Some Specific and Non-Specific Effects of Thiamine Deficiency in the Rat.* (17729)

FLOYD R. SKELTON.† (Introduced by Hans Selye.)

From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal.

Deficiencies of the vitamin B-complex and of thiamine have been known to cause adrenal enlargement since first reported by McCarrison(1), while only recently has attention been directed to the thymus atrophy also present in these deficiency states(2,3). The possibility that these apparently unrelated phenomena could be manifestations of the same physiological process was not recognized until Deane and Shaw(4) demonstrated that the adrenal and thymus changes which occurred during the course of some vitamin deficiencies were typical of the general-adaptation-syndrome of Selye. The characteristic manifestations of this syndrome occur when an organism is subjected to non-specific stress and their development depends largely upon an increased functional activity of the pituitary-adrenal system. Thus, as adrenal hypertrophy and thymus atrophy are reliable indicators of adrenal-cortex hyperfunction, it seems apparent that certain vitamin deficiencies cause non-specific as well as specific alterations in the deficient animal.

As is well known the disorganization of carbohydrate metabolism is the biochemical effect of thiamine deficiency and other changes resulting from the avitaminosis may be secondary to the specific metabolic derangements *i.e.*, non-specific. Consequently this study was undertaken to establish whether the major anatomic manifestations of thiamine deficiency are specific or non-specific in nature.

Materials and Methods. Fourty-four male, hooded rats between 40-50 g in weight were divided into 3 groups and treated as follows: Group I served as absolute controls, the rats being allowed to eat the complete diet *ad lib.* The animals of Group II were fed the complete diet in sufficient quantity to maintain their weights essentially similar to the rats of Group III which received a thiamine deficient diet *ad lib.* Composition of the complete diet used in this experiment was as follows: Casein (vitamin free) 18%, Carbohydrate (1 part sucrose to 1 part starch) 71%, Fat (Shortening) 6%, Mineral Mixture (Steenbock) 4%, Cod Liver Oil 1%. A vitamin B mixture of the following composition was administered daily to each rat *per os*: Thiamine 40 μg, calcium pantothenate 250 μg, biotin 0.2 μg, P-aminobenzoic acid 250 μg, pteroylglutamic (folic) acid 10 μg, choline chloride 5 mg, inositol 2 mg. Thiamine was omitted from the vitamin mixture administered to the rats of Group III. Ten mg of α-tocopherol acetate was given to each rat orally every 10 days. Special cages were used to prevent coprophagy and the rats of Groups II and III were individually caged. All rats were weighed daily in order to rigidly control the food intake of Group II. After 36 days, the majority of the thiamine deficient rats showed signs of polyneuritis and disturbances of equilibrium. The experiment was then terminated, the animals being killed by decapitation. The organs to be studied were removed immediately and placed in Suza fixative for hematoxylin and eosin sections, while the adrenals were fixed in 10% neutral formalin and stained for fat with Sudan IV. Ascorbic acid determinations (method of Carruthers as modified by Moya *et al.*(5)) were made on the adrenals of 6 rats from each

* This work was supported by a research grant from the Commonwealth Fund of New York.

† This work was done while the author was holder of a Research Fellowship from the Hoffmann-LaRoche Co., Nutley, N. J.

1. McCarrison, R., *Indian J. Med. Research,* 1919, v6, 275.

2. Stoerk, H. C., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1944, v56, 151.

3. Christensen, K., and Griffith, W. H., *Endocrinology,* 1942, v30, 574.

4. Deane, H. W., and Shaw, J. H., *J. Nutrition,* 1947, v34, 1.

5. Moya, F., Prado, J. L., Rodriguez, R., Savard, K., and Selye, H., *Endocrinology,* 1948, v42, 223.

group. All organs were weighed on the chainomatic balance prior to sectioning.

Observations. A summary of the experimental observations is presented in Table I. It can be seen that the rats receiving the thiamine deficient ration (Group III) gained weight initially (maximum attained on the 14th day of the experiment) followed by a progressive decline until, by the end of the experimental period, the initial weight was almost reached. The body weight changes of Group II paralleled those of Group III thus adequately controlling the inanition factor involved in this type of experiment. Since the growth of Group I was satisfactory the composition of the synthetic diet seems nutritionally adequate.

