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Distribution and Excretion of ^{3}H-Venoms of *Crotalus adamanteus, Crotalus atrox,* and *Agkistrodon piscivorus* and ^{3}H-Serum of *Lampropeltis getulus* in Rats

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Abstract □ ^{3}H-Labeled king snake serum and three pit viper venoms were administered intraperitoneally to rats to study the tissue distribution and rate of excretion. The peak blood level of king snake serum corresponded to that of the three venoms. The liver played a major role in metabolizing the venoms and king snake serum. Fecal excretion was the major route of elimination of these substances.

Keyphrases □ Snake serum and venoms (king and pit viper), radiolabeled - tissue distribution, urinary and fecal excretion, rats □ *Crotalus adamanteus*, radiolabeled—distribution and excretion, rats □ *Crotalus atrox*, radiolabeled—distribution and excretion, rats □ *Agkistrodon piscivorus*, radiolabeled—distribution and excretion, rats □ *Lampropeltis getulus*, radiolabeled—distribution and excretion, rats

Many snake venoms of the pit viper family manifest proteolytic activities (1–3). The most characteristic pathological changes associated with proteolysis is hemorrhage (4, 5). The prominent findings on the activity of the pit viper venoms are the hydrolyses of casein, gelatin, denatured hemoglobin, and hemin. King snake serum has been demonstrated to counteract the proteolytic activity and lethal effect of pit viper venoms (6); however, the mechanism of action is not known. It is of interest to explore the *in vivo* behavior of the king snake serum and the snake venoms that might yield information as to the mechanism of interaction of these counteracting substances. The purpose of this paper is to describe the *in vivo* behavior of the venoms of pit viper, *Crotalus adamanteus*, *Crotalus atrox*, and *Agkistrodon piscivorus*, and king snake serum in rats.

MATERIALS AND METHODS[1]

Preparation of Tritiated Snake Venoms—Tritium labeling of the snake venoms was performed according to Wilzbach's method (7). One gram each of the lyophilized venom of *C. adamanteus*, *C. atrox*, and *A. piscivorus* was dissolved in a small amount of water and thinly spread and dried in a round-bottom flask by using a vacuum evaporator. Tritium gas (3 c.) was introduced into the flask and the container was sealed. The reaction was allowed to proceed at 4° for 2 weeks. After the seal was opened and excess tritium gas was removed, a small amount of water was added to the flask to dissolve the venom and lyophilized to remove labile tritium. The LD_{50} of the ^{3}H-venoms on albino mice was found to be approximately the same as that of the original venom before tritiation (*C. atrox*, 6 mg./kg. i.p.; *C. adamanteus*, 25 mg./kg.i.p.; and *A. piscivorus*, 10 mg./kg. i.p.). The specific activity of each tritiated venom was as follows: *C. adamanteus*, 37 μc./mg.; *A. piscivorus*, 40 μc./mg.; and *C. atrox*, 27 μc./mg. Similarly, 1 g. of king snake serum was tritiated and purified according to the same procedure. The specific activity was 45 μc./mg.

Urinary and Fecal Excretion—A dose equivalent to LD_{25} of each ^{3}H-venom was dissolved in normal saline solution and injected intraperitoneally to three albino rats weighing 200–240 g. The animals were housed in individual metabolic cages and fed with food and water *ad libitum*. Urine and feces specimens were collected every 8 hr. Urine specimens were evaporated to dryness in a vacuum evaporator. Then an aliquot (0.1 g.) was placed in a liquid scintillation bottle containing scintillator fluid[2], and the activity was recorded in a liquid scintillation counter[3]. No hyamine hydroxide was used for the urine specimens. Feces specimens were

[1] Snake venoms were obtained from Miami Serpentarium Laboratories, Miami, Fla. Albino rats were purchased from Southern Animal Farms, Prattville, Ala. King snakes (*Lampropeltis getulus*) were obtained from Charles Chase Co., Fla., and Miami Serpentarium Laboratories.

[2] 10 g. 2,5-diphenyloxazole, 0.25 g. 1,4-bis[2-(5-phenyloxazolyl)]-benzene, 200 g. naphthalene, and dioxane *q.s.* to 1 l.

[3] Tracerlab, Corumatic 100 a.

