A 5-HYDROXYTRYPTAMINE FROM Hippophae rhamnoides AS AN ANTITUMOR PREPARATION IN EXPERIMENTS ON ANIMALS

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Two years ago the first report was published of the positive results of a preliminary trial of an extract from the bark of the sea buckthorn (Hippophae rhamnoides, fam. Elaeagnaceae) for its antitumor activity [3]. Later work showed considerable inhibition of the growth of various tumors of animals by the action of this extract [4]. In May, 1958,* G. P. Men'shikov and M. F. Petrova isolated from the extract a group of bases, which was conventionally named preparation Hr, and which contained the active principle [2].

In 1959 the same workers succeeded in isolating one of the bases of this group with Rf-0.4 in crystalline form (as the hydrochloride) and identified it as 5-hydroxytryptamine. As we know, 5-hydroxytryptamine is also present in the body of man and animals as one of the physiologically active biogenic amines, and is called serotonin or enteramine [6-8]; this is the first time that it has been isolated from plants (M.F. Petrova and G. P. Men'shikov, 1960). A report of the method of its isolation will be given by the authors elsewhere.

In the present communication the results are described of experiments with 5-hydroxytryptamine hydrochloride from sea buckthorn, as a preparation which we have called "hippophain," in rats and mice with transplanted tumors.

EXPERIMENTAL METHOD

Hippophain is readily soluble in water. The solution has a weakly acid reaction and is readily absorbed after subcutaneous injection without causing irritation of the surrounding tissues. Since the solution darkens on heating, we prepared it in aseptic conditions without sterilization. Its toxic action was investigated in rats, which showed much greater sensitivity towards it than did mice. All the therapeutic experiments were carried out on equal numbers of animals (mice and rats): ten in the experimental group and ten in the control. For the treatment of the tumors we used only those doses which did not cause death when given by subcutaneous injection to animals of that particular species for a period of 14 days. The tumors were inoculated subcutaneously, with the exception of Ehrlich's tumor, which was inoculated intramuscularly. Treatment of the animals was started on the 5-7th day after inoculation of the tumor [except in experiment No. 8 (Table 1), which was started on the ninth day] and continued for 12-15 days. In these experiments the preparation was injected subcutaneously every day. The inhibiting action on the tumors was estimated by the difference between the average weight of the tumors in the control and experimental series, expressed as a percentage of the control weight.

EXPERIMENTAL RESULTS

The pharmacological action of serotonin has been described by a number of authors [5-8]. Since its principal toxic effect is known to be its action on the vascular system and the smooth muscle of the intestine, and also on the kidneys, we were particularly interested in the reactions of these organs to the administration of a therapeutic dose, i. e., of a dose causing significant inhibition of growth of the tumor (see Table 1), and also in the upper limit of this dose. The subcutaneous or intraperitoneal injection of 10-20 mg/kg of hippophain in physiological saline into rats causes depression, restriction of mobility, changes in the rhythm and the strength of respiratory movements and an increase in the peristalis of the intestine with a transient relaxing

* The presence of unidentified alkaloids in the sea buckthorn was first mentioned by P. S. Massagetov [1].

TABLE 1. The Action of Hippophain on Tumors

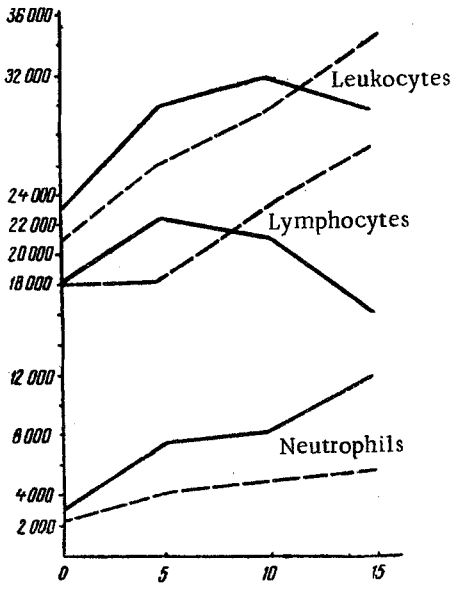
Expt. No.	Tumor	Dose (in mg/kg)	Mean wt. of tumor at end of expt. (in g)		Inhibition of tumor growth (in %)	P (by Student's method
			expt.	control		
	Mice					
1	Ehrlich's tumor (inoculated intramuscularly)	3	4.2	7.1	42	≤0.0001
2	The same	5	3.6	7.1	49	≤0.0001
3	" "	10	3.3	5.7	42	≤0.0001
4	" "	20	3.0	5.7	47	≤0.0001
5	" "	50	3.0	5.7	49	≤0.0001
6	" "	100	3.4	6.7	49	=0.001
7	" "	200	2.4	7.0	62	≤0.0001
8	" " (experiment begun on large tumors weighing 2 g)	20	2.6	4.9	47	=0.003
9	Hardening-Passey melanoma	10	3.5	6.3	44	=0.2
10	The same	20	3.8	6.8	44	=0.02
11	" "	5	3.2	6.8	53	≤0.0001
12	Crocker's sarcoma	50	1.2	6.2	81	≤0.0001
13	Sarcoma 37	50	1.8	4.1	56	=0.003
14	Carcinoma OZh-5 of the forestomach of the mouse	20	4.9	4.0	0	
15	Lymphosarcoma LIO-1	200	2.7	2.7	0	
	Rats					
16	Sarcoma 45	5	16.5	15.0	0	
17	The same	10	14.0	15.0	0	
18	" "	20	5.6	34.8	84	0.0001
19	Jensen's sarcoma	10	0.9	30.6	97	0.0001
20	Geurin's carcinoma	5	6.3	17.2	60	=0.058
21	The same	10	3.4	17.2	80	=0.008

