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EFFECT OF VITAMINS A, E, C, AND P ON INTENSITY OF EXPERIMENTAL INTRAVASCULAR BLOOD CLOTTING

A. Sh. Byshevskii, G. S. Solov'ev, S. L. Galyan, V. G. Solov'ev, and S. V. Solov'ev

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Vitamins A and E possess anticlotting, and vitamins C and P a vasoprotective action [2, 12], and they are all antioxidants. By lowering the blood clotting activity of biomembranes they limit thrombin production [10] and reduce the frequency of thrombotic complications in the postoperative period [9].

The aim of this investigation was to study the effect of these vitamins on disturbances of blood clotting and of the microcirculation in experimental exogenous thromboplastinemia.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats (150 ± 15 g), some of which received a mixed diet fortified with vitamins: A) 600 IU, E) 0.15, C) 45, and P) 20 mg/kg body weight daily (for 12 days). On the 13th day the aggregating activity of the platelets, the activated recalcification time (ART), the activated partial thromboplastin time (APTT), the prothrombin index (PI), total antithrombin activity (AA) and antithrombin III (AT-III), fibrinogen activity (FA), concentrations of fibrinogen (FG), PAF, and factor XIII (fXIII), activity of tissue thromboplastin in supramolecular particles of the blood plasma [1, 8], and deformability of the erythrocytes [11] were determined on the 13th day. The rats were then given an injection of a suspension of thromboplastin (0.5 ml/100 g body weight), and blood samples were again taken after 0.5 h. Some of the animals were killed by decapitation and their internal organs removed, fixed in Carnoy's fluid, and embedded in paraffin wax. Sections 5-7 μ thick were stained with Mayer's hematoxylin and eosin. Carbohydrates were revealed by Hale's reaction of colloidal iron binding and the PAS reaction after McManus.

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TABLE 1. Blood Clotting 0.5 h After Injection of Thromboplastin Suspension (0.5 ml/100 g body weight, activity 22 sec) into Animals Receiving Vitamins (12 rats in a group)

Parameters studied	Intact control	Thromboplastin injected	
		without vitamins	with vitamins
ART, sec	39,5±0,7	69,3±0,5*	48,7±0,4**
APTT, sec	28,3±0,2	95,7±1,5*	61,4±0,8**
PI, %	95,5±0,9	76,4±1,0*	90,4±1,4**
AA, %	100,0±0,9	100,0±1,1	89,5±2,0**
AT-III, %	77,0±0,7	247,0±3,8*	100,0±2,4**
FG, g/liter	3,4±0,3	0,9±0,2*	1,1±0,2*
FA, mm^2	51,4±7,0	51,4±5,1	42,8±2,9
PAF, mg %	15,6±2,8	11,9±0,9	12,7±0,8
ET, %	0,0	83,3	0,0
fXIII, sec	52,0±4,6	23,8±0,5*	42,8±0,7°*

Legend. *) Difference compared with group 1 significant; **) differences significant relative to groups 1 and 2; °*) relative to group 2 only ($p < 0.05$).

EXPERIMENTAL RESULTS

Administration of vitamins reduced APTT by 15%, increased AA by 4% and increased activity of AT-III by 7% in intact animals. The aggregating activity of the platelets, namely the duration of aggregation waves I and II was reduced by 2.8 and 5.3 times, and the blood clotting activity of the erythrocytes was reduced from 3500 ± 58 to 2100 ± 10 activity units/ml ($p < 0.05$). The changes reached a maximum 0.5 h after injection of thromboplastin (Table 1): in animals not receiving vitamins ART (by 77%) and APTT (by 239%) were lengthened, PI fell (by 21%), AT-III was activated (by 221%), and concentrations of FG (by 74%) and fXIII (by 55%) fell. Positive ethanol test (ET) appeared (83.3%). The changes in animals receiving vitamins were significantly limited, i.e., the intensity of ET was weaker.

