TABLE III. Distribution of Vitamin B_{12} in the Blood of Various Animals.

Animal	Hemato-crit (%)	Whole blood vit. B_{12} (μg/ml)	Plasma vit. B_{12} (%)	Erythro-cyte vit. B_{12} (%)
Human	38	.26	38.4	61.6
Dog	45	.22	40.9	59.1
Calf	40	.21	28.6	71.4
Rabbit	36	40.6	48.5	51.5
Chicken	29	6.53	11.6	88.4
Alligator	21	1.66	1.2	98.8

vitamin contained in the leukocytes and young forms of red cells. This is especially true of the calf since it is known that immature mammals may contain large numbers of immature, nucleated erythrocytes(10).

Summary. Vit. B_{12} activity of whole blood and plasma from the human, dog, calf, rabbit, chicken and alligator was studied before and after alkaline hydrolysis. The data show a wide variation of blood and plasma vit. B_{12} content between the different species. In mammals, the vitamin is almost equally distributed between the erythrocytes and plasma. In chicken and alligator, the major portion of the vitamin activity is associated with the nucleated erythrocyte.

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Pharmacological Studies with Rescinnamine, a New Alkaloid Isolated from *Rauwolfia serpentina.* (21027)

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The favorable clinical results obtained with preparations of *Rauwolfia serpentina* have created considerable interest in the active principles of this root and their mechanism of action. While at least 8 alkaloids had been found and investigated by various Indian and European workers, the only highly potent alkaloid found was reserpine, first isolated and studied by Müller, Schlittler, and Bein (1,2). In a recent communication from our Laboratories(3) it was stated: "Reserpine is the most potent single alkaloid so far examined. Available evidence suggests that other active alkaloid(s) are present in *Rauwolfia serpentina.*"

Since the known compounds account for only about 50% of the alkaloids in Rauwiloid®,* it was logical to look for additional active principles in the amorphous portion of this preparation. From it Klohs and coworkers(4) succeeded in isolating and identifying a new crystalline alkaloid, the trimethoxycinnamic ester of methylreserpate (M.P. 238-9°, $[\alpha]_D^{24}$ –97 ± 2, c, 1.0 in $CHCl_3$). As will be shown in this communication, rescinnamine has all the typical pharmacologic properties of Rauwiloid® and reserpine. The new al-

* The alseroxylon fraction of *Rauwolfia serpentina,* is a concentrate of the active alkaloids of the root, free from non-alkaloidal matter.

TABLE I. Observations in Dogs after a Single Intravenous or 5 Daily Oral Doses of Rescinnamine or Rauwiloid®.

Dose (γ/kg) and route	No. of dogs	HR*	MAP*	Sed.†	NM†	Dia.†
Controls	56	145 ± 37	117 ± 18	0/56	0/56	0/56
Rescinnamine						
250 i.v.	3	98 ± 19	69 ± 8	3/ 3	3/ 3	3/ 3
50 "	3	106 ± 25	81 ± 9	3/ 3	3/ 3	3/ 3
25 "	3	136 ± 25	93 ± 9	0/ 3	0/ 3	1/ 3
10 "	3	104 ± 12	99 ± 12	0/ 3	1/ 3	1/ 3
5 "	3	104 ± 18	106 ± 26	0/ 3	1/ 3	0/ 3
50 oral	10	76 ± 23	80 ± 9	9/10	10/10	10/10
15 "	10	83 ± 25	85 ± 8	4/10	10/10	9/10
5 "	9	91 ± 25	76 ± 15	0/ 9	4/ 9	5/ 9
1 "	10	112 ± 25	104 ± 20	0/10	6/10	3/10
Rauwiloid®						
1000 oral	15	93 ± 25	76 ± 15	13/15	13/15	13/15
250 "	13	85 ± 23	84 ± 16	5/13	12/13	12/13
50 "	10	111 ± 24	95 ± 13	0/10	3/10	1/10
5 "	10	120 ± 24	103 ± 12	0/10	7/10	7/10

* HR (heart rate in beats/min.) and MAP (mean arterial pressure in mm Hg) measured under pentobarbital and expressed as mean ± stand. dev.

