DEVELOPMENT OF EXPERIMENTAL AMYLOIDOSIS IN ANIMALS PRELIMINARILY ADAPTED TO HYPOXIA

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In the clinical practice of pulmonary tuberculosis cases are often seen in which, in the absence of active tuberculosis of the lungs but in the presence of pneumosclerosis and emphysema, accompanied by hypoxemia, the patients develop extensive amyloidosis of the internal organs [6]. This has led to the suggestion that hypoxemia may play a definite role in the pathogenesis of the amyloidosis found in tuberculous patients [9, 10]. Relatively short periods of adaptation to hypoxia are known to raise the general resistance of the organism not only to hypoxia, but also to several other agents [2, 4].

An attempt was made to determine experimentally the importance of hypoxia in the pathogenesis of amyloidosis by studying the role, both of relatively brief adaptation to hypoxia and of more prolonged chronic hypoxia, in the development of experimental amyloidosis. The duration of adaptation of the animals in the different series of experiments to hypoxia accordingly varied.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male albino mice weighing 20-29 g. Adaptation to hypoxia was achieved in a pressure chamber in which the atmospheric pressure was rarefied to 354 mm Hg, equivalent to an altitude of 6000 m. The animals of group 1 (24 mice) were kept at this "altitude" daily (except Sundays) for 4 h for 1 month, and those of group 2 (also 24 mice) likewise for 3 months. The control groups of animals (24 mice in each) were not exposed to the action of hypoxia and were kept in the vivarium in the same conditions as the experimental animals. At the end of the experiments the red and white blood composition was studied in all the mice. Next, in all the animals adapted to hypoxia, the general resistance to acute hypoxia was determined. For this purpose the animals were placed one by one under the bell of a vacuum pump, and the atmospheric pressure was quickly lowered. The "altitude" at which clonic convulsions began or respiration ceased altogether was recorded. In this way the "altitude ceiling" of the animals was measured. Both groups of animals were then transferred to a diet (oats and water ad lib.) and they began to receive subcutaneous injections of 5% casein solution, which was prepared weakly in sterile conditions without the addition of antibiotics and conservants. The injections of 5% casein solution were given to the mice subcutaneously in the dorsal region, in a dose of 0.5 ml daily except Sundays. Altogether 28 injection were given. To keep them in training, the animals adapted to hypoxia were placed in the pressure chamber for 4 h at an "altitude" of 6000 m on alternate days instead of daily. At the end of this series of injections, the red and white blood pictures were again studied in the mice of both groups, after which the animals were sacrificed. The liver, spleen, and kidneys were fixed in 10% formalin solution. From 4 to 6 sections were then cut from each organ and stained with hematoxylin-eosin, by Van Gieson's method, and for amyloid (with Congo red).

EXPERIMENTAL RESULTS

As in the authors' previous experiments [2], training for 1 month to hypoxia increased the general resistance of the animals to acute hypoxia of a high degree, as revealed by the increase in the "altitude ceiling from 11,520 ± 0.311 to 12,800 ± 0.302 m ($P < 0.01$). Marked stimulation of erythropoiesis was observed, as shown by an increase in the number of erythrocytes and reticulocytes in the peripheral blood. Meanwhile the leukocyte count was reduced. After injection of a 5% solution of casein for 1 month, a slight increase in the leukocyte count was observed in the blood of all the animals, but a particularly high leukocytosis (up to 29,500) was found in the control group of