There is a significant degree of adrenal hypertrophy and thymus involution in both the inanition (Group II) and thiamine deficient rats (Group III) compared to the normal controls (Group I). These changes probably indicate increased activity of the pituitary-adrenal system. Since there is also a significantly greater adrenal hypertrophy and thymus atrophy in Group III than in Group II, $P < .05$ and $< .01$ respectively) it is apparent that thiamine deficiency itself can function as a stressing agent, although inanition caused by the anorexia, invariably present in the avitaminosis, undoubtedly contributes to the actual degree of non-specific stress. Both kidney and liver were smaller by actual weight in the deficient and paired weight control rats than in the normal controls (Group I). The corrected weights of these organs expressed in the table, however, indicate that both partial inanition and thiamine deficiency produced a relative renal hypertrophy. In contrast the paired weight group showed hepatic atrophy while the thiamine deficient group evidenced no change in liver weight. It should be noted that the renal enlargement produced by the deficiency is very significantly greater than that caused by the equal degree of inanition in Group II. Although a relative increase in cardiac weight was present in both Groups II and III, the increase in the thiamine deficient group was significantly greater than in the paired weight controls. This suggests an actual cardiac

TABLE I.
Experimental Data on Thiamine-Deficient Rats and Their Controls.

Group	No. rats	Body wt in g		Organ wts expressed in mg/100 g body wt.								Adrenal ascorbic acid† mg/100 g tissue
		Initial	Highest	Final	Adrenal*	Thymus	Kidney	Liver	Heart	Testes	Seminal Vesicles	Spleen	Brain	Pituitary	
I Normal Controls	14	42±.4		138±6	17.8± .4	213±11	954±37	6180±202	330±14	1647± 36	168 ±11	582±37	1117± 77	3.6±.16	299±21
II Paired Weight Controls	15	42±.4	71±3	55±2	42.9±2.6	53± 5.1	1150±34	5423±230	380± 4	1790±148	19.6± 3.7	495±67	2321± 67	4.7±.06	275±22
III Thiamine Deficient	15	43±.3	70±2	53±2	50.4±2.5	28± 2.2	1793±86	6210±169	430±17	1902±106	20.0± 1.3	413±39	2608±142	5.2±.25	212±18

* Values obtained from 7 rats.
† " " 6 "

enlargement due to thiamine deficiency. The uncorrected weights of the testes and seminal vesicles showed developmental failure in both thiamine deficiency and chronic inanition groups. When the weights of these organs were corrected to 100 g of body weight the testicular atrophy seemed to be proportional to the retardation of growth while the seminal vesicle atrophy was apparently much more pronounced. This evidence could be interpreted to mean a greater pituitary secretion of follicle stimulating hormone (F.S.H.) than luteinizing hormone (L.H.). The spleen was equally atrophic in both the experimental and inanition control rats. The brain and pituitary did not change in actual weight which results in the relative increase in size shown by the corrected weights in the table.

Histologically, the adrenals of the thiamine deficient and paired weight control rats contained a greater amount of sudanophilic material than the normal controls. The adrenal content of lipid was greatest in thiamine deficiency, in distinct contrast to the ascorbic acid content which was lowest in this group. The involution of the thymus was more pronounced in the thiamine deficient group where the gland consisted almost entirely of connective tissue stroma. In the paired weight controls it was of almost normal structure although greatly reduced in size. Cross-section of the fixed kidneys from the thiamine deficient group showed an increase in cortical width with a well defined white band in the region corresponding to the inner cortex. No such zone was seen in the kidneys of the inanition or normal controls. Microscopically this zone showed some tubular and glomerular enlargement which probably caused the observed increase in width of the cortex. The kidneys of the inanition controls had no comparable changes; indeed the cortex was thinner glomeruli and tubules smaller than in thiamine deficiency. The liver of the normal and thiamine deficient rats had essentially the same microscopic appearance. The cells were large with vesicular nuclei and fluffy vacuolized cytoplasm. In contrast, the cells of the paired weight controls were small with compact nuclei and dense, homogeneous, eosinophilic cytoplasm.

Both testes and seminal vesicles of the thiamine deficient and paired weight groups showed developmental failure as compared to absolute controls. In the testes, there was only partial failure of spermatogenesis but a marked atrophy of the Leydig cells. The seminal vesicles were very atrophic with a low, infolded lining epithelium and only a slight amount of secretion. The microscopic structure of the heart, brain and spleen of both thiamine deficient and inanition control rats was normal.

