# *In vivo* Release of Histamine from Rabbit Blood by Reserpine. (22767)

T. Phillip Waalkes and Herbert Weissbach (Introduced by R. W. Berliner)

*Laboratory of Chemical Pharmacology, National Heart Institute, N.I.H., P.H.S., U. S. Department of Health, Bethesda, Md.*

Reserpine is a widely employed and useful drug in the treatment of a variety of clinical conditions, particularly hypertension and certain psychiatric disorders. One of the most common and consistent side effects of reserpine therapy in man has been nasal stuffiness. This effect may be relieved in most patients by the use of vasoconstrictive substances and in some by oral medication with antihistaminics(1). In addition, reserpine has been found to cause an increase in the volume and free acidity of gastric secretion following both oral and intravenous administration of this drug to humans(2). These secondary findings suggested the possibility that reserpine could cause the release of histamine from its body sources. Recent studies have shown that reserpine liberates serotonin (5-hydroxytryptamine) from its depots in animals(3), and from human platelets *in vivo*. Both histamine and serotonin are present in rabbit blood platelets in relatively large amounts as compared to other amines. Previous studies had indicated that reserpine incubated *in vitro* with rabbit platelets caused a release of platelet serotonin but no histamine release.

The following study was done to determine whether or not reserpine liberated histamine *in vivo* from rabbit blood.

*Methods and materials.* *Male rabbits* (1.5-2.0 kg) were given a single intravenous injection of reserpine (5 mg kg). A control blood specimen was taken prior to the injection, and experimental samples at various intervals after the injection. All samples were drawn by cardiac puncture into siliconed glassware using heparin as anticoagulant. Both histamine and serotonin were determined chemically on whole blood. For each determination, 1 ml of whole blood was diluted to 8 ml with water, 1 ml of 10% zinc sulfate solution added and, after shaking, 0.5 ml of 1 N sodium hydroxide. Following centrifugation at high speed, a 1 ml aliquot of the supernatant was analyzed directly for serotonin by

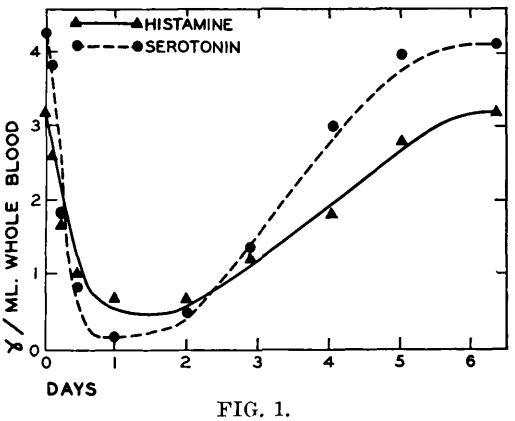


FIG. 1.

measuring its specific fluorescence in strong acid(4). Five ml of the remaining supernatant were analyzed for histamine employing modifications of the methods of McIntire *et al.* (5,6) and Lowry *et al.*(7). The details of the methods employed here, whereby both serotonin and histamine can be determined on the same supernatant after protein precipitation will be reported later. Recoveries, standards and blanks were run along with each determination. For both serotonin and histamine, recoveries were over 80%. The rabbit whole blood values obtained in this work compare favorably with those previously reported for histamine(6,8) and serotonin.*

*Results.* Composite data from 5 animals given reserpine are shown in Fig. 1. The reduction in whole blood histamine was similar in rate and degree to the reduction in whole blood serotonin. The return of histamine and serotonin values to control levels was also parallel. The curves for each individual animal were substantially the same, reaching a minimal level at about 20 hours, remaining low until about 48 hours, and returning to normal in 5 to 7 days. The minimum histamine level at 40 hours varied from 7% to 25% of the control value in the animals. All the rabbits showed typical reserpine effects. Previous

* Unpublished observations.

TABLE I. Effect of Reserpine on Rabbit Blood, and Platelet Serotonin and Histamine.

| | Serotonin | | Histamine | |
|---|---|---|---|---|
| | Control | 24 hr | Control | 24 hr |
| Whole blood, γ/ml* | 4.6 | .5 | 3.0 | .6 |
| Platelet, γ/mg protein† | 6.8 | .7 | 3.7 | .7 |

* Avg of 5 animals. † Avg of 2 animals.

work has shown that platelet counts are essentially the same in rabbits before and 16 hours after the intravenous injection of 5 mg/kg of reserpine.* Since in the rabbit both histamine and serotonin are found in platelets, the decrease in whole blood histamine and serotonin cannot be accounted for by a reduction in whole blood platelet count. Instead, a release from platelets is postulated with subsequent metabolism of both serotonin and histamine to account for the reduction of these constituents in whole blood.

In support of a release phenomenon to account for the drop in whole blood histamine and serotonin, platelets were isolated according to the method of Dillard *et al.*(9) from rabbit whole blood before and again 24 hours after an intravenous injection of reserpine. Both histamine and serotonin were determined and related to the amount of platelet protein(10) as shown in Table I.