Table I—Urinary and Fecal Excretion of ^{3}H-Labeled Venoms of *C. atrox* (Western Diamondback Rattlesnake), *C. adamanteus* (Eastern Diamondback Rattlesnake), and *A. piscivorus* (Eastern Cottonmouth Moccasin), and King Snake Serum-^{3}H (*Lampropeltis getulus*) in Rats (Expressed in Terms of Percent of Administered Activity)

Materials	Hours 8	20	32	44	56	68	80
C. atrox							
Urine	9.25 ±1.60	4.43 ±1.02	1.59 ±0.32	3.05 ±0.31	0.58 ±0.06	0.79 ±0.08	0.98 ±0.27
Feces	3.75 ±0.11	21.98 ±3.06	0.75 ±0.07	0.48 ±0.05	0.15 ±0.01	0.21 ±0.02	0.09 ±0.01
C. adamanteus							
Urine	10.95 ±1.21	3.76 ±0.95	1.67 ±0.12	0.59 ±0.08	0.63 ±0.13	0.58 ±0.08	0.67 ±0.04
Feces	3.21 ±0.35	11.02 ±1.42	3.41 ±0.87	3.34 ±1.14	1.18 ±0.47	1.26 ±0.47	0.74 ±0.03
A. piscivorus							
Urine	6.30 ±0.05	10.20 ±0.55	1.98 ±0.14	0.99 ±0.04	0.69 ±0.07	0.59 ±0.06	0.19 ±0.01
Feces	2.13 ±0.30	13.05 ±2.38	3.45 ±0.90	2.40 ±0.77	1.20 ±0.46	1.00 ±0.12	0.87 ±0.26
King snake serum							
Urine	3.46 ±0.92	12.88 ±0.36	1.01 ±0.45	1.18 ±0.23	0.51 ±0.02	0.71 ±0.04	0.48 ±0.05
Feces	12.65 ±2.23	11.82 ±1.54	2.54 ±0.65	0.60 ±0.09	0.91 ±0.12	0.50 ±0.07	0.13 ±0.01

dried and pulverized. An aliquot was weighed out, macerated with water, and evaporated to dryness *in vacuo*. The residue was placed in a scintillation bottle and digested with hyamine hydroxide at 50° overnight, and a small amount of hydrogen peroxide was added to bleach the colored substances. Scintillator fluid was added in the container, and the mixture was subjected to liquid scintillation counting.

Tissue Distribution—Five milligrams each of ^{3}H-venoms was injected intraperitoneally to five rats weighing 220–240 g. The animals were sacrificed at the intervals of 0.5, 1, 2, 4, and 8 hr. The organs (blood, brain, bone, heart, intestines, kidneys, lungs, liver, muscles, spleen, stomach, and urine) were removed, rinsed with normal saline (except blood and urine), and briefly dried, and the weights recorded. Approximately 0.5 g. of the organs except bone was placed in a bottle containing 5 ml. of hyamine hydroxide (30%) and digested at 50° for 12 hr. A few drops of hydrogen peroxide was used to bleach the turbidity before the scintillator liquid was added. The bone (femur) was treated with sulfuric acid and diluted with the scintillator solution for the activity recording. Tritiated water (0.1 ml.) was added to each bottle as an internal standard. The serum and the urine specimen (1 ml.) were evaporated to dryness in a bottle, dissolved in the scintillator solution, and subjected to activity counting in a liquid scintillation counter.

RESULTS

The radioactivity of the four materials recovered in the urine and feces is shown in Table I. The peak urinary excretion for *C. atrox*, *C. adamanteus*, and king snake serum was at 8 hr.; *A. piscivorus* had its peak at 20 hr. Fecal excretion of the three snake venoms was consistent with the peak level at 20 hr. and then declined thereafter. King snake serum showed a peak fecal excretion at 8 hr. and maintained a relatively high level of activity until 20 hr. The total activity recovered in the urine and feces during the 80-hr. period was 42–48% of the administered activity for the three venoms and 49% for king snake serum.

The tissue distribution data of ^{3}H-venoms of *C. atrox*, *C. adamanteus*, and *A. piscivorus* are shown in Tables II, III, and IV, respectively. Evidently, the intraperitoneally administered ^{3}H-venoms were well absorbed, as reflected in the high blood levels during the first 60 min. (6). The liver was the major organ concentrating the venoms and excreted them into the intestines *via* the bile secretion. The brain concentration of *C. adamanteus* was much higher than that of the other two venoms. The excretion process by the kidneys was quite active throughout the 8-hr. period. *A. piscivorus* venom seems to have a high affinity for the bone, showing the highest concentration in the bone after 8 hr. The activity in the muscles was fairly consistent during the experimental period.

The tissue distribution of ^{3}H-king snake serum is shown in Table V. The distribution pattern appeared to be similar to that of the snake venoms; that is, the majority of the radioactivity was concentrated in the liver and excreted into the intestines. The kidneys also played a considerable role in the excretion of king snake serum. The blood level of king snake serum was considerably high during the 8-hr. period.

DISCUSSION

Pit viper venoms are known to attack blood cells to cause hemolysis. King snake serum is able to neutralize the lethal effect of the pit viper venoms *in vivo* and to counteract the proteolytic effect of these venoms *in vitro*. The excretion study indicated that the majority of the administered venoms and king snake serum was excreted in feces. This fact was also reflected in the tissue distribution study, which showed that the liver had the highest activity followed by the intestines. The excretion pattern was similar among the three venoms and king snake serum. The high blood level of king snake serum may account for the fairly rapid neutralization effect of king snake serum against these venoms. The liver was the major organ that concentrated the venoms and king snake serum, because it appeared to be the major organ that metabolized these substances.

