CERTAIN PATHO-MORPHOLOGICAL DISTURBANCES WITH EXPERIMENTAL VITAMIN B_1 DEFICIENCY IN DOGS*

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In the literature, there are references to the fact that any vitamin deficiency in the animal organism may bring with it disturbances in the metabolism of another vitamin. Thus, for example, M. S. Levinson [3] refers to the works of Raduan and Misho, who induced in rats (animals not sensitive to absence of vitamin C in food) the disease of scurvy by depriving them of all the water-soluble vitamins.

Dogs, widely used for experimental purposes, like rats do not require intake of vitamin C [2]. In the dogs, as is known, one cannot induce scurvy by excluding vitamin C from the food. Nonetheless, with induced thiamine or vitamin B complex deficiency, investigators, as a rule, always add to the diet vitamin C in natural or synthetic form [1, 3, 5].

We decided to find out whether there is change in the course of the experimental polyneuritis in the dogs if vitamin C is excluded from their diet. As was to be expected, the general clinical picture of the diseased dogs with vitamin B_1 deficiency did not differ from that described in the literature [1, 3, 5]. However, in the last days of the life of the dogs (3-5 days before death), we found in the experimental animals hemorrhagic diarrhea and vomiting of blood—symptoms not characteristic of vitamin B deficiency.

It was necessary to carry out patho-morphological investigation of the dead animals in order to identify the causes of the phenomena observed.

As is indicated in the literature, with the development of thiamin or vitamin B complex deficiency in dogs, one observes the following patho-morphological changes: hyperemia, extravasates, occasional signs of edema in the tissues of the brain, lungs, liver, kidneys, etc; in the cortex of the brain, the diencephalon and the cerebellum, one finds degenerative changes of the nerve cells and neurophage. As regards the cardio-vascular system, one notes enlargement of the right heart, edema, and colloid swelling of the vascular muscle, congestion in the lesser circulation, increased capillary permeability. In the stomach, no special changes are usually noticed. In the intestines, one occasionally observes manifestations of subacute enteritis; the mucous membrane is usually poor and swollen, and in the submucous layer, one sees at times accumulation of small lymphocytes [3]. In the peripheral section of the nervous system, one finds degenerative changes in the ganglia and nerve fibers (the latter are by no means found in all cases).

Analyzing the changes of the gastro-intestinal tract of the dead dogs in our experiments, we found the following special features.

EXPERIMENTAL

In our experiments, two dogs were used—females; 3-4 years of age, weighing about 20 kg. The deficiency in thiamine was achieved by feeding the animals with autoclaved food (4 hours at 125°-130°). The daily

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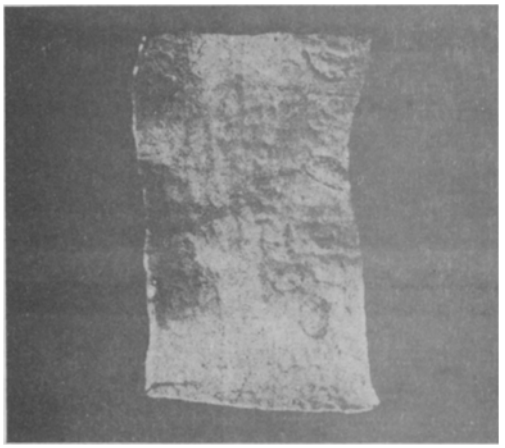

Fig. 1. Frontal section of the small intestine in dog Volchok. 3 ulcers in mucous membrane. (Reduced).

ration consisted of: bread (250 g), groats millet (250 g), meat (250-300 g), vegetables (100-200 g), and milk (200 g). To the food was daily added 4 mg riboflavin and nicotinic acid, and one tablespoonful of fish oil. No ascorbic acid was introduced into the food.

The richness of the diet was ascertained on the control dog of the same weight and sex as the experimental ones, fed on the same amount of food, not subject to autoclaving. Fall in body weight and deviations from the normal in the condition of the control dog were not observed.

In order to check the complete destruction of thiamin on autoclaving, pigeons were used, which died with signs of marked vitamin B_1 deficiency when fed on autoclaved food.

