tive 480–500-day-old male NZB mice were incapable of inducing graft-versus-host reactions when injected into (NZB×A) F1 hybrids. This occurred even when the total number of nucleated cells injected was adjusted to contain 15–20 $\times$ 10^6 lymphoid cells. Younger, 45 and 150 day old, NZB mice were capable of inducing graft-versus-host reactions in a normal fashion.

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Received Dec. 6, 1967. P.S.E.B.M., 1968, Vol 127.

Effects of Vitamin B_6 and B_1 Deficiencies and Restricted Caloric Intake on Tissue Zinc Levels* (32911)

STANLEY N. GERSHOFF (Introduced by F. J. Stare)
Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115

Studies from this laboratory have suggested that there is a decreased availability of insulin in vitamin B_6 deficient rats (1). Although the role of zinc on insulin metabolism has never been determined, associations of zinc and insulin have been reported for more than 30 years. Recent *in vitro* studies (2,3) using isolated epididymal adipose tissue have clearly shown that zinc in concentrations in the range of those observed in human and rat plasma and higher inhibit insulin activity. Hsu (4) has reported that vitamin B_6 deficiency in rats results in decreased zinc content of plasma, liver, pancreas, and heart tissues. Work in this laboratory using a different experimental protocol appeared in disagreement with Hsu's conclusions. This report contains a study of the effects of vitamin B_6 deficiency on tissue zinc concentrations.

Since some pyridoxal enzymes are magnesium dependent and it has been shown that magnesium supplementation of vitamin B_6 deficient rations for rats prevents at least in part some effects of vitamin B_6 deficiency, [protects against calcium oxalate urolithiasis, increases urinary citrate and decreases xanthurenic acid excretion (5)] the effects of vitamin B_6 deficiency on tissue Mg levels

* Supported by USPHS Grant AM03056 and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

were also investigated. To partially determine how specific the effects observed were, studies of the effects of thiamine deficiency and restricted feeding on zinc tissue levels and the effects of restricted feeding and vitamin B_6 deficiency on potassium tissue levels were also undertaken.

Experimental. Weanling male Charles River CD rats were used in these studies. They were fed semipurified diets containing in percent: casein, 25; sucrose, 70.7; corn oil, 9; cod liver oil, 1; salts IV (6), 4; and choline, 0.3. Eight mg of riboflavin, 40 mg of niacin, 20 mg of calcium pantothenate, 1 mg of folic acid, 1 mg of menadione, 0.2 of biotin, 0.05 mg of vitamin B_{12} and when used 4 mg of thiamine hydrochloride, and 4 mg of pyridoxine hydrochloride were added to each kilogram of diet. The basal diet contained 40 mg of magnesium and 1.4 mg of zinc/100 gm. The rats were housed in galvanized iron cages and were either fed *ad libitum* or were pair-fed. The Zn and Mg analyses were done by atomic absorption flame spectrophotometry and K by flame photometry on perchloric–nitric acid digests of the tissues.

Results. Table I shows the results of feeding groups of 12 rats the basal diet with and without vitamin B_6 for 23 days on tissue zinc, magnesium, and potassium levels. Half of the rats in each group were fasted during the final 24 hours of the experimental period. Among the fed animals there were increased levels of tissue zinc in the vitamin B_6 deficient rats. These differences were statistically significant in the pancreata, sera, and kidneys and were particularly impressive in the pancreata where those of the vitamin B_6 deficient rats contained over 100% more zinc than those of the controls. The effects of vitamin B_6 deficiency on tissue levels of potassium and magnesium in fed rats were not nearly as extensive. In the fed deficient rats there was a significant decrease in pancreatic magnesium and rises in liver magnesium and potassium and kidney potassium. No statistically significant differences in tissue zinc, magnesium, or potassium were observed when fasted control rats were compared to fasted vitamin B_6 deficient animals.

