PHARMACOLOGY

EFFECT OF A PREPARATION OF VITAMIN P ISOLATED FROM TEA LEAVES ON BLOOD MORPHOLOGY AND ON SOME ASPECTS OF BLOOD AND TISSUE BIOCHEMISTRY

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Vitamin P prepared from tea leaves is recommended for the treatment of patients presenting symptoms of heightened permeability of blood vessels, such as edema other than of cardiac or renal origin, hemorrhagic diathesis, etc. [2]. In such cases the vitamin is taken repeatedly over a long time.

We have been unable to find in the literature any references to the experimental study of the pharmacological action of tea-leaf vitamin P, or of its effect on blood morphology and metabolism.

The present paper describes the effect of repeated administration of vitamin P on blood morphology, on blood clotting in experimental hypoprothrombinemia, on the adenosinetriphosphate, creatine phosphate, and inorganic phosphate contents of heart muscle, on the glycogen content of heart and skeletal muscle and liver, on the oxygen and carbon dioxide tensions of arterial and venous blood, and on the activity of some enzymes in vitro.

Effect of the Vitamin P Preparation on Blood Morphology

A 0.5% solution of vitamin was injected subcutaneously into 8 albino mice, at a dosage level of 100 mg of vitamin per kg body weight, daily for 11 days.

Examination of the blood initially and after 11 days showed no change in the hemoglobin content, in the erythrocyte, reticulocyte, and leucocyte counts, or in the leucocyte differential count, but an increase in the thrombocyte count.

Effect in Experimental Hypoprothrombinemia

Prothrombin times were determined for a group of 14 albino rats weighing 200-213 g, by B. A. Kudryashov's modification of Quick's method. The rats were made hypoprothrombinemic, as a result of obstructive jaundice caused by ligation of the common bile duct, [1]. In obstructive jaundice, as well as in biliary fistula, secretion of bile acids into the intestine is prevented; this interferes with absorption of vitamin K from the intestine, and so leads to depletion of blood prothrombin, and to development of hemorrhagic diathesis.

The common bile duct was ligated under aseptic conditions and under anesthesia, and after the operation 30,000 to 50,000 units of penicillin were given intraperitoneally. Jaundice developed on the sixth day, when the skin of the ears became icteric, and the urine becomes yellowish-brown.

Blood was taken from the jugular vein; attempts at taking blood from the heart resulted in hemorrhage into the pericardium, followed by death from occlusion of the heart.

All the rats developed hypoprothrombinemia after ligation of the common bile duct. Ten rats were given a daily subcutaneous injection of 100 mg of vitamin P per kg body weight, as 1% solution, for 6 days, and prothrombin time was again determined on the sixth day; the remaining 4 rats served as controls. Most of the rats receiving vitamin P had a normal prothrombin time, and showed less tendency towards hemorrhage, so that it became possible to take blood from the heart (death did not result from hemorrhage into the pericardium, as was the case with control rats not given vitamin P).

The results of this experiment are presented in Table 1.

TABLE 1

Effect of Vitamin P on the Prothrombin Content of the Blood of Hypoprothrombinemic Rats

No. of animal	Before operation		6 days after ligation of the common bile duct		6th day of vitamin administration	
	clotting time (sec.)	prothrombin index (%)	clotting time (sec.)	prothrombin index (%)	clotting time (sec.)	prothrombin index (%)
1	14	100	29	49.5	14	100
2	12	100	29	41.2	12	100
3	15	100	40	37.5	15	100
4	13	100	70	18.5	13	100
5	14	100	*		14	100
6	12	100	*	*	12	100
7	13	100	*	*	13	100
8	14	100	70	20	19	74
9	12	100	16	75	12	100
10	14	100	70	20	14	100
					Controls (vitamin P not given)	
11	14	100	40	35	70	20
12	14	100	52	27	*	*
13	14	100	70	20	*	*
14	14	100	*	*	*	Died

Note: The prothrombin index is calculated as % of controls, taken as 100.

*No clotting for 24 hours.

The experiments show that repeated administration of our preparation of vitamin P shortens the clotting time in hypoprothrombinemia due to bile duct ligation.

Effect of Vitamin P on Blood Gases, on the Glycogen Content of Various Organs, and on the Content of Phosphorus Compounds in Heart Muscle.

Six rabbits were taken for the experiments. Blood was taken from ear arteries and veins an hour after a single injection of vitamin, at a dosage level of 20 mg per kg body weight, and blood gases were determined in a Van Slyke apparatus (venous blood from 4 rabbits, arterial from 2; the remaining three served as controls). Thereafter all animals received a daily dose of 20 mg of vitamin per kg body weight, for 10 days, and blood samples were again taken on the 11th day. A further dose of vitamin was then again given, and the rabbits were killed by decapitation an hour later. Tissue fragments (heart, skeletal muscle, and liver) were minced and frozen in liquid nitrogen, and the glycogen content was determined by Pflüger's method, and the content of phosphorus compounds by the method of Severin and Meshkova. The results of the experiments are presented in Tables 2 and 3.

