THE EFFECT OF HIGH DOSES VITAMIN A (100 000 IU) ON THE ACTIVITY OF ACID HEPATIC PHOSPHATASE (β-GLYCEROPHOSPHATE PHOSPHATASE) IN NORMAL, HEMITHYROIDECTOMIZED AND TREATED WITH EXTRACT OF THE THYROID MALE ALBINO RATS

D. S. Kalitzin, T. K. Pencheva

After adding high vitamin A doses to tissue cultures of chicken embryos, Dingle (10) established acid protease liberation from the tissues. According to de Duve and co-workers (6), the acid proteases of the cell, together with other hydrolytic enzymes, are concentrated in small organelles, contained within the cytoplasm. These particles were denominated with the term «lysosomes». The treatment of isolated lysosomes «in vitro» with large doses vitamin A causes break down of the lysosome membrane and release of proteolytic enzymes and other hydrolases (10, 12).

More recent studies by Dingle and co-workers (9) showed that vitamin A exerts identical effect also under «in vivo» conditions. Thus, the authors found out that lysosomes from the liver of rats, in which hypervitaminosis A was induced by way of administering toxic vitamin A doses (50 000 IU daily), are more unstable than the lysosomes of control animals.

In the experiments of de Duve and co-workers (7), in vitro, with vitamin A addition to isolated lysosomes from liver of rat, as well as in the investigations, in vivo, of Weissmann and co-workers (20) on guinea pigs, subjected to acute avitaminosis A, an increase has been established in the activity of acid hepatic phosphatase. Changes in the activity of the enzyme towards normal animals were proved by Koels and assoc (17) in animals with hypovitaminosis A.

Literature (15) and personal data (3, 4) show that the thyroid gland hormones influence the acid hepatic phosphatase activity.

In the works of Zaleskaya (1), Ascarelli and co-workers (5), Kitano and co-workers (14) the relationship existent between the function of the thyroid and the utilization and action of vitamin A in the organism is pointed out.

Based on the above data, we undertook investigations on the effect of high vitamin A doses on the acid hepatic phosphatase of rats, in which hypo- and hyperthyroid conditions were experimentally induced.

Material and Methods

The investigations were conducted on 76 unbred young, sexually mature, male albino rats, distributed into 6 groups: group I — control (intact) — 15 rats; II — injected with vitamin — 10 rats; III — treated with thyroid gland extract — 10 rats; IV — treated with vitamin A and thyroid gland extract — 18 rats; V — hemithyroidectomized — 11 rats and VI — hemithyroidectomized, and injected with vitamin A — 12 rats.

In the animals of groups III and IV, experimental hyperthyroid condition was induced by daily administration, over a period of 10 days, via probe per os, of thyroid gland extract at dose 15 mg/100 g body weight in the form of water emulsion.

In the animals of groups V and VI, hypothyroid condition was provoked by means of hemithyroidectomy. The animals were investigated one and a half months after the operation.

Vitamin A was injected by intramuscular route in oil solution over a period of 10 days, at three-fold administration and total dose amounting to 100 000 IU per rat.

The animals of all the groups were fed on a diet consisting of bread and milk ad libitum. They were killed with a blow on the occiput at the same hour of the day (11 a. m.), previously being kept on a fasting regime for 15 hours, as already described in earlier works by the same authors (2, 3).

The activity of acid phosphatase (β-glycerophosphate phosphatase) was determined in total hepatic homogenates according to a method elaborated by Kalitzin (2, 4). After the killing of the animal, the liver was promptly removed, weighed and subjected to homogenization at 0° for 1—2 minutes in glass homogenizer, type Potter, with 0° water at a 1 : 5 ratio.

The reaction was carried out at 37° over a period of 60 min at pH=5.5 with 0.1 M acetate buffer in the presence of β-glycerophosphate (0.3 M). The inorganic phosphate freed during the reaction was determined by employing ascorbic acid as a reductor. The activity of the enzyme was demonstrated on the basis of the difference between the amount of inorganic phosphate after the incubation and before the incubation. This difference was expressed by the increase of inorganic phosphate in micrograms per 100 mg fresh hepatic tissue.