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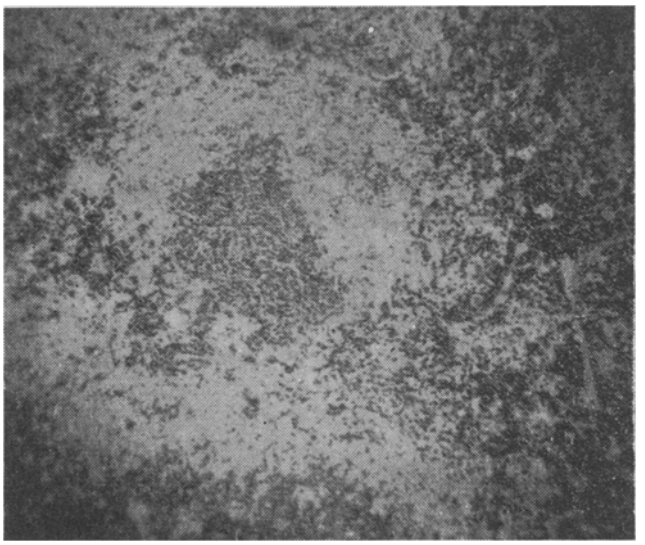

Fig. 1. Massive deposition of amyloid around the follicles and in the red pulp of the spleen. Congo red. 80 ×.

mice with amyloidosis of the spleen, liver, and kidneys. In all the animals a tendency was observed for the erythrocyte count to fall, but in the controls this was more marked than in the mice adapted to hypoxia for 1 month.

After the beginning of the casein injections, 3 of the 24 mice adapted to hypoxia and 5 of the 24 control mice died from shock. The results of a histological investigation of the liver, kidneys, and spleen of the animals sacrificed after the end of the course of casein injections showed that in 5 of the 21 (23.8%) mice adapted to hypoxia initial amyloidosis was present in the spleen, characterized by slight deposition of amyloid material around the follicles only. In the control group of animals amyloidosis of the spleen was found twice as often – in 10 of the 19 animals (52.6%), and it was more severe than in the animals adapted to hypoxia: in 4 mice the deposition of amyloid was around the follicles only, in 5 others a more massive deposition of amyloid was observed both around the follicles and in the red pulp (Fig. 1), and in one continuous areas of amyloid were found, with disappearance of the cellular structure of the spleen. Besides amyloidosis of the spleen, in 5 of these 10 animals amyloidosis of the liver was also present, with deposition of amyloid in the vessel walls and in some places between the trabeculae, and in 2 mice an initial deposition of amyloid was seen in the walls of the large blood vessels of the kidneys.

The results of training of the mice for 3 months in hypoxic conditions differed appreciably from the results obtained after training in the pressure chamber for 1 month. First, the "altitude ceiling" of the animals after training for 3 months in the pressure chamber not only did not exceed normal, as was found after training for 1 month, but in most animals it was lowered from 11,500 to 9000 m. It must be assumed that training for 3 months in this severe hypoxia caused by lowering the atmospheric pressure to 354 mm Hg (pO_2 71 mm Hg) led ultimately to collapse of the adaptation, to untraining, and to a lowering of the animals' general resistance.

So far as the changes in the peripheral blood were concerned, these animals did not show an erythrocytosis and their erythrocyte count was within normal limits, while the leukocyte count was slightly raised. After the beginning of the casein injections the leukocyte count in the blood of some control animals fell substantially (to 30,500), while in the group of mice with hypoxia it rose to 52,500 or even to 60,000, which was associated with the development of marked amyloidosis of the internal organs in these animals.

The results of clinical examinations also showed that in patients with severe forms of amyloidosis a hyperleukocytosis was present, without a shift of the leukocyte formula to the left [5]. The erythrocyte count was unchanged in these mice after injection of casein, but the reticulocyte count was raised by comparison with the controls ($P < 0.001$). Since this stimulation of erythropoiesis did not lead to any marked increase in the number of erythrocytes in the blood, it must be concluded that the destruction of the erythrocytes in the blood of the animals with hypoxia was intensified, and this constitutes an unfavorable factor.