The dogs were subjected to dissection 1 to 3 days after death. The material obtained was fixed in a 10% solution of neutral formalin, and after this, placed in celloidin according to the generally accepted method. The solidified

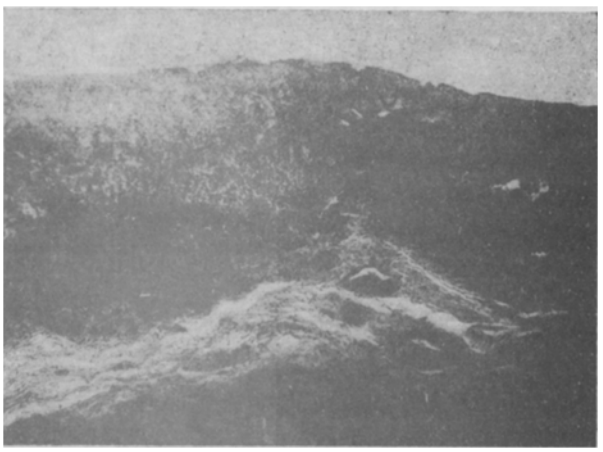

Fig. 2. The base of one of the ulcers depicted in Fig. 1. Necrosis of the mucous membrane, lymphoid cellular infiltration at the border between the mucous and submucous membranes, enlargement of the vessels. (Microphotography (Approx. 7, ob. 8).

microscopic sections were subjected to partial staining. We conducted histological examination of the internal organs (liver, spleen, kidneys, lungs, heart, stomach and intestines), of the central nervous system, and peripheral ganglia. In order to show up the histological changes, we stained with hematoxylin-eosin, Sudan III and picrofuchsin, according to Nissl, and iron-containing pigment.

EXPERIMENTAL RESULTS

The experimental dog Sharik died at the end of the 5th month after commencement of the experiment, although during the final weeks it received with the food increasing doses of vitamin B_1 (from 0.15 to 30 mg per os, subcutaneously and intravenously). Death was accompanied by frequently recurring convulsive fits, gastro-

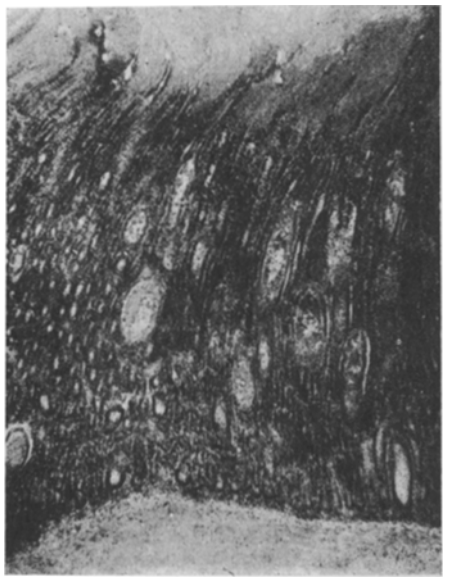

Fig. 3.. Small intestine of Dog Volchok. Hemorrhagic enteritis ; cyst-like distended glands, filled with formal elements of blood.
(Microphotography (ocular %7, ob. 8).

intestinal disturbances (vomiting and sanguineous dysentery) and complete exclusion of conditioned reflex activity. The loss in weight in the last 5 months of life was 19%

In the second dog Volchok, the first clinical symptoms of disease appeared in the same period as for Sharik, i. e., at the end of the 4th month of the experiment. However, after receiving with the drinking water 0.15 and 0.5 mg thiamin (two administrations at intervals of three days) all the signs of thiamin deficiency disappeared. Later, Volchok, as before, received autoclaved food, deprived of vitamin B_1.

The first symptoms of thiamin deficiency after this appeared within 5 weeks in the form of periodic lessening of appetite and sporadic disturbances of the intestine. 13 weeks later, the dog died with signs of disturbed coordination of movement, sanguineous dysentery and blood vomiting. The loss of weight in the final 2 months was 18.5%.

On examination of the dead animals, we found in Volchok traces of blood vomiting in the form of reddish-brown, foul smelling liquid filling the oral cavity. In Sharik, before death, copious unceasing salivation was seen.

In both dogs, the cells of the subcutaneous fatty tissue were very well developed. Considerable deposits of fat were seen in the mesentery, the peri-renal tissue cells, around the heart, and in other organs. In the

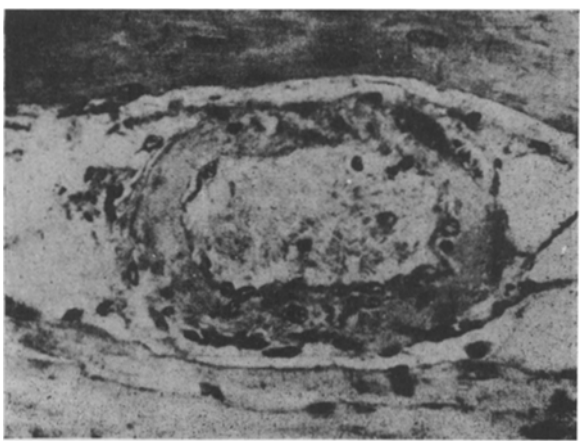

Fig. 4. Proliferation of endothelium cells and plasmo hemorrhagia of the wall of small blood vessel of the heart in dog Volchok.
Microphotography (ocular 7, ob. 8).

abdominal cavity, a small amount of light yellow, slightly turbid liquid was found.