The protective effect of vitamins was confirmed by an integral test, namely the mortality rate after injection of thromboplastin in a dose of LD_{50} (0.5 ml, activity 14 sec): of 42 rats receiving vitamins 16 died, of 27 not receiving vitamins 18 died, i.e., rates of 38 and 49% respectively ($p < 0.05$). Since the cause of death of the animals from thromboplastinemia is intravascular thrombosis and disturbance of the microcirculation [5, 6], a histologic study of the internal organs with a well-developed capillary network (lungs, kidneys, liver, spleen, adrenals, testes) was carried out 0.5, 3, 6, and 24 h after the injection of thromboplastin (six rats in a group).

Spasm, occlusion, and thrombosis of branches of the pulmonary arteries accompanying the small bronchi and terminal bronchioles were discovered in animals receiving vitamins 0.5 h after the injection. The changes developed in stages: narrowing of the lumen of the arteries due to local contraction of muscle cells and pavementing of the blood cells, deposition of fibrin threads, juxtamural fixation of blood cells (Fig. 2a) and thrombus formation (Fig. 2b). The disturbance of function increased after 3 h: hyperemia, stasis in the small venous vessels and in the capillary bed of the respiratory sectors, disturbance of the integrity of the walls of the capillaries and venules, diapedesis of erythrocytes into the interalveolar tissue, hemorrhage into the alveolar cavity (Fig. 2c), activation of tissue macrophages, and their migration with erythrocytes, lymphoid cells, and monocytes into the alveoli were discovered. After 6 h spasm of the arteries increased, hemorrhages arose from whole acini and even lobules, the integrity of the wall of the capillaries and venules was disturbed, permeability (diapedesis of the cells and profuse hemorrhages into the alveoli) increased, and desquamation of the alveolar epithelium in the zone of diapedesis continued. After 24 h

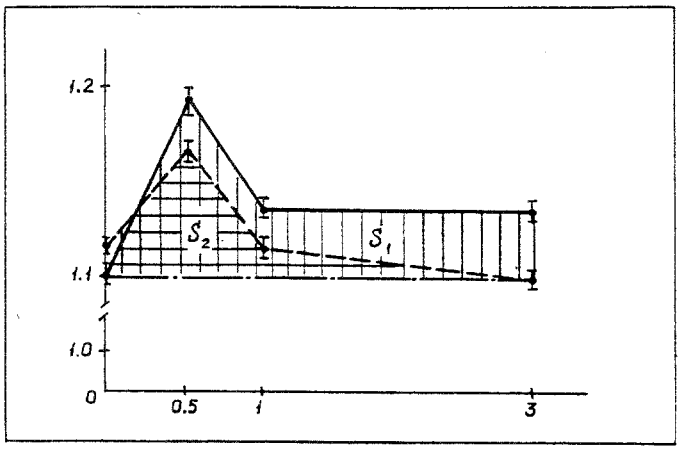

Fig. 1. Coefficient of deformation of erythrocytes (ordinate) as a function of time after injection of thromboplastin (abscissa). Broken line, degree of deformation in intact animals; short vertical and horizontal lines indicate areas bounded by curves characterizing control and vitaminized animals (S_1 and S_2 respectively).