† Animals showing sedation (Sed.), nictitating membrane prominence (NM), or diarrhea (Dia.)/animals tested. These data were recorded before anesthesia was induced.

kaloid when given orally or intravenously produces in normotensive dogs bradycardia, hypotension, and at higher doses also sedation. At higher doses, nictitating membrane prominence and diarrhea are also seen in animals. Rescinnamine also produces the same alterations in cardiovascular responses which have been demonstrated for Rauwiloid® by Gourzis *et al.*(5). In brief, these involve reduction or abolition of pressor responses to certain classical laboratory stimuli. The prolongation of pentobarbital-induced sleeping time of mice which has been suggested as an index of sedative activity of Rauwiloid®(6) is also observed with rescinnamine.

Methods. Dogs, unselected as to sex, weight or breed were medicated orally or intravenously. For oral use, the drug was given in capsules daily for 5 days. For parenteral administration the drug was dissolved in a few drops of glacial acetic acid and diluted to the required volume with distilled water. The pH of the final solution varied from 3 to 5. Twenty hours after the intravenous injection or on the sixth day of oral feeding, sedation was estimated using spontaneous activity and response to stimulation as criteria. Diarrhea and nictitating membrane prominence were noted. The dogs were then anesthetized with pentobarbital sodium intravenously, disappearance of the swallowing reflex serving as the end point. Twenty minutes after anesthesia had been established, a needle was inserted into the femoral artery and connected to a Sanborn electromanometer for recording of arterial pressure and heart rate. The recordings were made 10 minutes after the arterial needle had been inserted. In some of the orally medicated animals, responses to certain cardiovascular stimuli were determined using the procedure of Gourzis *et al.*(5). In another experiment dogs, anesthetized with urethane intravenously, were prepared for recording of MAP on the mercury manometer. Various reflex responses were measured before and after bilateral vagotomy. Rescinnamine was then administered intravenously and the responses were determined at hourly intervals up to 7 hours. For the sleeping time experiments, mice of both sexes (17-21 g) were used. Rescinnamine solution was injected intraperitoneally (0.01 ml/g of body weight). Two hours later pentobarbital sodium was given intraperitoneally at a dose of 65 mg/kg in 0.65% aqueous solution. The duration of sleeping time was judged by the righting reflex of the animals. All experiments were made in groups of 10 animals, including a

TABLE II. Cardiovascular Responses of Dogs under Pentobarbital Anesthesia following Oral Medication with Rescinnamine (50 γ/kg/Day for 5 Days).

	Medicated (9) dogs			Control (15) dogs†		
	Pre-ex. MAP, mm Hg	Max. MAP response mm Hg	Max. MAP response %	Pre-ex. MAP, mm Hg	Max. MAP response mm Hg	Max. MAP response %
Epinephrine, 1 γ/kg	104 ± 17	82 ± 23*	80 ± 24	127 ± 20	32 ± 13	25 ± 10
3 γ/kg	99 ± 17	126 ± 38*	127 ± 19	121 ± 15	70 ± 21	58 ± 17
Isuprel, 3 γ/kg	97 ± 22	−46 ± 21	−46 ± 14	133 ± 18	−54 ± 13	−41 ± 10
Carotid occlusion (bilateral, 30 sec.)	89 ± 26	10 ± 9*	11 ± 9	133 ± 18	36 ± 23	27 ± 17
Hypoxia (100 N_2, 45 sec.)	90 ± 21	−22 ± 13*	−25 ± 14	134 ± 16	+34 ± 23	25 ± 17

All figures given as mean ± stand. dev.
* Indicates significant difference ($p = 0.05$ or less) from the controls.
† Control values from Gourzis, *et al.*(5).

control group. The sleeping time of the medicated groups was expressed as percentage of that of the corresponding control group. This reduced uncontrolled daily fluctuations.

Results. The direct effects of rescinnamine after a single intravenous dose or repeated oral doses ranging from one to 250 γ/kg were bradycardia, hypotension, and, at higher doses, sedation (Table I). Nictitating membrane prominence and diarrhea were frequently observed. For comparison, results of 5-day oral Rauwiloid® tests are presented. The control data were accumulated over a 2-year period.

The new alkaloid was, on a weight basis, considerably more potent than Rauwiloid®. The changes in cardiovascular responses to epinephrine, Isuprel, carotid occlusion and hypoxia (Tables II and III) were also evoked by a smaller dose (50 vs. 500 γ/kg/day). Thus, rescinnamine is one of the highly active principles of Rauwiloid® and is of the same order of activity as reserpine(6).