The mucous membranes of the mouth, glottis and esophagus did not have macroscopic changes, and were not subjected to histological examination.

The stomach was moderately full of reddish-brown liquid. Its serous membrane did not have visible changes; many folds were seen in the mucous membrane. In Sharik, in the fundus of the stomach together with curvature, there were many regions of hyperemia, spotted hemorrhages, and some small surface erosions.

The entire length of the small intestine in both dogs was filled with liquid sanguineous contents. The wall of the intestines was thickened, the mucous membrane smooth and swollen with diffuse scattered hemorrhage in the form of spots. In the iliac intestine, a small number of hemorrhage-free regions were found. In general, we noted a gradual decrease in the amount of hemorrhage in the direction from the frontal to the hind part of the small intestine.

In the frontal section of the thinnest intestine, we found some ulcers (in Sharik 3 and Volchok 5), oval and well outlined, with a diameter of 10 to 15 mm (Fig. 1).

On histological examination of the pit of the stomach, hemorrhages in the surface layer of the mucous membrane were found. Hemorrhages were also noted in the pancreas.

The intestinal ulcers were regions of necrotic mucosa, demarcated from the submucosa by lymphoid cellular elements. In the central part, the ulcers of the cellular infiltration were less marked. The vessels of the submucosa were enlarged (Fig. 2). In the submucosa, signs of atrophy were seen; in the muscular and serous layers of the intestines, no changes were observed.

The mucosa of the small intestine was widely infiltrated with erythrocytes with an admixture of lymphoid cells; a well marked hemorrhagic diapedesis in the lumen of the glands, some of which as a result were cyst-like distended (Fig. 3).

In other internal organs, we found the following changes: liver of a dark reddish color, elastic, on transverse cut, well visible "nutmeg" and some granulation. The gall bladder was overfilled with a very thick olive colored bile. Histologically, in the liver, we discerned enlargement of the lumen of the central veins, and of the adjacent capillaries which were filled with blood cells.

In the hepatic cells - granular degeneration - fine-drop fatty dystrophy, pycnosis of the nuclei; in the kupfer cells there were signs of swelling and some hemosiderosis.

The spleen was reduced in size, wrinkled, flat, with a surface of grey-steel color; on transverse cut dry, cinnamon-reddish color. Microscopically, atrophy of the lymph follicules, reduction of peri-follicular zone, decrease in number of cellular elements of the blood, and insignificant hemosiderosis were noted. The kidneys were slightly swollen in the cortical and medullary layers, there were many pointed and spotted hemorrhages, microscopically we found marked enlargement of some small blood vessels, especially in the cortical layer, proliferation of the epithelium cells in the renal bundles, turbid swelling, pycnosis of the nuclei, and focal fine-drop fatty dystrophy of the epithelium of the renal tubules. The urinal bladder was empty; its mucosa had no visible changes.

In the pleural cavities, there was about 200 ml of a transparent fluid of a faint yellowish color. The lungs were filled with air. Histological examination showed thickening of the alveolar walls, and in the interstitia, an insignificant deposit of grains of a dark cinnamon pigment, not giving an iron reaction.

In the cardiac stem there was a small amount of transparent reddish fluid. The heart was dilated to the size of man's fist. On the epicardium, there were many spotted hemorrhages assuming a diffuse character in the auricles. The right heart was greatly enlarged. In Volchok, the thickness of the wall of the right ventricle was 3-5 mm, of the auricle 1.5-2 mm; of the left ventricle 16 mm and the auricle 4 mm. The spotted hemorrhages were noted under the endocardium, and deeper in the myocardium of the right and left heart, in the papillary muscles and at the base of the arterio-ventricular valves. We also found edema of the tricuspid valve to 4-5 mm and less expressed of the bicuspid valve. Microscopically, fresh and traces of old hemorrhagic foci were found in the cardiac muscle, turbid swelling and disappearance of the transverse striations of the muscular fibers.

Particular attention was evoked by changes in the blood vessels in the heart of Volchok. In the small vessels, signs of plasmorrhagia were observed–the penetration of the walls of the vessels by an albumen substratum, selectively stained with eosin (Fig. 4). We noted frequent continuous infiltration of the walls of the vessels by cellular elements of the blood, and proliferation of the epithelium cells. These changes were of a focal character, affecting sections of the individual vessels. In the foci of the hemorrhages, contiguous with such parts, proliferation of the histiocytes was witnessed.

Macroscopically, the changes in the central nervous system were noted only in Sharik in the form of insignificant dilation of the cavities of the lateral chambers of the brain. Histologically, in the brain and spine, in the intervertebral ganglia, no prominent changes (with the use of the above-mentioned method of investigation) were seen. In Volchok, pycnosis of the Purkin cells in the cerebellum were seen.

