1.3	10.46 ± 1.4	27.8 ± 3.0
Group B	Normal diet + virus	1	16.5 ± 1.3	18.29 ± 2.5	950 ± 150
50		2	16.08 ± 2.0	18.37 ± 2.2	720 ± 110
Group C	Thiamine deficient, no virus	1	16.80 ± 1.5	10.01 ± 1.2	26.0 ± 5.0
50		2	16.04 ± 1.5	10.71 ± 1.4	27.6 ± 4.5
Group D	(Aliquot of group A)	1[b]	10.34 ± 1.1	17.84 ± 1.4	1100 ± 132
50	refed thiamine	2[c]	10.41 ± 1.2	17.51 ± 2.0	824 ± 80

[a] Abbrev.: MIBW = mean initial body weight; MTBW = mean total body weight; and MSW = mean splenic weight.

[b] 43 survivors.

[c] 40 survivors.

(Group A) and retained for further dietary manipulation (Group D). All other animals were sacrificed and both total body weight and splenic weight were determined. Histologic examination was performed on all spleens. The remaining 50 (Group D) previously maintained on a thiamine-free diet were subsequently fed a regular chow diet and water with added thiamine (0.1 mg/ml) *ad libitum*. These animals were then sacrificed 21 days after being placed on the thiamine-enhanced diet for total body weight, splenic weight determinations, and histologic examination.

As seen in Table II, the animals on a thiamine-free diet all lost a sizable proportion of total body weight (MTBW) and had markedly diminished splenic size (MSW). The differences in total body weight loss and splenic size between Group A and Group C are not significant. In contrast, the animals of Group B, which were continuously fed a normal diet after virus inoculation, showed an anticipated mean total body weight gain and

an approximate 40 times greater mean splenic weight increase. The largest proportionate weight gains were seen in Group D, however, which was subsequently refed a high thiamine diet after having been virus infected and originally maintained on a thiamine-free diet.