Among the control rats, fasting resulted in a marked increase in pancreatic, serum, and liver zinc and a slight but statistically significant rise in kidney zinc. With the exception of a small rise in liver potassium, fasting did not significantly effect tissue magnesium or potassium levels. In the vitamin B_6 deficient rats, fasting did not result in significant changes in the tissue levels of any of the

TABLE I. The Effect of Vitamin B_6 Deficiency and a 24-Hour Fast on Rat Tissue Zinc, Magnesium, and Potassium Levels.[a]

Treatment	Pancreas	Kidney	Liver	Serum
		Zn (μg/gm or ml)		
$+B_6$, fed	29 ± 4	20 ± 0.8	27 ± 4	1.77 ± .09
$+B_6$, fasted	47 ± 6	23 ± 0.6	43 ± 3	2.65 ± .23
$-B_6$, fed	67 ± 19	33 ± 5	45 ± 9	2.11 ± .13
$-B_6$, fasted	70 ± 18	28 ± 3	47 ± 6	4.18 ± .77
		Mg (μg/gm)		
$+B_6$, fed	249 ± 5	182 ± 4	215 ± 10	
$+B_6$, fasted	264 ± 14	191 ± 7	238 ± 9	
$-B_6$, fed	220 ± 7	191 ± 10	264 ± 15	
$-B_6$, fasted	222 ± 13	179 ± 4	257 ± 10	
		K (mg/gm)		
$+B_6$, fed	3.96 ± .04	2.96 ± .03	3.62 ± .04	
$+B_6$, fasted	3.84 ± .07	2.88 ± .02	3.81 ± .04	
$-B_6$, fed	4.07 ± .13	3.25 ± .05	3.86 ± .06	
$-B_6$, fasted	3.96 ± .11	3.23 ± .18	3.90 ± .02	

[a] Values represent means ± SE for 6 weanling rats fed the experimental diets for 23 days.

TABLE II. The Effect of Vitamin B_6 Deficiency and Pair Feeding on Rat Tissue Zinc Levels.[a]

Treatment	Zn (μg/gm or ml)			
	Pancreas	Kidney	Liver	Serum
$+B_6$	14.7 ± .5	16.0 ± .4	18.3 ± .6	1.19 ± .07
$+B_6$, pair-fed	20.2 ± 2.1*[b]	21.5 ± 1.4***	17.8 ± .8	1.20 ± .06
$-B_6$	19.5 ± 1.7**	18.6 ± .5***	22.1 ± 2.4	1.35 ± .04

[a] Values represent means ± SE for groups of 8 weanling rats fed the experimental diets 17 days.
[b] *, **, *** = $p < .05, .02, .01$ when compared to *ad libitum* fed control rats.

cations studied except for a rise in serum zinc.

Table II shows the effects of feeding the basal diet with and without vitamin B_6 on rat tissue zinc levels for 17 days. In this experiment a pair-fed control group was also used. The data indicates that both the feeding of the vitamin B_6 deficient diet and pair-feeding the control diet in this study resulted in a significant rise in pancreatic and kidney zinc levels with the result that there were no significant differences in the zinc levels of the tissues studied in the deficient and pair-fed control groups.

Table III shows the effect of feeding the basal diet with and without thiamine to weanling rats for 23 days on their tissue zinc levels. It can be seen from these data that thiamine deficiency resulted in increased levels of pancreatic kidney and liver zinc levels but did not effect serum values. As in the previous experiments the effect was most marked in the pancreas with the zinc content of pancreatic tissue almost tripled in the deficient animals.

Discussion. It is apparent from these studies that the effects of deficiencies of vitamin B_6 or B_1 or restricted caloric intake on tissue levels of the cations studied may be of considerable magnitude and may vary with the cation being measured. Thus in these studies vitamin B_6 deficiency resulted in increased pancreatic, serum, and kidney zinc levels. When control rats were pair-fed or fasted for 24 hours, there was a rise in their tissue zinc content which was particularly marked in the fasted rats. Except for serum, the effect of fasting on zinc levels was not significant in vitamin B_6 deficient rats. These data probably explain the results of Hsu (4) who pair-fed his rats and then fasted them overnight before determining the zinc content of their tissues. Thus, his conclusion that vitamin B_6 deficiency in rats reduced tissue zinc concentrations was in great part a reflection of the high tissue levels of zinc in his fasted control rats. In the current studies thiamine deficiency resulted in a large increase in pancreatic, kidney, and liver zinc levels. As in vitamin B_6 deficient rats and fasted controls the rise in zinc appeared greatest in the pancreata.

