

# THE MECHANISM OF ACTION OF CERTAIN ESCULETIN DERIVATIVES

(UDC 612.015.643+615.32:577.164.3-092.259)

I. S. Rudakova and M. Yu. Ladinskaya

Department of Chemotherapy (Head—Professor A. M. Chernukh), Institute of Pharmacology and Chemotherapy (Director—Active Member of the Academy of Medical Sciences of the USSR Professor V. V. Zakusov), Academy of Medical Sciences of the USSR, Moscow  
(Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Zakusov)  
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 10, pp. 65-68, October, 1965  
Original article submitted April 13, 1964

The natural flavonoids (rutin, citrin, catechin) and their synthetic derivatives are widely used in various branches of medicine as substances with a vitamin P action. However, the mechanism of their effect on the permeability and stability of the capillaries has so far received little study. On the basis of data in the literature and of his

TABLE 1. Antihyaluronidase Activity of Esculamine in Experiments on Rabbits  
(Area of stain in mm<sup>2</sup> after intradermal injection of trypan blue, M±m)

Time of measurement (in min)	Before injection of esculamine		Time after injection of esculamine in a dose of 10 mg/kg		
			10 min	30 min	
After 20	143 ± 9	222 ± 13 <sup>1</sup>	196 ± 5 <sup>1</sup>	186 ± 3 <sup>1</sup>	130 ± 7
After 60	172 ± 16	239 ± 17 <sup>1</sup>	230 ± 13 <sup>1</sup>	224 ± 13 <sup>1</sup>	156 ± 3

\*Trypan blue was injected along with hyaluronidase.

TABLE 2. Effect of Esculamine on the Ophthalmoreaction of Guinea Pigs to Injection of Histamine

Animal No.	Weight (in g)	Severity of ophthalmoreaction				
		before injection of preparation	at interval after injection of preparation of			
			1½ h	3 h	24 h	48 h
1	230	++++	±	++	++++	++++
2	220	++++	+++	+	++++	++++
3	270	+++++	++++	++	++++	++++
4	260	+++++	±	+	+++	++++
5	240	++++	++	+	++++	++++
6	200	++++	+	+	++	+++
7	200	++++	++	+	++	++++
8	250	++++	+++	+++	+++	++++
9	250	++++	++	++	+++	++++
10	200	++++	++	++	++++	++++

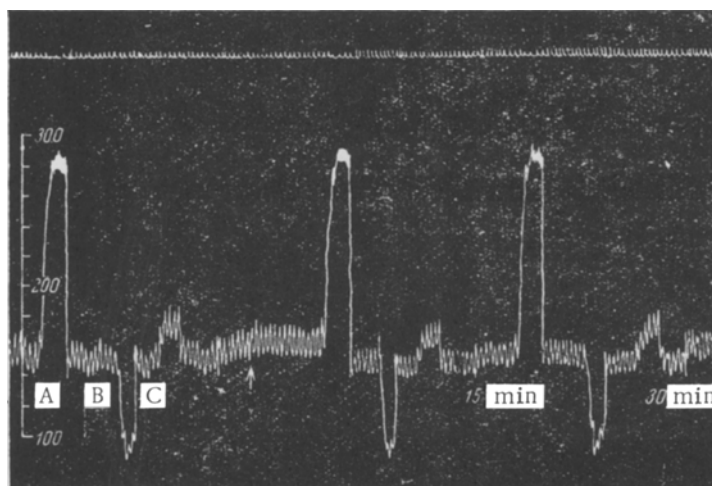


Fig. 1. Effect of preparation 33-0-50 (esculamine) in a dose of 10 mg/kg on the reaction of the arterial pressure to injection of adrenalin (A) in a dose of 40  $\mu$ g/kg, of acetylcholine (B) in a dose of 0.5  $\mu$ g/kg, and to compression of the carotid artery for 30 sec (C) in an acute experiment on an anesthetized cat.