The pressor response to carotid occlusion was significantly reduced on direct comparison. Applying the formula of Prochnik *et al.* (7), the orally medicated animals gave a response of 39 ± 24% vs. 51 ± 38% for the controls. For a group of 9 dogs, this difference is not significant at the 95% level.

The characteristic latency of Rauwiloid® action was shown by rescinnamine. After the drug was given intravenously to dogs anesthetized with urethane several hours had to elapse before significant changes occurred (Table III). About 3 to 4 hours after a single intravenous dose of 300 γ/kg the MAP had significantly dropped and the primary pressor response to central vagal stimulation disappeared. With doses of 100 γ/kg the delay was 4 to 6 hours. The hypoxia test was performed only once, 7 hours after injection of rescinnamine, when the pressor response to 100% nitrogen for 45 seconds was blocked in 2/4 and in 3/4 dogs following doses of 100 and 300 γ/kg, respectively. The response to bilateral carotid occlusion after 300 γ/kg was smaller at the end of 7 hours than it had been before the injection or in the controls. However, no change occurred according to the formula of Prochnik *et al.*(7). This type of experiment did not demonstrate bradycardia.

The prolongation of pentobarbital-induced sleeping time in mice (Table IV) was significant after 5 mg/kg of rescinnamine and it increased with larger doses. With the same doses, voluntary motor activity was greatly reduced within one hour after injection of rescinnamine.

The incidence of eyelid ptosis in mice has been suggested by Rubin and Burke(8) as the basis for a bioassay of reserpine-like substances in *Rauwolfia serpentina*. Rescinnamine produced ptosis in mice within 2 hours after intraperitoneal administration of a minimum dose of 2 mg/kg. Probably more than 30 mg/kg are required to reach a maximum effect as indicated by complete closing of the eyes.

When given intraperitoneally to rats at a dose of 10 mg/kg, rescinnamine produced within 15 minutes a marked sedation and a continuous, copious nasal discharge in 3 animals tested.

TABLE III. Cardiovascular Responses of Dogs under Urethane Anesthesia Measured at Hourly Intervals following Intravenous Injection of Rescinnamine.

Test	Dose (γ/kg)	No. of dogs	Before medication: Pre-vagot.	Before medication: Post-vagot.	After injection of rescinnamine: 1 hr	2 hr	3 hr	4 hr	5 hr	6 hr*	7 hr*
Mean arterial pressure, mm Hg	100	4	154 ± 23	156 ± 34	147 ± 30	151 ± 39	168 ± 16	142 ± 24	139 ± 27	128 ± 37	119 ± 36
	300	5	142 ± 10	145 ± 8	146 ± 16	143 ± 11	131 ± 18	117 ± 18	106 ± 35	101 ± 40	91 ± 35
	C	7	134 ± 16	142 ± 26	131 ± 11	138 ± 17	146 ± 17	150 ± 17	151 ± 16	151 ± 8	144 ± 10
Bilat. carot. occlusion resp., mm Hg	100	4	50 ± 27	57 ± 25	63 ± 37	69 ± 33	92 ± 11	69 ± 26	65 ± 26	58 ± 36	49 ± 30
	300	5	66 ± 38	72 ± 24	83 ± 24	82 ± 22	54 ± 37	39 ± 23	47 ± 33	37 ± 27	23 ± 17
	C	7	36 ± 14	51 ± 17	58 ± 30	61 ± 30	69 ± 34	71 ± 36	72 ± 28	62 ± 31*	55 ± 13
Central vagal stimulation†	100	4		4/4 32 ± 27	4/4 29 ± 27	4/4 20 ± 12	—	2/4 9 ± 12	1/4 13	1/4 7	0/4
	300	5		5/5 74 ± 28	5/5 55 ± 15	5/5 33 ± 19	3/5 26 ± 11	3/5 16 ± 20	1/5 36	1/5 34	1/5 24
	C	7		7/7 35 ± 16	7/7 44 ± 29	7/7 41 ± 19	7/7 42 ± 23	7/7 45 ± 21	7/7 35 ± 14	7/7 48 ± 11	7/7 45 ± 9

All responses given as mean ± stand. dev.
* Only 3 control animals followed for 6 and 7 hr.
† No. of dogs with pressor rise / No. of dogs tested and mean pressor rise.
C = Control.