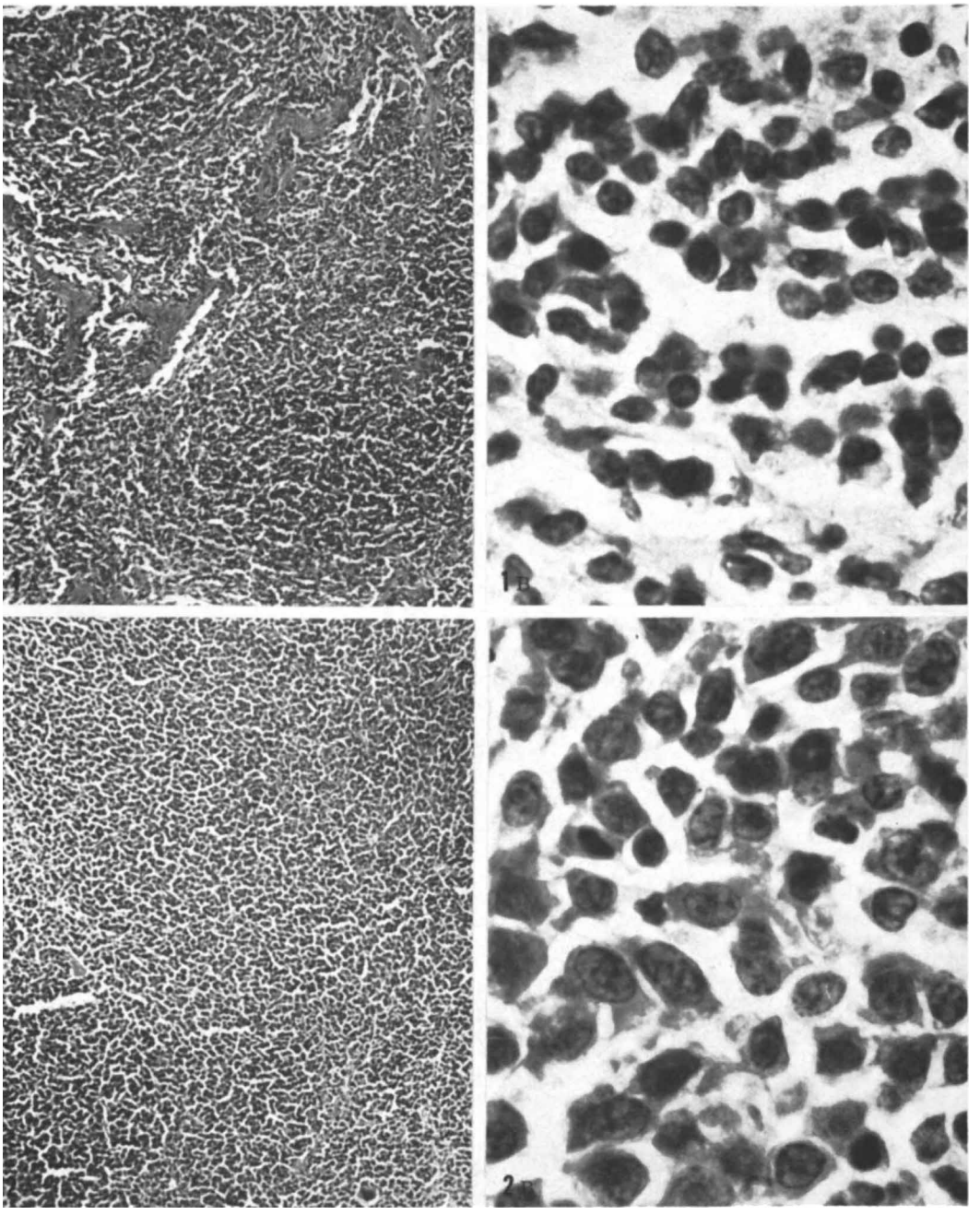

FIG. 1A. Splenic section Group C; hematoxylin and eosin × 100. B. Splenic section Group C; hematoxylin and eosin × 1200.

FIG. 2A. Splenic section Group B; hematoxylin and eosin × 100. B. Splenic section Group B; hematoxylin and eosin × 1200.

The splenic weights were also greatest in the animals from Group D.

All thiamine deprived mice exhibited hyperirritability, scruffy coat and ataxia with a few developing hind leg paralysis characteristic of beri-beri in this species; none of these animals showed histologic evidence of leukemia.

Figures 1a and b show photomicrographs of hematoxylin and eosin stained histologic sections of spleen from Group C at 100 and 1200 magnifications, respectively. This control group exhibits a typical follicular pattern in the lower magnification with a regular arrangement of uniform small cells. The higher magnifications show more distinctly the uniformity of individual cells with characteristic large basophilic nuclei and indistinct cytoplasm. These sections are those typically seen in normal mouse spleen.

Figures 2A and B are photomicrographs taken at 100 and 1200 magnifications of histologic sections of spleen from Group B. The lower magnification shows complete loss of follicular pattern and a diffuse pattern of pleomorphic cells of considerable variation in size and chromatin density. The higher magnification more clearly shows the marked variability of cell types including very large blast-like cells which have vacuolated nuclei and prominent nucleoli. Smaller hyperchromatic cells are also seen. These changes are consistent with typical histologic patterns seen in the spleens of experimentally induced Friend virus leukemia.

Figures 3a and b are again photomicrographs of hematoxylin and eosin stained histologic sections of spleen from Group A animals taken at 100 and 1200 magnifications. Despite virus inoculation, these splenic sections from thiamine-deprived animals retain a typically normal follicular pattern composed of regular small rounded cells with large basophilic nuclei. The higher magnification reveals only a small variation among the individual cells and a lack of mitoses or hyperchromaticism. These sections are completely compatible with normal mouse spleen.