action. On intraperitoneal injection, all these phenomena occur at once, but on subcutaneous injection they arise after 10-15 min. After intraperitoneal injection of 50 mg/kg, death of the animal occasionally ensues. An increase of the dose to 300-500 mg/kg, injected intraperitoneally, causes ataxia and, in certain rats, a severe spasm of the vessels of the optic fundus ("white eyes"); these phenomena all pass off after 4-5 hr. With these doses, death also supervenes occasionally in animals most highly sensitive to the action of the preparation. They die within 24 hr after the injection, showing signs of disturbance and gradual weakening of respiration and of cardiac activity. At necropsy the dilated, flabby heart and the parenchymatous organs of the abdominal cavity are overfilled with venous blood. We did not establish with accuracy the value of LD_{50} for rats, but it is considerably more than 500 mg/kg. After administration of therapeutic doses of 5 and 10 mg/kg for 14 days to mice and rats we observed no dystrophic changes whatsoever in the internal organs. A dose of 20 mg/kg at the end of the experiment caused in individual rats the development of necrotic areas in the kidneys. This also took place as a result of the administration of a dose of 10 mg/kg to rats for a period of 30 days. Attention must be drawn to the marked individual sensitivity to the toxic action of the preparation. One of the authors tried out a single injection of the preparation on himself (40 mg as a 4%

solution intramuscularly). Ten minutes after the injection a reaction of the cardiovascular system developed, in the form of considerable reddening of the skin of the face and neck,, a feeling of heaviness in the head and, in particular, in the region of the heart, All these phenomena passed off after 15 min. The arterial pressure and the pulse rate were not altered during this reaction.

The action of hippophain on hemopoiesis was studied in rats in which a sarcoma 45 had been inoculated subcutaneously, in a therapeutic experiment lasting 2 weeks on 10 animals, at the end of which growth of the tumors was retarded under the action of the preparation to the extent of 84% of the control values (see Table 1, experiment no. 17). The controls used were ten rats with tumors but not receiving treatment. The preparation was injected subcutaneously in a dose of 20 mg/kg daily. The blood was examined once before treatment and three times in the course of treatment, every 5 days. From determinations of the number of leukocytes (in a counting chamber) and of the leukocyte formula (from blood films), the number of lymphocytes and neutrophils in 1 mm^3 of blood was determined separately, after which the arithmetic mean values of these indices for all the rats were calculated, and these were plotted on a graph. The total leukocyte count in the control animals (see top graph in figure, broken line) rose continuously in accordance with the growth of the tumor and with the increasing destruction of its central part. In the experimental series (continuous line), in spite of the considerable inhibition of the tumor growth by the action of the preparation, a moderate leukocytosis was also found, and this diminished slightly on the 15th day. As is shown by the middle graph (see figure, lymphocytes), this decrease results from a slight fall in the number of lymphocytes (by 15% below the initial level) in the blood of the treated animals. On the other hand, however, the preparation has a stimulating action on the segmented neutrophils: their number rose considerably, with no shift to the left, and at the end of the experiment was 70% above the original level (see figure, neutrophils). This is seen particularly demonstratively in the rats, in whose blood there are far more lymphocytes than neutrophils. Under the action of the preparation, at the end of the experiment their blood closely resembled human blood in the proportions of these cells. No other abnormalities were observed during the examination of the films and calculation of the leukocyte formulae. No action of the preparation on the number and external appearance of the red cells could be found. The injection of the same dose per kg body weight into mongrel mice (without tumors) for the same period of time gave rise to no changes in the total leukocyte count and in their relative proportions.