The data obtained were statistically elaborated after Student.

Results

The data of the investigations performed are illustrated in Table 1:

The data of Table 1 show that:

1. After treatment of normal male rats with high doses vitamin A, statistically reliable changes in the activity of the acid hepatic phosphatase do not occur.

2. The administration of thyroid extract in male rats causes a tendency towards increase of the enzyme activity (statistically unreliable — $t=1.4$; $p=0.18$).

Table 1

The activity of acid hepatic phosphatase in male albino rats, treated with thyroid extract and high vitamin A doses

Group	Number	Activity of acid phosphatase (inorganic P increase in mcg/100 mg hepatic tissue)
I — controls (intact animals)	15	416.9± 5.3
II — treated with vit A	10	403.3±10.0
III — treated with thyroid extract	10	431.1± 9.5
IV — treated with vit A plus extract	18	449.3± 6.4
V — hemithyroidectomized	11	414.7±10.3
VI — hemithyroidectomized plus vit A	12	429.0± 5.0

3. Upon treatment of male rats with high doses vitamin A+extract of thyroid gland, a statistically reliable increase of activity is established with 7.9% ($t=3.95$; $p<0.001$).

4. In hemithyroidectomized rats the activity of the enzyme is not altered as compared to the controls.

5. Vitamin A, introduced in hemithyroidectomized rats does not account for a statistically reliable change of the enzyme activity.

Discussion

The studies carried out demonstrate that vitamin A at dose 100 000 IU per rat (administered three times over a period of 10 days) does not alter the activity of acid phosphatase in the liver of normal and hemithyroidectomized rats. Against the background of the experimentally induced hyperthyroid state (at simultaneous administration of thyroid extract), the same vitamin A dose causes an increase of the enzyme activity.

In accordance with the data of Weissmann (20) and Koels and co-workers (17), the changes in the activity of acid hepatic phosphatase are related, first and foremost, to the changes in the membranes of the lysosomes. On account of its hydrophil and hydrophobe groups, vitamin A is endowed with a strongly pronounced surface activity (16). In the review of Dingle and Lucy (11), the hypotheses are analyzed about the primary effect of vitamin A upon the membranous systems, and it is furthermore emphasized that in hypo- and hypervitaminosis A, induced both «in vivo» and «in vitro», the membranes are less stable than under normal conditions. In physiological conditions, vitamin A accounts for stabilization of the membranous structures (13).

In the opinion of Dingle and Lucy (11), certain additional side factors, such as vitamin E and the hormones, play a role in the effect of vitamin A upon the membranes.

The data reported by Verity (19) in the past few years show that the reactivity changes of the thyroid gland cause changes in the lysosomes.

According to our data, whenever only thyroid extract is being administered, it fails to alter reliably the activity of the acid hepatic phosphatase.

Such an action was established during the combined effect exerted by vitamin A and thyroid extract, explainable by the referred in the literature (1, 5, 14) interrelationship between the function of the thyroid gland and the action of vitamin A in the organism.

Inferences

1. Vitamin A at dose 100 000 IU per rat, introduced with threefold injections over a period of 10 days, does not account for changes in the activity of acid hepatic phosphatase.
2. The thyroid extract at dose 15 mg/100 g weight, given daily per os over a period of 10 days, accounts for a tendency towards an increase of the enzyme activity.
3. The simultaneous administration of vitamin A and thyroid extract results in statistically reliable increase of the acid phosphatase activity.
4. In hemithyroidectomized rats, the activity of the enzyme is not changed under the influence of vitamin A.