Discussion. In a previous publication(6), it was shown that most of the gross anatomical changes studied in B-complex deficiency were manifestations of the general-adaptation-syndrome. Many of the characteristic findings of this syndrome, such as adrenal hypertrophy, thymus atrophy and failure of testicular and seminal vesicle development have been reported also for thiamine deficiency. Indeed Deane and Shaw(4) have followed the adrenal and thymus changes through all three stages of alarm, resistance and exhaustion. In the above experiment the activation of the pituitary-adrenal system in thiamine deficiency was greater than that seen in equal inanition controls. However, as the partial starvation itself produced evidence of increased adrenal activity, part of the picture of thiamine deficiency is therefore due to the anorexia induced by the avitaminosis. It is of interest to note that at this stage of severe thiamine deficiency the adrenal sudanophilic lipids were increased above that seen in the normal or paired weight controls, yet the ascorbic acid content was significantly decreased. Apparently, as the adrenal cortical activity became exhausted in extreme thiamine deficiency, the ascorbic acid content decreased before the lipids were altered. This is another instance, besides that previously reported(7), where there is a dissociation between the adrenal content of stainable lipids and ascorbic acid.

The renal hypertrophy reported in B-complex and thiamine deficiency is probably another manifestation of non-specific damage. Constantinides(8) in unpublished experi-

6. Skelton, F. R., *Am. J. Physiol.*, in press.

ments has shown that even after a stress of 48 hours duration there is enlargement of the kidney, while Dugan(9) has observed a marked enlargement after prolonged exposure to cold. Jiménez Diaz *et al.*(10) have reported a negative nitrogen balance during severe thiamine deficiency and Sandberg, Perla and Holly(11) have demonstrated that the nitrogen excretion during the deficiency exceeds that in inanition controls by 28%. Thus the renal hypertrophy seen in this experiment is probably due to the increased excretion of nitrogen metabolites which result from the protein breakdown caused either by a direct damaging effect of thiamine deficiency, increased gluconeogenesis from protein caused by the increased corticoid secretion or a specific effect of the avitaminosis on protein metabolism.

The relatively slower loss of liver and heart weight in thiamine deficiency as compared to inanition controls suggests a specific effect of the vitamin deficiency on these organs. This is not inconceivable in the light of known effects of the avitaminosis on carbohydrate metabolism and cardiac physiology. The distinct tendency to splenic atrophy in thiamine deficiency is in contrast to the tendency to hypertrophy previously reported for B-complex deficiency(6). Likewise the microscopic appearance of the spleen is strikingly different in thiamine deficiency than in B-complex avitaminosis. In the former the germinal centres of the follicles were of normal appearance while in the latter they have lost all sign of proliferative activity. In both experiments the inanition control rats have had a normal appearance of the splenic lymphatic follicles. These observations suggest that in thiamine deficiency one of the B factors present in the diet maintained either directly or indirectly the normal lymphatic activity of the spleen while its absence in B-complex deficiency caused failure of lymphopoiesis in the germinal centers of the follicles.

In thiamine deficiency as in B-complex deficiency, the evidence suggests that the secretion of follicle stimulating hormone (F.S.H.) by the pituitary is greater than the secretion of luteinizing hormone (L.H.) so that the seminal vesicles become much more atrophic than the testes because the development of the former depends on androgen production which in turn is controlled by the pituitary secretion of L.H.

Summary and conclusions. A detailed study was made of the gross and microscopic organ changes in the thiamine deficient rat as compared to normal and paired weight controls. It was apparent that the deficiency produced the essential changes characteristic of non-specific stress such as adrenal hypertrophy, thymus involution and decreased adrenal ascorbic acid. Marked renal hypertrophy was also observed in the deficient rats and was considered to be due to the prolonged negative nitrogen balance known to be associated with thiamine deficiency. The reduced gonadotrophin and increased adrenocorticotrophine secretion as indicated by the developmental failure of the sex organs and hypertrophy of the adrenals illustrates the so-called "shift" of pituitary hormone production under these circumstances. In contrast to the non-specific changes above, thiamine deficiency showed specific effects on the liver and heart. The activity of the germinal centres of the splenic lymphatic follicles was normally maintained in contra-distinction to the failure of germinal activity previously observed in B-complex deficiency.

Received February 14, 1950, P.S.E.B.M., 1950, v73.

7. Skelton, F. R., Fortier, G., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 120.

8. Constantinides, P., personal communication.

9. Dugal, L. P., and Thérien, M., *Endocrinology*, 1949, v44, 420.

10. Jiménez Diaz, C., Vivanco, F., Palacios, J. M., Buylla, A., and Picatoste, R., *Rev. Clin. espanola*, 1947, v25, 249.

11. Sandberg, M., Perla, D., and Holly, O. M., PROC. SOC. EXP. BIOL AND MED., 1937, v27, 35.