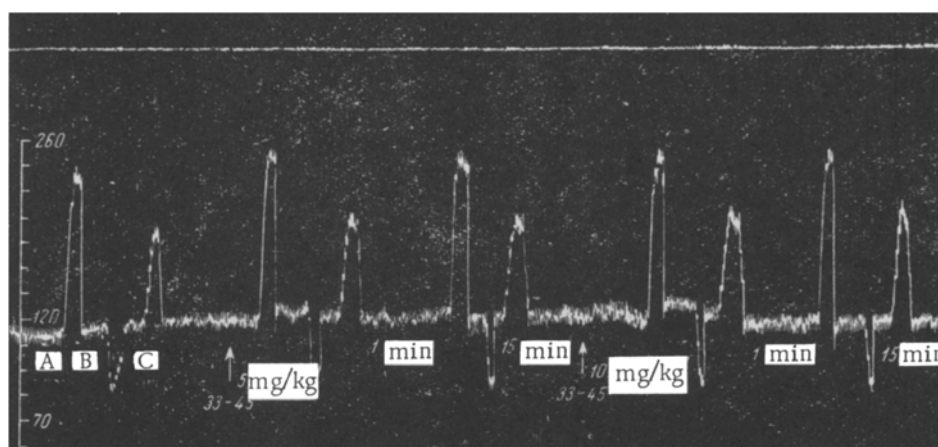


Fig. 2. Effect of preparation 33-0-45 in doses of 5 and 10 mg/kg on the arterial pressure following injection of adrenalin and acetylcholine in the same doses and compression of the carotid artery in an acute experiment on an anesthetized cat. Legend as in Fig. 1.

own investigations, Martin [7] postulates that "the deep physiological action of the flavonoids can evidently be explained only by the presence of a series of interconnected effects," such as a direct action on the capillaries, depression of the action of hyaluronidase and histamine, inhibition of the oxidation of adrenalin, and an influence on the bleeding time and clotting time of the blood, etc.

The authors have studied synthetic derivatives of esculetin and 4-methylesculetin and have demonstrated the considerable vitamin P activity of certain compounds in experiments on both healthy animals and animals exposed to a single dose of whole-body x-ray irradiation [5]. They observed that nitrogen-containing derivatives of 4-methylesculetin (8-dihydroxydiethylaminomethyl-4-methylesculetin hydrochloride, called esculamine, and 8-dimethylaminomethyl-4-methylesculetin hydrochloride: designated as preparations 33-0-50 and 33-0-45 respectively)

TABLE 3. Effect of Preparation 33-0-50 (Esculamine) on the Prothrombin Time in Healthy and Irradiated Rabbits (M±m)

Group	Num- ber of rab- bits	Prothrombin index before injection of preparation	After one injection of the preparation					After three injections of the preparation					Day of experiment after administration of prep- aration					Day after irradiation			
			1 h	2h	4h	15 min	1 h	3 h	5 h	7 h	2nd	3rd	6th	8th	1st	2nd	3rd	5th			
Experimental	7	100	106 ± 2,4	107 ± 3	114 ± 4,4	110 ± 1,3	116 ± 3,3	128 ± 3	127 ± 2,4	119 ± 3,4	112 ± 4,1	108 ± 2,8	103 ± 1,5	106 ± 2	109 ± 4,1 97 ± 2,1	105 ± 4,9 92 ± 1,0	100 ± 3 86 ± 3,9	103 ± 3,1 81 ± 3			
Control	5	100																			

lower the permeability and increase the stability of the blood vessels, factors of considerable importance in the development of the hemorrhagic syndrome, especially if caused by x-ray irradiation. In the present investigation the effect of the above-mentioned active compounds was examined on the action of adrenalin and acetylcholine, hyaluronidase, and histamine, and on the prothrombin time of the blood.