Discussion. The new alkaloid, rescinnamine, has the typical pharmacological properties of Rauwiloid® and reserpine. The only apparent discrepancy is that while the absolute pressure response to bilateral carotid occlusion was reduced by rescinnamine, the changes were not significant when related to the pre-existing MAP according to Prochnik *et al.*(7). This is in contrast to results with Rauwiloid®(5). The discrepancy may be explained by one or several alternatives: 1. The number of animals was too small. 2. The dose of rescinnamine was too small. 3. At the time when the pressor response started to decrease markedly, the pre-existing MAP in some animals was below 80 mmHg and, therefore, the formula was no longer applicable.

An accurate potency relation between Rauwiloid®, rescinnamine, and reserpine is difficult to establish. Several criteria may be considered: 1. Hypotension in dogs has a very flat dose response curve and wide standard deviations. 2. Alterations in cardiovascular responses present even greater problems of quantitative evaluation. 3. Sedation in dogs is very difficult to quantitate. 4. Prolongation of pentobarbital-induced sleeping time in mice. 5. Ptosis of eyelids in mice. The last two criteria have apparently a more satisfactory dose response curve, but their correlation to the therapeutic use of these drugs has yet to be established. However, the available data indicate that, in animals, rescinnamine is considerably more potent than Rauwiloid® and of the same order of activity as reserpine.

It is not known whether different potency relations exist between Rauwiloid®, rescinnamine and reserpine for various indices.

Summary. 1. The pharmacological properties of a new alkaloid, rescinnamine, recently isolated from Rauwiloid®, have been described. Rescinnamine produced typical pharmacological effects of *Rauwolfia serpentina*: bradycardia, hypotension, and sedation, as well as characteristic alterations in cardiovascular responses. 2. Rescinnamine caused augmentation of pressor response to epinephrine, reversal of pressor response to hypoxia, diminution of pressor response to bilateral

TABLE IV. Effect of Rescinnamine on Pentobarbital-Induced Sleeping Time of Mice.*

Dose, mg/kg	No. of groups (10 mice ea.)	Sleeping time, % of controls
2.5	2	126
5.0	4	144 ± 17†
10.0	4	167 ± 3†
20.0	2	213 †

* Rescinnamine given intraperitoneally 2 hr before standard dose of pentobarbital. See text.
† Significant increase ($p = .05$ or less).

carotid occlusion, and blockade or reversal of primary blood pressure rise elicited by faradization of afferent vagus. 3. The sedative effects of rescinnamine were demonstrated by gross observation in dogs, rats and mice and by prolongation of pentobarbital-induced sleeping time in mice. 4. Rescinnamine produced marked eyelid ptosis in mice and a copious nasal discharge in rats. 5. On a weight basis, rescinnamine appeared to be several times as potent as Rauwiloid® and similar to reserpine. 6. Rescinnamine is the second highly potent alkaloid derived from *Rauwolfia serpentina.*

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Cultivation of Human Tissues in Media Containing Bovine Allantoic and Amniotic Fluids. (21028)

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Allantoic and amniotic fluids have been used in tissue cultures by a number of workers. Moppett(1), Wendrowsky, and Zapolska(2), and Grossfeld(3) employed amniotic fluid from the embryonated egg in drop cultures of chick tissues. Fedorow(4) used the fluid surrounding the embryo of the cephalopod Rossia as the medium in which to culture nervous tissue from this organism. Thomas and his coworkers(5) described the use of deproteinated bovine amniotic fluid for cultivation of the virus of foot and mouth disease in suspended cell cultures of foetal calf skin; and Enders(6) used whole bovine amniotic fluid in roller tube cultures of various human tissues, propagating a number of viruses in these cultures. Moppett(7) also advocated the use of chick embryo allantoic fluid in cultures of chick tissues. Since the healing of wounds appeared to be stimulated by extracts of embryonic tissues from species possessing a well-developed allantois, Robinson(8) considered that solutions of allantoin might be of benefit in the treatment of wounds, and clinical trials with commercially-prepared allantoin confirmed his view. As a result of his observation, Shipp and Hetherington(9) studied the effect of allantoin on drop cultures of chick heart tissue, but found no significant stimulation of fibroblast growth. Chu(10) used allantoin in greater concentration than these workers, and concluded that it had a slightly retarding action on the growth of chick epithelial cells and chondroblasts in culture.

During the collection of bovine embryonic fluid in this laboratory, it was frequently noted that blind puncture of the gravid uterus by means of a hollow trocar might result in the withdrawal of 2 kinds of fluid, differing in

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