Figures 4a and b are photomicrographs of hematoxylin and eosin stained splenic sections from Group D at 100 and 1200 magnifications respectively. The lower magnification shows marked pleomorphism, loss of follicles and a dense collagenous stroma. Cellular fragmentation is discernible in several areas and occasional large multinucleated cells are seen. In the higher magnification the great variety of cells are more easily seen including large vacuolated cells with distinct nucleoli as well as normal appearing cells with a small amount of cytoplasm. The chromatin derangements and pleomorphism exhibited here is fully compatible with Friend disease. Comparison of these changes exhibited here from Group A (Group D being an aliquot of Group A) shows the transformation which took place when thiamine was reintroduced into the diet of these animals.

Discussion. These data tend to support the premise that while thiamine certainly does not enhance neoplastic proliferation, its apparent absence seems to exert some type of protective effect against it. The similarity between the two groups which were both thiamine deficient but differed in that one group was virus-inoculated, is quite striking. It has been previously reported (7) that controlled starvation alone modified the leukemic manifestation in mice infected with Friend virus. It is difficult to separate the effects of the role of inanition from those of thiamine deficiency in these experiments, but a relative thiamine deficiency cannot be excluded in the studies of Sidwell *et al.* (7). Animals with full-blown symptomatology of beri-beri are quite lethargic and do not eat or drink as much as their virus-inoculated counterparts on a normal diet. It therefore is obvious that in the present study, inanition and thiamine deficiency coexisted. The surprising fact is, however, that *no* evidence of malignancy was found in the virus-inoculated, thiamine-deficient group, and only when an aliquot group was refed thiamine did leukemia become manifest. Obviously the viruses were still present, as shown by subsequent development of leukemia in this latter group, but the cellular mechanisms and the capabilities of the cells to respond to "malignant transformation" while being dietarily deprived, ostensibly only of thiamine, were impaired. The question of whether shellfish extracts,

which have been shown to be oncostatic, exert this effect via a thiamine degradation process, also previously demonstrated, is still hypothetical.

An interesting corollary which is not directly related but which illustrates the potential of thiamine inhibition or deprivation is the work of Guthrie *et al.* (8) who showed that