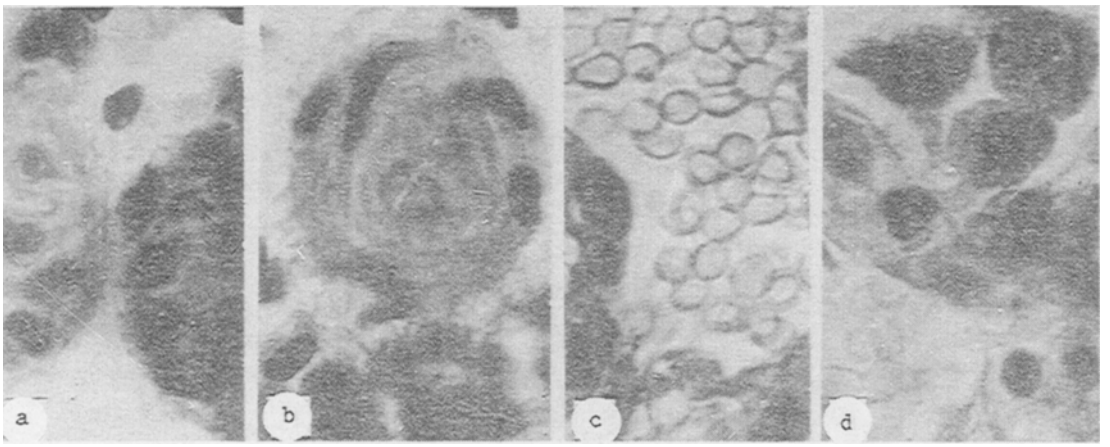

Fig. 2. Reaction of the lung to injection of tissue thromboplastin: deposition of fibrin threads in region of artery in spasm, 0.5 h of experiment; b) thrombosis in artery, 0.5 h of experiment; c) hemorrhage into alveolar cavity, 3 h of experiment; d) macrophages during lysis of alveolar contents, 24 h of experiment. Stained with Mayer's hematoxylin and eosin, magnification 630×.

the drainage function of the bronchopulmonary tree was activated, with lysis of erythrocytes, utilization of the alveolar contents by macrophages (Fig. 2d), and their transport into the lumen of alveolar bronchioles and terminal and small bronchi. Individual alveoli were filled with tissue macrophages, and stasis was present in the postcapillary venules. Diapedesis of erythrocytes almost ceased, but migration of the leukocytes and lysis of components of the blood in the alveoli continued.

In the kidney the lumina of the capsules of the renal corpuscles were dilated 0.5 h after injection of thromboplastin: the blood flow in the arterial glomerulus was reduced because of spasm of the afferent arterioles. No appreciable changes could be seen in the larger vessels and the transport function remained intact. After 6 h the

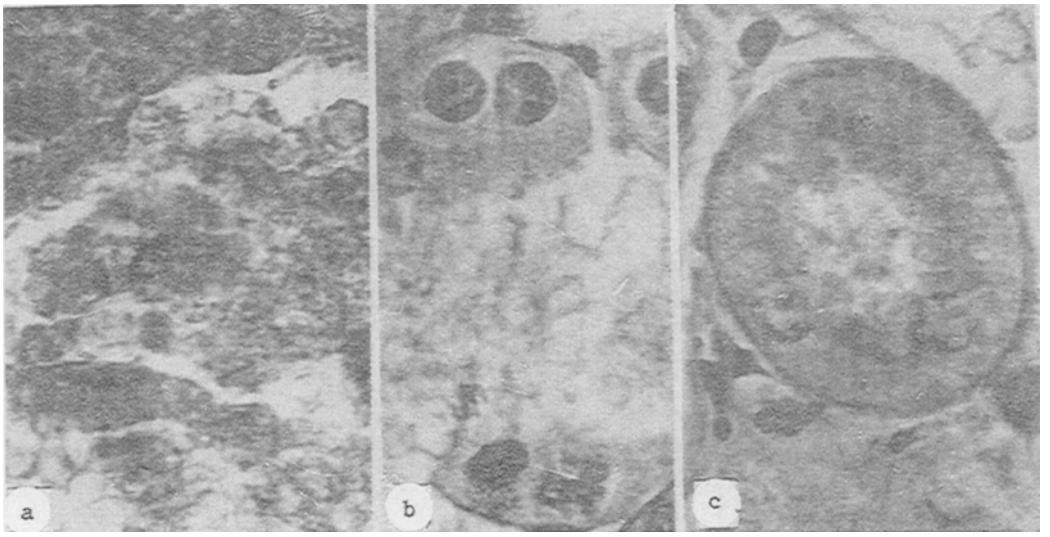

Fig. 3. Reaction of kidney to injection of tissue thromboplastin: a) hemorrhage into lumen and destruction of renal corpuscle, 24 h of experiment; b) destruction of proximal tubule of animal not treated with vitamins, 24 h of experiment; c) reaction of proximal tubule of animal receiving vitamins, 0.5 h of experiment. PAS reaction after McManus, magnification 630×.