The action of hippophain on tumors. The antitumor action of the preparation is distinguished by a wide spectrum, for as a result of its action on 9 strains of transplanted tumors, 7 were found to show some degree of inhibition of growth (see Table 1). No inhibition whatsoever of growth of a squamous cell carcinoma OZh-5 of the forestomach of the mouse (line C_3HA) and of a lymphosarcoma LIO-1 (in a mouse of line Afb) took place. However, those strains of tumor which were found to be sensitive to the action of this preparation varied very considerably in the character of their sensitivity to it. This is clear from a comparison of the inhibiting action of different doses on growth of the Ehrlich's tumor and of the sarcoma 45 (see Table 1). Inhibition of growth of the Ehrlich's tumor, caused after treatment with a daily dose of 5 mg/kg for 12 days, amounted to about 50%. Subsequent experiments in which the daily dose was increased to 200 (!) mg/kg gave no significant increase in the strength of the inhibiting action. In contrast to this, sarcoma 45 gave no inhibition of growth when injected in doses of 5 and 10 mg/kg; inhibition developed only after a dose of 20 mg/kg. The other rat tumors – Jensen's sarcoma and Guerin's carcinoma – were more sensitive to this preparation, for they reacted in varying degree to doses of 5 mg/kg (Guerin's carcinoma) and 10 mg/kg (Jensen's sarcoma). The same applies to the melanoma of mice (Harding-Passey strain). Only the action of a large dose has so far been tested on other tumors. Administration of the preparation by mouth had no antitumor effect.



The action of hippophain (20 mg/kg) on the blood of rats with sarcoma 45. Along the axis of ordinates – mean number of leukocytes in 1 mm^3 blood. Along the axis of abscissas – time in days from the beginning of treatment. – – – control; —— experiment.

TABLE 2. Comparison of the Antitumor Action of Two Therapeutic Forms of Serotonin: 5-Hydroxytryptamine Hydrochloride (Hippophain) and 5-Hydroxytryptamine-Creatinine Sulfate (5-HTCS)

Expt. No.	Preparation	Dose (in mg/kg)	Tumor	Commence day of expt. (after transplantation)	Result: weight of tumor (in g) expt.	Result: weight of tumor (in g) control	Result: inhibition (in %)
1	Hippophain	5	Ehrlich's tumor, intramuscularly	5-th	3.4	5.9	42
	5HTCS[1]	10	The same	5-th	4.5		24
2	Hippophain	5	Jensen's sarcoma, subcutaneously	4-th	14.2	43.0	67
	"	10	The same	4-th	5.8		87
	5HTCS[2]	20	" "	4-th	13.4		71

[1]Synthesized by Prof. N. N. Suvorov (All-Union Chemo-Pharmaceutical Research Institute, Moscow)

[2]Synthesized by M. F. Petrova (Institute of Experimental and Clinical Oncology of the AMN SSSR, Moscow)

Since synthetic 5-hydroxytryptamine (serotonin), which is widely used in laboratory practice, is usually obtained as a complex with creatinine sulfate, we carried out experiments to compare the action of the hydrochloride of 5-hydroxytryptamine (hippophain) and of synthetic 5-hydroxytryptamine-creatinine sulfate on tumors. Adequate doses were computed according to the content of the pure base: the hydrochloride contains approximately $^2/_3$ of the pure base, whereas the complex with creatinine sulfate contains approximately $^1/_3$; we therefore gave twice the dose of the complex with creatinine sulfate.

The results showed (Table 2) that 5-hydroxytryptamine, in the form of a complex with creatinine sulfate, has a much weaker action on tumors than the hydrochloride. For instance, in an experiment on Jensen's sarcoma, 5-hydroxytryptamine-creatinine sulfate in a dose of 20 mg/kg caused almost the same degree of inhibition as that caused by hippophain in a dose of 5 mg/kg, i. e., the inhibiting activity of the former is approximately half † as strong as that of the latter.

There are reports in the literature of investigations into the action of synthetic serotonin (in the form of a complex with creatinine sulfate) on tumors in animals [9, 10]. The authors of these reports, however, obtained no antitumor effect with serotonin, but came to the opposite conclusion, namely that it has a stimulating action on the growth of experimental tumors. The discrepancy between these authors' findings and our own may, possibly, be explained by differences in the form of the preparation (see Table 2) or in the method of its administration, or, finally, by the use of different tumor strains for the investigation.

It follows from these results that hippophain may be of interest for trial in clinical oncological practice.

SUMMARY

5-hydroxytryptamine was recently isolated from the bark of Hippophae rhamnoides by G. P. Men'shikov and M. F. Petrova. The results of investigation of the action of the hydrochloride of the alkaloid upon the organism and tumors of mice and rats are presented. The preparation was named hippophain. The antitumor effect of hippophain is characterized by a wide range of action, the preparation inhibiting the growth of 7 out of 9 animal tumors (various types). However, the tumors do not resolve completely. Hippophain exercised no antitumor effect when administered per os.

The side effects of the preparation are connected with action upon the cardiovascular and respiratory systems.

The effects of a large dose of the preparation (20 mg/kg) upon hemopoiesis was studied during a 2-week treatment of sarcoma 45.

A moderate leukocytosis in the peripheral blood was observed under the action of hippophain. The absolute number of neutrophils per 1 mm^3 of the blood increases by 70% in comparison with the initial level (without any shift to the left) whereas the number of lymphocytes is slightly reduced.

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