#### EXPERIMENTAL

The effect of the substances on the blood pressure and respiration after intravenous injection of adrenalin and acetylcholine was studied in acute experiments on cats anesthetized with chloralose and urethane. The blood pressure was recorded by a mercury manometer and the respiration of the animals was recorded by means of an endotracheal cannula and syphygmometer.

The antihyaluronidase activity was judged by the effect of the preparation on the change in the area of spread of a 0.75% solution of trypan blue, injected intradermally, produced by hyaluronidase when injected along with the dye in a dose of 250 units (the method of I.I. Matasis [1]). The area of spread of the stain was measured after 20 min, for after longer periods its extent of spread diminished on account of the development of inflammatory edema. According to the size of the area measured 60 min after the injection of hyaluronidase, a conclusion was drawn regarding the effect of the preparation on the spread of the dye during inflammation [3]. The area of the stain was calculated from the formula:

$$\frac{D \times d \times \pi}{4},$$

where D is the largest diameter of the stain (in mm) and d the smallest.

The antihistamine action of the preparation was determined by the degree to which it prevented the development of edema of the eyelids and conjunctiva in a guinea pig (depression of the ophthalmoreaction) after instillation of a 2% solution of histamine into the eyes [6]. The intensity of the ophthalmoreaction was noted 15-20 min after injection of histamine.

The prothrombin time of the blood was determined in experiments on rabbits by Borovskii's drop method as modified by Khrushcheva (described by V. E. Predtechenskii [4]).

#### RESULTS

Following intravenous injection of compound 33-0-50 (Fig. 1), a transient and slight increase in the arterial pressure and a slight increase in the rate and depth of respiration were observed. The injection of preparation 33-0-45 caused a somewhat larger increase of pressure (Fig. 2). The preparations had no visible effect on the arterial pressure when adrenalin or acetylcholine was injected (see Figs. 1 and 2).

The antihyaluronidase activity of preparation 33-0-50 was studied in experiments on rabbits; it was more marked 30 min after injection of the preparation (Table 1), i. e., at that time of the investigation the area of the stain was least [4]. At the 60th minute after injection of hyaluronidase, against the background of the action of the preparation, a tendency for the spread of the dye to diminish was also observed.

The antihistamine action of preparation 33-0-50 was shown by a depression of the ophthalmoreaction in guinea pigs after the injection of histamine. The maximal effect was observed 3 h after the administration of esculamine, although the strength and duration of its action were slight (Table 2).

The changes in the prothrombin time of the blood after 3 subcutaneous injections of esculamine (preparation 33-0-50) in a dose of 50 mg/kg in healthy and irradiated rabbits are shown in Table 3. In the healthy animals the increase in the prothrombin index reached 128%, and in the same rabbits after x-ray irradiation in a dose of 800 R, the prothrombin index varied after daily injection of the preparation for 3 days between 109 and 100%, while in the control irradiated animals it fell to 81%.

Hence, among the investigated properties of the nitrogen-containing derivatives of 4-methylesculetin (using esculamine as example), the antihyaluronidase activity, the slight antihistamine action, and the increase in the coagulability of the blood may be important elements in the mechanism of their vitamin P activity.

#### LITERATURE CITED

1. I. I. Matusis, Nauchn. zapiski Gor'kovsk. Inst. dermatologii i venerologii, 14 (1950), p. 8.
2. I. A. Olivin, In the book: The Pathogenesis of Inflammation and Pathology of Vascular Permeability [in Russian], Dashanbe (1956), p. 195.
3. I. S. Rudakova, In the Book: Current Problems in Pharmacology [in Russian], Moscow (1963), p. 316.
4. V. E. Predtechenskii, Textbook of Clinical Laboratory Investigations [in Russian], Moscow (1960), p. 87.
5. D. A. Kharkevich, Farmakol. i toksikol., 6 (1957), p. 46.
6. G. Martin, In the book: Bioflavonoids and Capillary Permeability [Russian translation], Moscow (1957), p. 23.

---

**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**

---