nuclei in the renal corpuscles were swollen, cells of the outer layer of the capsule were increased in height, and PAS-positive substrate appeared in the cavity, but cells of the mesangium, podocytes, and epithelial cells of the outer layer preserved their vital properties, and the blood flow in the loops of the arterial glomerulus remained intact. After 24 h foci of necrosis of individual renal corpuscles were detected in the cortex, and the cavities of the capsules were filled with blood (Fig. 3a). Significant changes also were observed in the tubular structures of the nephron: after 6 h destruction of the epithelial cells in the proximal portions (separation of the brush border followed by pycnosis of the nucleus and destruction of the basement membrane), hemorrhages into the lumen of the necrotic tubules and into the stroma, edema of the connective tissue, and widening of the space between the convoluted tubules were found. The damage was mosaic in character (Fig. 3b).

On the whole the changes discovered are characteristic of disseminated intravascular clotting [5]. Changes in other organs will not be examined because of shortage of space – they were similar to those described above: disturbances of the microcirculation associated with spasm of arterioles, fibrin deposition, desquamation of the vascular epithelium and diapedesis of blood cells into the perivascular connective tissue.

The response to injection of thromboplastin was weaker after vitamin administration. In the lungs the circulation through the arteries accompanying bronchi of small caliber and terminal bronchioles was preserved, there was no sign of thrombosis, the vascular wall was impermeable to blood cells, the perivascular spaces contained cells of the fibroblast series, and amorphous material, and the fibrous structures were not dilated but gave a weak Hale-positive reaction, which was abolished by treatment with testicular hyaluronidase (a sign of a sufficiently high content of hyaluronates, i.e., of low endogenous hyaluronidase activity). Toward 6 h stasis and hyperemia were found only in certain parts of the capillary bed. Diapedesis of erythrocytes into alveoli and massive hemorrhages were not present, interalveolar connective-tissue bands were widened a little (seepage from adjacent capillaries), and active tissue macrophages were present. The lumen of the bronchi was moderately filled with PAS- and Hale-positive material, with solitary desquamated epithelial cells. Toward 24 h the capillary bed was restored to normal: the

number of macrophages in the interalveolar connective-tissue septa was increased, the alveoli were rid of their contents, and the epithelial lining had regenerated (Fig. 2).

Tubular necrosis was not observed in the kidneys of animals receiving vitamins, although the integrity of the epitheliocytes was disturbed – the brush border was detached in cells of the proximal tubule, with preservation of the basement membrane and nuclei of individual epitheliocytes underwent pycnosis (Fig. 3).

The severity of the morphological changes in other organs investigated also was reduced by preliminary vitamin treatment.

Desquamation of the vascular endothelium led to hyperthromboplastinemia [14]. Having separated fragments of the cell membranes from plasma, we found an increase in tissue thromboplastin activity in animals not treated with vitamins, from 14,000 ± 439 to 18,500 ± 432 activity units/ml ($p < 0.05$), but in animals receiving vitamin treatment this parameter was unchanged.

Limitation of disturbances of the microcirculation by administration of vitamins was confirmed by a study of deformation of erythrocytes, which increases during thrombinemia as a means of compensation to maintain the transport function of these cells. Deformation of erythrocytes in the course of 3 h after injection of thromboplastin into rats receiving vitamins was 2.3 times lower than in animals untreated with vitamins (Fig. 1). This cannot be considered to be associated with any negative effect of vitamins on the erythrocyte membrane: antioxidants, by activating Ca^{2+}-dependent ATPase and reducing the intracellular Ca^{2+} concentration, increase the conformation potential of spectrin [12] and inhibit lipid peroxidation in erythrocytes, by optimizing the microviscosity of the membranes [7]. Consequently, deformation of the erythrocytes, which increases in intact animals under the influence of vitamins, is not called for in thromboplastinemia because of limitation of the microcirculatory disturbances.

Preliminary administration of vitamins A, C, E, and P thus weakens blood clotting shifts and microcirculatory disturbances induced by thromboplastinemia – an inevitable intermediary of disseminated intravascular clotting of varied etiology [3, 8].

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