REFERENCES

1. Залесская, Ю. М. *Биохимия,* 1965, 6, 1132—1137.
2. Калицын, Д. С. *Вопросы мед. химии,* т. VI, 1960, 6, 631—634.
3. Калицин, Д. С. Под печат в *Медицински архив,* 1970.
4. Калицин, Д. С., Т. К. Пенчева. Под печат.
5. Ascarelli, I., P. Budowski, I. Nir, A. Bondi. *Poultry Sci.,* 43, 1964, 2, 370—377, цит. по РЖ Бх, 1965, 398.
6. De Duve, C., B. C. Pressman, R. Gianetto, R. Wattiaux, F. Appelmans. *Biochem. J.,* 60, 1955, 604.
7. De Duve, C., R. Wattiaux, M. Wibo. *Proc. I st. int. Pharmacol. Meet.,* vol. 5, p. 97, Oxford: Pergamon Press Ltd, 1963.
8. De Leo, T. A. Barletta, S. Di Mao. *FEBS,* 1969, Madrid, Abstracts, № 602.
9. Dingle, J. T., I. M. Sharman, T. Moore. *Biochem. J.,* 98, 1966, 476—484.
10. Dingle, J. T. *Biochem. J.,* 79, 1961, p. 509.
11. Dingle, J. T., J. A. Lucy. Vitamin A, carotinoids and cell function. *Biol. Rev.,* 40, 1965, 422—461.
12. Fell, H. B., J. T. Dingle, M. Webb. *Biochem. J.,* 83, 1962, p. 63.
13. Guha, A., O. A. Roels. *Biochem. Biophys. Acta,* 111, 1965, 364—374.
14. Kitano, W., W. Kuroda. *Exptl. Cell. Res,* 48, 1967, 8, 350—360.
15. Kobayashi, H., K. Maruyama, S. Kamara. *Endocrinology,* 57, 1955, 129.
16. Olson, J. A. *Pharmacological Rev.,* vol. 19, 1967, 4, 559—596.
17. Roels, O. A., M. Tront, A. Guha. *Biochem. J.,* 1964, 23 c.
18. Veil, Catherine, E. Triantaphyllidis, H. Farmand. *Semain Hopitaux Pathol. et biol. 9,* № 23—24, 1961, 2225—2294, цит. по Рж Бх 1962, 20 C 240.
19. Verity, M. A. *Calif. Med.* 103, 1965, 4, 267—276.
20. Weissmann, G., J. W. Uhr, L. Thomas. *Proc. Soc. exp. Biol. N. J.,* 112, 1963, 284.

ВОЗДЕЙСТВИЕ ВЫСОКИХ ДОЗ ВИТАМИНА А (100 000 ИУ) НА АКТИВНОСТЬ КИСЛОЙ ПЕЧЕНОЧНОЙ ФОСФАТАЗЫ (β-ГЛИЦЕРО-ФОСФАТ ФОСФАТАЗЫ) НОРМАЛЬНЫХ, ГЕМИТИРЕОИДЭКТОМИРОВАННЫХ И ПОЛУЧАВШИХ ЭКСТРАКТ ЩИТОВИДНОЙ ЖЕЛЕЗЫ САМЦОВ БЕЛЫХ КРЫС

Д. С. Калицын, Т. К. Пенчева

РЕЗЮМЕ

Авторы провели исследование на 76 самцах белых крыс, разделенных на следующие группы: I — контрольные (интактные); II — получавшие инъекционно витамин А; III — получавшие экстракт щитовидной железы; IV — получавшие витамин А с экстрактом щитовидной железы; V — гемитиреоидэктомированные; VI — гемитиреоидэктомированные, получавшие экстракт щитовидной железы. Животные V и VI групп исследовались спустя полтора месяца после операции.

Витамин А вводился внутримышечно в общей дозе 100 000 ИУ на крысу, трехкратно в течение 10 дней.

Экстракт щитовидной железы вводился через зонд per os в течение 10 дней, в суточной дозе 15 мг/100 г веса.

Одновременное введение крысам витамина А и экстракта щитовидной железы вызывает повышение активности кислой печеночной фосфатазы. Витамин А сам по себе не вызывает изменений активности энзима у нормальных и гемитиреоидэктомированных крыс.

Введение экстракта щитовидной железы у нормальных половозрелых крыс не вызывает повышения активности энзима.