The reasons that the control pancreatic zinc levels reported in Table II are lower than those in Tables I and III are not readily apparent. The studies were done several months apart. The data in Tables I and III were obtained from animals fed the experi-

TABLE III. The Effect of Thiamine Deficiency on the Zinc Content of Rat Tissues.[a]

Diet	Zn (μg/gm or ml)			
	Pancreas	Kidney	Liver	Serum
$+B_1$	25.7 ± 2.0	21.5 ± 1.0	17.3 ± .6	2.2 ± .1
$-B_1$	70.6 ± 14.1*[b]	27.1 ± 1.5*	39.8 ± 4.5**	2.3 ± .1

[a] Values represent means ± SE for 12 $+B_1$ rats and 13 $-B_1$ rats fed the diets for 23 days.
[b] *, ** represent p values of $< .01$ and $.001$, respectively.

mental diets 23 days, while the data in Table II are considerably smaller, since they were obtained from rats which had been on the diets for only 17 days. In any case, the effect of the dietary deficiencies studied on pancreatic zinc were consistent in each study.

Whether the increased zinc levels observed in thiamine and vitamin B_6 deficient rats are simply a reflection of the inanition which accompanies the production of these deficiencies states cannot be determined from these data.

Compared to the effects of vitamin B_6 deficiency and caloric restriction on zinc content of the tissues examined, their effects on tissue magnesium and potassium levels were either not significant or of relatively small magnitude.

It is of some interest to speculate whether the increased tissue zinc levels produced by nutritional deficiencies in these studies might serve some physiological purpose. Insulin plays a major role in glucose transport and is a primary hormone involved in lipogenesis and the deposition and retention of fat in tissue.

To protect animals in a wide variety of metabolic states including nutritional deficiencies, it seems likely that control mechanisms regulating the activity and availability of insulin must exist in animals. It has been shown that 0.048 μg of zinc/ml of Krebs–Ringer–bicarbonate buffer containing 3 mg of glucose and 0.5 mU of crystalline insulin inhibits the lipogenic activity of isolated rat epididymal adipose tissue. This inhibition increased as the concentration of zinc increased (3). In the present studies the mean serum zinc values ranged from 1.19 to 4.18 μg/ml. Fasting and deficiencies of vitamin B_6 and B_1 resulted in increased tissue levels of zinc which were most marked in the pancreata. Whether these changes in zinc concentrations are reflected in altered insulin metabolism remains to be studied.

The author wishes to thank Mr. and Mrs. Wynfield Hendrickson for their help in taking care of the animals and Miss Alice Carney for her technical assistance.

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Received Dec. 6, 1967. P.S.E.B.M., 1968, Vol. 127.

Reovirus Type 2: Production of and Sensitivity to Interferon in Human Amnion Cells (RA)* (32912)

HERBERT K. OIE[1] AND PHILIP C. LEH (Introduced by S. Baron)
Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822

Several RNA- and DNA-containing animal virions have been found capable of inducing the production of viral inhibitors called interferons in different animal and cell species. Among these virions are myxoviruses (1), arboviruses (2), papovaviruses (3), herpes viruses (4) and picornaviruses (5). The present report will describe the production of an interferon-like substance in a stable line of human amnion cells (RA) by the double-stranded RNA containing reovirus type 2.

After the completion of these studies a report appeared describing the production of interferon under *in vivo* conditions by the isolated double-stranded RNA component of reovirus type 3 (6).

Methods and Materials. Cell cultures. Monolayer cultures of RA (human amnion),

* This investigation was supported by research grant AI-04685 from the National Institute of Allergy and Infectious Diseases.

[1] NIH Trainee 5TI AI-243-03.