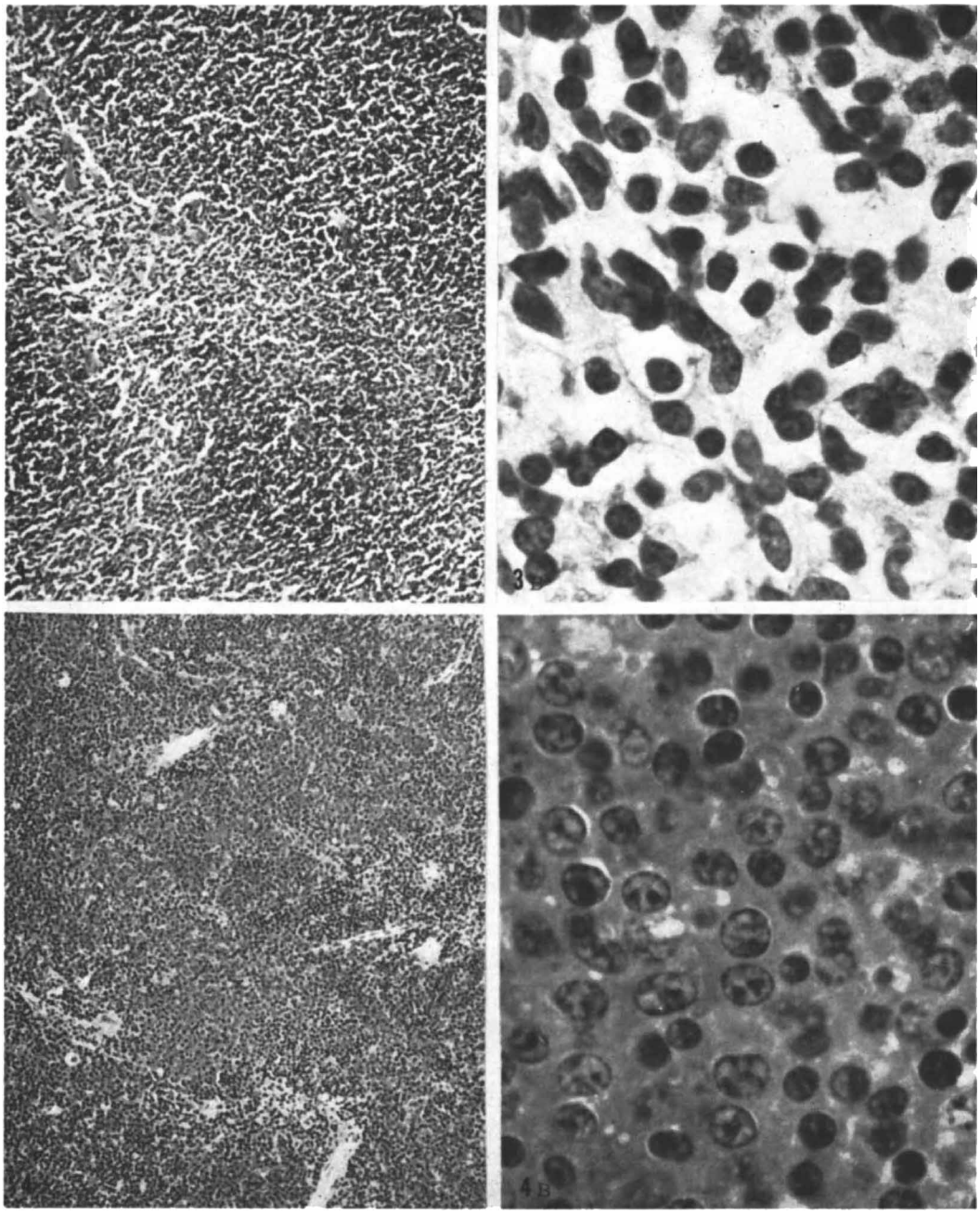

FIG. 3A. Splenic section Group A; hematoxylin and eosin × 100. B. Splenic section Group A; hematoxylin and eosin × 1200.

FIG. 4A. Splenic section Group D; hematoxylin and eosin × 100. B. Splenic section Group D; hematoxylin and eosin × 1200.

pyrimidine analogues of thiamine are very active in growth inhibition of microorganisms shown to be resistant to both amethopterin and purine antagonists.

It is a reported clinical observation (9) that patients suffering from Hodgkin's disease, leukemia, and alimentary tract carcinoma, all have two to three times normal amounts of thiamine in their sera. The exact meaning of this fact is unclear, yet it may well represent a reflection of increased metabolic activity associated with a concomitant increased mobilization and/or utilization of thiamine. Hypothetically, this may fit in with the interpretation that certain tumor cells may mobilize and require more thiamine than normal cells.

The increasing reports in the literature regarding the use of various enzymes as antitumor agents lends credence to the hypothesis that a "thiaminase" may be the modality of action of various seafood extracts as oncostatic or oncocidal agents. Further investigations utilizing thiamine analogues as well as degradation of thiamine in various model tumor systems are being conducted.

Summary. The DBA/2 mice infected with Friend leukemia virus and maintained on a thiamine-free diet showed no histologic evidence or clinical manifestations of leukemia compared with counterpart controls maintained on a normal diet. Aliquot groups originally thiamine-deprived and subsequently refed thiamine developed gross splenomegaly and histologic evidence of leukemia. Similarities in preparation of materials from shellfish extracts in experiments to produce a "thiaminase," ichthiamin, and those used in antineoplastic experiments suggest a correlation in mechanism of action: thiamine inhibition.

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Delay of Hereditary Muscular Dystrophy of the Chicken by Oxygen Therapy: Histology*† (32953)

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Hereditary muscular dystrophy in the chicken is a myopathy most easily characterized by the loss of functional ability of the breast muscles (1). Using the exhaustion test as a measure of this functional ability, we have consistently observed that dystrophic birds, from our stock, reach peak scores at about 2.5 to 3.5 weeks of age and then fall

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