*Discussion.* It is well substantiated that reserpine liberates serotonin from the body depots in animals, and from platelets in man. In the study presented here, histamine has been shown to be liberated from rabbit blood *in vivo*. Recently Carlsson(11) has shown epinephrin release from rabbit adrenals by reserpine, but further work* has shown that this release does not occur in a cord sectioned animal which indicates an indirect effect. These studies suggest that reserpine is capable of liberating several potent biologically active substances from blood and tissues, although its action may not necessarily be a direct one.

Histamine is known to cause the release of catechol amines(12), particularly in patients with pheochromocytomas, and in turn is released by a variety of primary amines(13,14), including epinephrine(15,16). Feldberg and Smith(17) have shown the release of histamine from tissues by serotonin using bioassay. It is therefore possible that these compounds are mutually able to release each other from blood and tissues although perhaps to a variable degree and extent. Reserpine may be capable of a primary liberation of histamine, serotonin and epinephrine, or by liberating one of these cause a secondary release of the others. That reserpine may liberate other substances also becomes a possibility. Finally, one can speculate that the therapeutic effects as well as the side effects of this drug may be due to the release of these basic compounds, their attendant interaction, and resultant loss. However, preliminary experiments indicate that reserpine does not release histamine from other animal tissues *in vivo*.

Studies on the release of histamine from tissues in animals, and blood in humans are in progress in this laboratory.

*Summary.* Intravenous reserpine in rabbits has been demonstrated by chemical means to release histamine from whole blood. 1. The minimal histamine levels were reached in one to 2 days with complete recovery to control values in 5 to 7 days. 2. Simultaneous serotonin determinations, revealed that the rate and degree of liberation, and the recovery of both histamine and serotonin were very similar. 3. Platelets, isolated 24 hours after reserpine, showed a marked reduction in both histamine and serotonin compared to control platelets.

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## Electrophoretic Behavior of Some Animal Hemoglobins. (22768)

V. P. SYDENSTRICKER, R. OLIVER, B. M. CHANDLER, AND O. SYDENSTRICKER
*Department of Internal Medicine, Medical College of Georgia, Augusta*

The authors have been unable to find any report of results of paper electrophoresis of hemoglobins other than human. The present communication presents findings in 6 species of animals.

*Methods.* Electrophoresis was performed on "sandwich" type plate glass apparatus using Whatman 3MM paper in sheets 36 × 16 cm and 36 × 12 cm. Barbiturate buffer at pH 8.6 and M 0.025 was used in all preliminary experiments. Current was applied to 36 × 16 cm sheets at 300 volts and 5 milliamperes for 6 hours and to 36 × 12 cm sheets for 4 hours, constant temperature of 20°C was maintained. Under these conditions normal human hemoglobin (Hb. A) migrated approximately 5 cm during the run. In addition to electrophoresis alkali denaturation tests(1) and solubility tests(2) were carried out on all specimens (Table I). Rat hemoglobin was obtained from the pooled blood of adult Wistar rats killed at the end of experiments. Dog hemoglobin was from mongrel dogs used in experimental surgery. Rabbit hemoglobin was from albino rabbits used for pregnancy tests. Chicken hemoglobin was from pooled blood obtained from a poultry farm. Turtle hemoglobin was from several specimens of the common land turtle, *Gopherus polyphemus*. Alligator hemoglobin was from a single small specimen of *Alligator missippiensis*. Hemoglobin solutions were prepared in the usual manner and adjusted to approximately 5%, 3 lambda of the solution were applied to the paper.

TABLE I. Resistance to Alkali Denaturation and Solubility as COHb of Animal Hemoglobins.

| Species | % alkali resistant | % soluble as carbonmonoxy-hemoglobin |
|---|---|---|
| Dog | 0 | 100 |
| Rabbit | 36 | " |
| Rat | 100 | " |
| Chicken | 35 | " |
| Alligator | 0 | " |
| Turtle | 0 | " |

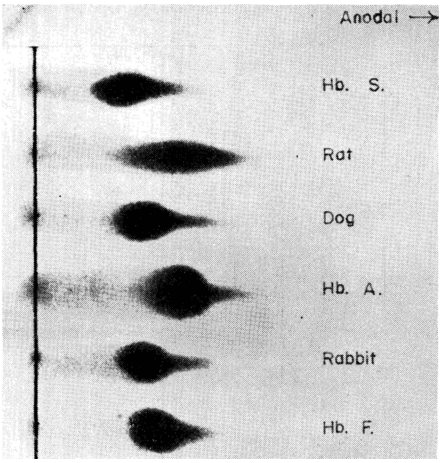


FIG. 1. Patterns of rat, dog and rabbit hemoglobins with Hb. S., Hb. A. and Hb. F. for comparison.

*Results.* As shown in Fig. 1, dog and rabbit hemoglobins move as homogenous compounds at a rate slightly slower than fetal hemoglobin but faster than Hb. S while rat hemoglobin produces a trailing spot suggest-