COMPARATIVE BIOCHEMICAL AND HISTOCHEMICAL STUDY OF THE LIPIDS OF THE LIVER AND HEART IN EXPERIMENTAL HYPERVITAMINOSIS B_1 IN PIGEONS

Yu. M. Ostrovskii and V. S. Nikitin

Department of Biochemistry (Head, Yu. M. Ostrovskii) and Department of General Biology (Head, V. S. Nikitin), Grodno Medical Institute
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 56, No. 10, pp. 32-35, October, 1963
Original article submitted July 4, 1962

Thiamine is closely related to lipid metabolism through its fundamental co-enzyme functions. For instance, the formation of acetyl residues from carbohydrate precursors is brought about by pyruvate dehydrogenase. The complete utilization of these two-carbon residues in the three-carbon cycle is closely bound up with another enzyme containing thiamine diphosphate– ketoglutarate dehydrogenase. Thiamine diphosphate is associated with transketolase, a constituent of the cycle supplying the bulk of the $NADP \cdot H_2$ for the reductive biosynthesis of the higher fatty acids and cholesterol [2, 6]. A thiamine derivative – thiamic acid – has been identified as a coenzyme of α-glycerophosphate dehydrogenase [5].

It has previously been shown [4] that essential hypertension is characterized by changes in thiamine metabolism, which may be considered to be a manifestation of endogenous hypervitaminosis B_1, and injections of thiamine into such patients often cause undesirable changes in lipid metabolism [1, 3]. In the present investigation the effect of experimental hypervitaminosis B_1 was studied on the state of the lipid metabolism in certain tissues of the pigeon.

EXPERIMENTAL METHOD

Pigeons (22) were kept on a mixed balanced diet of grain. The birds of the experimental group (11) received, besides their diet, thiamine chloride in a dose of 1-2 mg per bird daily for 20-30 days. After decapitation, the internal organs were extracted in the cold, pieces of the heart and liver were excised for histological investigation, and homogenates were prepared for the quantitative estimation of cholesterol, phospholipids, and total lipids. The pieces of tissue were fixed in Zenkerformol and formalin with calcium chloride. Sections were stained with hematoxylin-eosin, iron hematoxylin, azure II-eosin, and by Mallory's method. Frozen sections were treated with Sudan III by Daddi's method to demonstrate lipid inclusions.

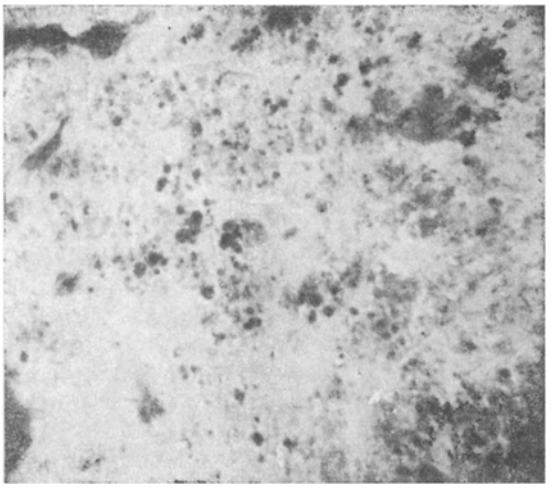
EXPERIMENTAL RESULTS

In the pigeons of the experimental group a small increase was noted in the mean weight (from 304 ± 6.8 to 329 ± 7.8 g; $t = 2.42$). A corresponding increase took place in the weight of the heart (from 3.66 ± 0.08 to 3.98 ± 0.08; $t = 2.86$), as a result of which its relative weight remained unchanged (1.21 and 1.22 %). Macroscopically, deposition of lipids in the heart along the course of the coronary arteries was observed more frequently in the pigeons of the experimental group than in the controls, but nevertheless inconstantly.

In the microscopic preparations no significant, constant changes in the structural elements of the heart muscle or liver were observed. In some cases marked vacuolation of the liver cells was found, with a change in the relative proportions of dark and light cells. However, these findings could not be related to the excessive utilization of thiamine by the animals. In the sections of heart tissue groups of fat cells were found in all the animals, in the epicardium and along the course of the vessels. In the animals of the experimental group fat cells were encountered more frequently than in the controls, in the myocardium along the course of the connective-tissue septa, particularly towards the apex of the heart. In sections of the liver of the control group of animals only single cells containing lipid granules were

Content of Various Fractions of Lipids in the Liver and Heart of the Pigeons

Index (in mg %)	Heart			Liver		
	control	experiment	t	control	experiment	t
Total lipids	4 687 ±314	5 781 ±525	0,70	4 844 ±173	5 625 ±228	2,30
Cholesterol	165 ±11	175 ±13	0,59	299 ±13	308 ±11	0,58
Phospholipids	2 403 ±29	2 556 ±52	2,57	2 810 ±178	3 075 ±142	1,16



Hypervitaminosis B_1 in the liver of a pigeon. Granular deposition of lipids. Photomicrograph. Sudan III. Objective 90, ocular 7, filter SS-8.

found as a rule. In most animals of the experimental group fat was found in the liver cells in the form of large granular deposits. Many cells were tightly packed with lipid granules or contained 1 or 2 large granules and also a finely dispersed sudanophilic granularity, merging with a diffuse staining of the cytoplasm (see figure).

The results of histological examination of the sections agreed with the biochemical findings in the liver tissue, and to a lesser degree with the corresponding findings in the heart (see table).

The accumulation of sudanophilic granules revealed morphologically in the liver cells was evidently associated with only one fraction of the lipids – the triglycerides. This fraction was evidently responsible for the main quantitative difference in the total lipid concentration in the experimental and control pigeons.

The morphological investigation revealed no significant differences in the lipid concentration in the heart muscle. Yet such differences were detected by biochemical methods. It may be postulated that the differences were concerned with the presence of lipids accumulated around the coronary vessels. It was difficult to make a reliable quantitative comparison between the lipid deposits in this region by microscopic examination, for relatively small pieces of the heart wall were excised for study.

SUMMARY

Administration of 100 times the physiological doses of thiamine to pigeons for 20-30 days caused considerable shifts in the lipid metabolism. The total lipid content increases in the liver; microscopically this is manifested in the form of intensified intracellular deposition of fat granules. The phospholipid content in the heart rises with a tendency for accumulation of total lipids. Because of the uneven fat distribution in this organ a comparative microscopic examination gave no definite results. The cholesterol content of the heart and liver did not differ in experimental and control birds.

LITERATURE CITED

1. I. A. Myasnikova, Transactions of the Naval Medical Academy [in Russian], Vol. 8, p. 140. Leningrad (1947).
2. Yu. M. Ostrovskii, Ukr. biokhim. zh., 4, 617 (1959).
3. Yu. M. Ostrovskii, Byull. éksper. biol., 7, 62 (1961).
4. Yu. M. Ostrovskii, in book: Abstracts of Sectional Proceedings of the 5th International Biochemical Congress [in Russian], Vol. 2, p. 186. Moscow (1961).
5. J. Eys, Fed. Proc., Vol. 19, No. 1, Pt. 1, p. 26 (1960).
6. D. S. Goodman, in book: Fifth International Biochemical Congress. Biosynthesis of Lipids (Symposium 7) [in Russian], Book 8, p. 19. Moscow (1961).