More marked histo-pathological changes were to be found in Volchok, which died as a result of the development of repeated thiamine deficiency.

On analysis of the patho-morphological and clinical material, an underlying connection between the clinical picture of the disease and the morphological distrubances of the corresponding organs was noted. Obviously, the cause of the intestinal hemmorrhage in the experimental animals was a highly pronounced hemorrhagic diathesis, connected with diapedesis, in particular chronic ulcers, forming in the small intestine.

The patho-morphological and clinical picture did not give sufficient grounds to enable one to speak of disease of the animals involving scurvy alongside polyneuritis. One could only note an unusual extensive hemorrhagic affection in our dogs, compared with what has been described in the literature. This particularly applied to the hemorrhagic affection of the intestine. In addition, what was new was the development of numerous ulcerations in the gastro-intestinal tract.

In the literature, we have not found descriptions of ulcerous lesions of the digestive tract in dogs experimentally deprived of vitamin B_1. Only M. S. Levinson [3] indirectly refers to the fact that in a series of experiments with polyavitaminosis in one dog, he observed a manifestation of ulcer in the intestine. He tentatively associated this fact with vitamin A deficiency. However, our experiments do not bear out this hypothesis, since we regularly gave fish oil to the experimental animals.

In addition, we succeeded in finding references [4] to the development of ulcerous involvement of the gastro-intestinal tract in colts with an experimental deficiency of vitamin B complex and C. In the experiments of A. P. Onegov et al, as in our work, the experimental animals did not have an intake of supplementary vitamin C. The colts also belong to the group of animals which in natural conditions do not require vitamin C in their food. The authors of the work [4], like us, did not succeed in inducing marked symptoms of scurvy in the colts.

Among the lesions of other internal organs, particular attention was aroused by changes in the heart in our dogs, which were at the root of the severe disturbances of cardiac activity in the experimental animals. In the first stage of development of avitaminosis, bradycardia was present (60-90 beats a minute). With a detioration in the condition of the animals, bradycardia was replaced by tachycardia (up to 200 beats a minute). Respiratory arrhythm which was normally weakly expressed in the dogs rose sharply in the period of bradycardia, and disappeared with the manifestation of tachycardia. In the E. C. G. readings recorded at various stages of development of vitamin B1 deficiency, at first a sharp intensification of the tonus of the vagus nerve (bradycardia, respiratory arrhythm) was noted and then its paralysis occurred or the heart somehow evaded its influence (tachycardia, complete disappearance of respiratory arrhythm). On the electrocardiagram, the most common reading was seen in inversion or di-phasing of the T wave. In Volchok, a steady break down of the R waves was witnessed. The tonus of the vessels in the final days before death of the animals fell heavily. The veins collapsed. The arterial pulse was felt with difficulty.

The above-described patho-morphological disturbances in the cardiac muscle (hypertrophy, edema, hemorrhage; degenerative changes of the muscle fibres) represented typical consequences of vitamin B_1 deficiency. What was new in our experiments in comparison with the literature findings was the discovery of signs of plasmorrhagia, endo-and mesoarteritis of the cardiac vessels.

As for the central and peripheral nervous systems, the disturbances proved to be typical of polyneuritis. The development stages of clinical thiamin deficiency in our animals, as also the time of their death, did not deviate from the normal.

However, there is no doubt that the development of ulcerous affections of the gastro-intestinal tract with vitamin B deficiency in the dogs in our experiments was not fortuitous. It is possible that here, absence from the diet not only of thiamin, but of ascorbic acid is of significance. In the given case, it is not clear whether on a marked thiamin deficiency in the dogs, there takes place in absence of vitamin C intake disturbances of the functions of the organism in relation to auto-synthesis of vitamin C.

This question must be the subject of further detailed and thorough study.

It is possible that study of this question will enable one to make progress in clarifying certain aspects of the etiology and pathogenesis of ulcerous diseases.

LITERATURE CITED

[1] Zevald, L. O., "Influence of vitamin B_1 Deficiency on Conditioned Reflex Activity in Dogs.,". Works of the I. P. Pavlov Physiological Laboratory (Moscow-Leningrad, 1948), Vol. 14, pp. 159-165.

[2] Kudryashov, B. A., Biological Bases of Our Knowledge of Vitamins* (Moscow, 1948).

[3] Levinson, M. S., Clinical Aspects of Experimental Vitamin BDeficiency * (Rostov-on Don, 1939).

[4] Onegov, A.P., Lagunov, M.P., and Mitrofanov, M. V., Veterinariya 1949, No. 12, pp. 41-43.

[5] Razenkov, I.P., and Shekun, L. A., Byull. Biol. i Med., Vol. 28, No. 9, pp. 220-225 (1949).

* In Russian.