Our experiments show that administration of vitamin P has no effect on the glycogen content of the heart and skeletal muscles and liver, on the creatine phosphate, adenosinetriphosphate, and inorganic phosphate contents of heart muscle, or on the carbon dioxide content of venous blood and the oxygen content of arterial blood.

TABLE 2

Glycogen Content of Heart and Skeletal Muscle and Liver, and Content of Phosphorus Compounds in Heart a Muscle on the 11th Day of Administration of Vitamin P.

No. of rabbit	Glycogen, %						Phosphorus compounds in heart muscle (mg-%)					
	heart		skeletal muscle		liver		inorganic phosphate		creatine phosphate		adenosinetri-phosphate	
	control	after vitamin P administration	control	after vitamin P administration	control	after vitamin P administration	control	after vitamin P administration	control	after vitamin P administration	control	after vitamin P administration
1	0.410	0.454	0.242	0.264	6.708	3.894	24.5	25.0	5.2	5.2	18.8	18.8
2	0.300	0.246	0.300	0.100	3.500	2.000	27.5	24.0	5.4	3.9	15.7	26.1
3	0.380	0.326	0.170	0.316	1.970	4.304	24.5	23.0	4.9	10.4	19.0	15.0
4	—	0.365	—	0.542	—	6.744	—	27.5	—	7.5	—	14.8
Mean	0.363	0.345	0.258	0.308	4.059	4.232	25.5	24.9	5.2	6.8	17.5	18.7

TABLE 3

Carbon Dioxide and Oxygen Contents of the Blood of Rabbits Given Single Injections of Vitamin P at a Dosage Level of 20 mg per kg., and Given Daily Injections for 10 Days.

Nature of sample	No. of rabbit	Oxygen (volumes %)		Carbon dioxide (volumes %)	
		before injection	after injection	before injection	after injection
Venous blood	1	I 15.6	—	33.1	—
		II 15.6	—	33.1	—
Control (repeated sampling)	2	I 11.0	—	27.8	—
		II 11.0	—	34.5	—
	3	I 13.7	—	36.8	—
		II 13.7	—	34.8	—
Venous blood	4	a 19.5	18.1	37.2	37.2
		b 16.7	14.2	23.7	26.1
	5	a 17.2	15.8	29.7	35.2
		b 14.8	14.8	28.2	24.6
	6	a 13.7	12.7	36.8	34.5
	7	b 13.5	11.8	30.7	35.2
Arterial blood	8	a 15.9	15.3	28.3	32.6
		14.6	15.0	17.0	17.0
	9	16.8	16.8	41.5	28.9

Explanation: I signifies first sampling, II second sampling, 1 hour later; a) single, b) repeated injection of vitamin.

We found a fall in oxygen content of venous blood in all cases after single or multiple injections of vitamin, without rise in arterial blood oxygen content. This may indicate increased uptake of oxygen by the tissues after administration of vitamin; this possibility requires further experimental confirmation.

Effect of the Vitamin Preparation on the Activity of Enzymes in Experiments In Vitro.

The activity of various enzyme preparations in presence of various concentrations of vitamin was determined by the following methods: cholinesterase by the method of Pravdich-Neminskaya, urease by determining production of ammonia from urea, amylase by its action on strach as shown by the iodine test, and catalase by a

manganometric method. The results are presented in Table 4.

TABLE 4

Effect of Vitamin P on Activity of Enzymes.

Conditions of experiment	Activity of			
	cholinesterase (ml $\frac{H}{100}$ NaOH)	urease (ml $\frac{H}{10}$ NaOH)	catalase (mg of undecomposed H_2O_2)	amylase, as limiting dilution of extract
Inactivated enzyme	0.01	5.8	4.58	—
Enzyme without addition of vitamin	2.8	3.9	0.29	1:480
Enzyme + vitamin in a concentration of				
0.00025 mg (ml)	2.7	3.8	0.29	1:480
0.0025 "	2.8	4.05	0.29	1:480
0.025 "	1.9	4.1	0.29	1:480
0.25 "	1.6	4.25	0.43	1:240
2.5 "	1.4	4.3	0.57	1:60
25.0 "	0.8	5.8	3.58	1:60

It appears from the experiments that vitamin P in concentrations of 0.025 mg per ml, and higher, lowers the in vitro activity of the enzymes studied.

LITERATURE CITED

[1] B. A. Kudryashov, P. D. Ulitina, and A. A. Pugacheva, Bull. Exptl. Biol. Med., 11, 2, 99-101 (1941).

[2] F. Bicknell, and F. Prescott, The Vitamins in Medicine, New York, 1946.