During the first days after the beginning of the casein injections, six mice in each group died, so that the histological investigation of the internal organs for amyloidosis after the end of the experiment was carried out on 18 mice with hypoxia and 18 control animals. In all 18 mice with hypoxia amyloidosis of the spleen was observed,

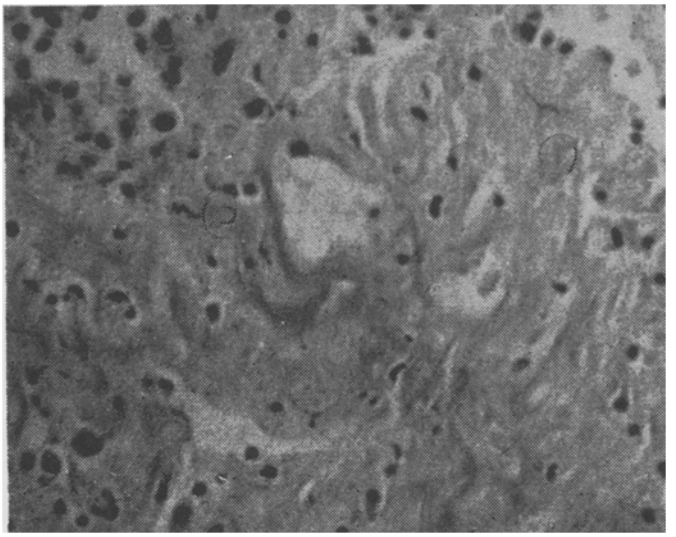

Fig. 2. Massive deposition of amyloid in the spleen with disappearance of its cell structure. Congo red. 40×.

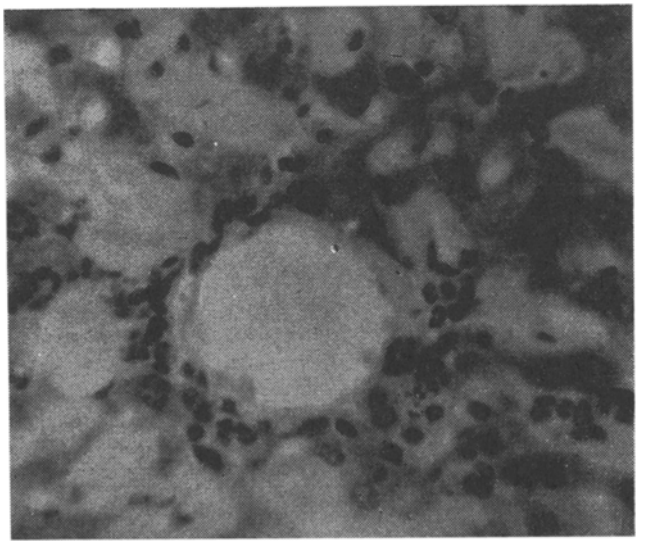

Fig. 3. Deposition of amyloid in the wall of a vein and along the course of the intralobular capillaries with atrophy of the hepatic trabeculae in the central portion of a lobule of the liver. Congo red. 40×.

and in 4 of them it was severe, in the form of continuous zones of amyloid (Fig. 2). In addition, in 8 of these 18 animals marked amyloidosis of the liver was found, with deposition of amyloid between the hepatic trabeculae and with atrophy of the trabeculae themselves (Fig. 3), and in 4 animals amyloidosis of the kidneys was found, with deposition of amyloid beneath the tunica propria of the straight renal tubules and, in 2 animals, in some of the renal glomeruli besides. In the control group amyloidosis of the spleen developed in 10 of the 18 mice (in 55%), the liver was involved in 5, and the kidneys in only 1 of these 10 mice. The deposition of amyloid in these animals just mentioned was in its initial stages or moderately severe.

These experiments thus demonstrated that preliminary training for 1 month of animals to hypoxia, by increasing their general resistance, inhibits the subsequent development of experimental casein amyloidosis.

It is technically very difficult to create a state of constant hypoxia in animals experimentally which would correspond to chronic hypoxemia found in patients with cardio-pulmonary insufficiency [7]. However, it has been shown that, as a result of the prolonged, intermittent action of hypoxia, various physiological and biochemical changes develop in acclimatized animals [2, 7, 8, 11], just as in chronic hypoxemia.

The other group of mice was exposed to training for 3 months in severe hypoxic conditions caused by a fall in atmospheric pressure to 354 mm Hg (pO_2 71 mm Hg), and this evidently led to a collapse of adaptation, for it has been shown that as a result of the prolonged action of harmful factors and, in particular, of hypoxia the resistance of the body is lowered, not raised [2].

The results of experimental investigations have shown that the synthesis of ascorbic acid in the body is disturbed under the influence of chronic hypoxia [3] and its content in the adrenal tissues is reduced [1, 13]. It has also been found that after exposures of long duration to high degrees of hypoxia experimental animals may develop exhaustion and marked functional insufficiency of the adrenals [12, 14]. The depression of adrenal cortical function, which plays an important role in the regulation and the normal course of protein metabolism, provides a favorable background for the development of amyloidosis, regardless of the causes responsible for it. This may evidently be the explanation of the development of casein amyloidosis of the spleen in all the mice exposed to prolonged and severe hypoxia, whereas in the control group it was found in only 55% of the animals and was less marked.

SUMMARY

The purpose of the research was to ascertain the influence of a relatively brief (month-long) and longer (3 month-long) hypoxia on the subsequent development of experimental casein amyloidosis. The experiments were carried out on 96 albino mice. It was shown that a month-long training for adaptation to hypoxia, while increasing the general resistance of the animals, inhibited the subsequent development of amyloidosis, which was manifested by a noticeable decrease in spleen amyloidosis in animals trained for hypoxia (23.8%) as compared to the control group (52.6%). Three month-long training of animals for such hard hypoxia as rarefaction of the air to 354 mm Hg(pO_2 71 mm Hg) thwarted adaptation and contributed to the development of spleen amyloidosis in all animals (100 per %), whereas in the control group it was noted much less frequently (55%). Apparently, chronic hypoxia causing a number of changes in the body, in particular, hypofunction of the adrenal cortex regulating protein metabolism, thereby contributes to the development of casein amyloidosis in all test animals.

LITERATURE CITED

1. G. A. Arutyunov and N. S. Yarusova, In: Proceedings of the 7th All-Union Congress of Physiologists, Biochemists, and Pharmacologists [in Russian], Moscow (1947), p. 638.
2. Z. I. Barbashova, Acclimatization to Hypoxia and Its Physiological Mechanisms [in Russian], Moscow–Leningrad (1960).
3. V. N. Borsuk, Proceedings of the I. P. Pavlov Institute of Physiology, AN SSSR [in Russian], Vol. 4, Moscow–Leningrad (1949), p. 197.
4. V. M. Davydova, Byull. Éksp. Biol., No. 10 (1963), p. 35.
5. Z. I. Kostina and V. P. Grigor'eva, Probl. Tuberk., No. 7 (1961), p. 34.
6. Z. I. Kostina, Terap. Arkh., No. 9 (1962), p. 62.
7. E. M. Kreps, N. A. Verzhbinskaya et al., Fiziol. Zh. SSSR, No. 1 (1956), p. 69.
8. M. E. Lobashev, Doklady Akad. Nauk SSSR, Vol. 68, No. 4 (1949), p. 793.
9. A. Ya. Tsigel'nik, Z. I. Kostina et al., Klin. Med., No. 12 (1961), p. 14.
10. A. Ya. Tsigel'nik and B. Ya. Shapiro, Sov. Med., No. 6 (1963), p. 87.
11. P. D. Aitland and B. Highman, Am. J. Physiol., Vol. 161 (1951), p. 261.
12. H. G. Armstrong and J. W. Heim, J. Aviat. Med., Vol. 9 (1938), p. 45.
13. G. Aschan, Acta Soc. Med. Upsalien., Vol. 58 (1953), p. 265.
14. H. J. Saha, Indiana Med. Assoc., Vol. 23 (1954), p. 428.