Table II—Tissue Distribution of ^{3}H-Labeled Venom of *C. atrox* in the Rat (Expressed in Terms of nc./g. Tissue of One Animal at Each Time Interval)

Organs	Hours 0.5	1	2	4	8
Blood	ns[a]	70.7	24.6	14.1	9.9
Brain	2.5	4.0	7.1	7.7	2.9
Bone	96.0	127.4	158.9	179.4	259.3
Heart	5.4	3.4	2.3	2.1	1.4
Intestines	87.2	128.2	449.0	580.8	583.0
Kidneys	10.5	30.3	7.3	25.7	16.5
Liver	62.6	568.3	276.8	50.5	46.1
Lungs	7.0	42.0	52.9	8.0	13.8
Muscle	115.3	100.6	110.4	85.2	136.2
Spleen	1.1	3.4	1.9	9.5	2.9
Stomach	36.1	11.9	7.5	9.4	7.0
Urine	ns	ns	ns	73.7	114.0
Feces	ns	ns	ns	39.0	68.2
Abdominal washings	620.6	33.3	7.8	5.9	2.9

[a] ns = no specimen.

Table III—Tissue Distribution of ^{3}H-Labeled Venom of *C. adamanteus* in the Rat (Expressed in Terms of nc./g. Tissue of One Animal at Each Time Interval)

Organs	Hours				
	0.5	1	2	4	8
Blood	22.8	23.9	10.0	5.0	3.8
Brain	6.1	47.0	106.9	143.7	58.5
Bone	43.1	109.2	146.0	162.0	172.3
Heart	166.0	172.9	109.3	92.6	53.1
Intestines	383.3	597.3	917.6	650.6	986.3
Kidneys	178.6	26.9	22.7	23.3	33.9
Liver	154.2	76.4	55.6	79.5	96.0
Lungs	7.1	11.6	14.8	32.0	99.0
Muscle	138.5	172.2	149.9	96.8	183.9
Spleen	50.1	93.2	117.4	100.0	84.4
Stomach	103.6	49.3	41.1	13.9	84.4
Urine	ns[a]	ns	ns	13.8	13.7
Feces	ns	ns	ns	169.3	287.8
Abdominal washings	644.2	429.1	191.6	134.4	26.8

[a] ns = no specimen.

In the distribution study, the overall recovery of the radioactivity of the ^{3}H-venoms and ^{3}H-king snake serum from the tissues of the animals 8 hr. after the administration was 79–98 and 83%, respectively. The activity excreted in the urine and feces during the same period was 5.1–16.6% for the ^{3}H-venoms and 26.5% for the ^{3}H-king snake serum. In the excretion study, the recovery of the radioactivity of the ^{3}H-venoms and ^{3}H-king snake serum in the urine and feces during the 80-hr. period was 42–48 and 49%, respectively.

The tritium labeling of snake venoms and king snake serum by the Wilzbach method (7) was quite effective and yielded high specific activity products. The tritium atoms in the protein molecules seemed to be quite stable during storage at $-20°$ because the loss of potency of the venoms and king snake serum was insignificant during the 3-month period. There was an indication that tritium exchange occurred when these tritiated materials were distributed in aqueous media *in vivo*. Therefore, it is imperative to treat the specimens with water and subsequently remove the solvent in order to eliminate the labile tritium in the specimens. The possibility of an isotopic exchange taking place between these compounds and the normal body metabolites, hormones, and neuroamines cannot be ruled out; however, the control experiment indicated that this type of isotopic exchange *in vivo* was negligible.

Table IV—Tissue Distribution of ^{3}H-Labeled Venom of *A. piscivorus* in the Rat (Expressed in Terms of nc./g. Tissue of One Animal at Each Time Interval)

Organs	Hours				
	0.5	1	2	4	8
Blood	94.9	147.4	70.5	23.3	22.0
Brain	6.5	14.7	19.6	12.8	12.5
Bone	226.5	258.1	393.0	607.9	807.2
Heart	14.5	10.1	12.9	13.2	11.8
Intestines	391.8	531.0	756.8	970.9	855.3
Kidneys	40.0	89.1	53.1	76.1	61.3
Liver	127.4	205.7	257.3	94.2	90.0
Lungs	164.5	170.7	257.5	135.9	88.7
Muscle	205.6	225.8	140.5	118.9	107.2
Spleen	104.8	96.1	91.2	69.4	55.3
Stomach	89.5	105.8	87.0	67.1	62.6
Urine	ns[a]	ns	5.0	41.8	68.4
Feces	ns	ns	ns	ns	49.3
Abdominal washings	1510.8	440.6	127.6	64.4	15.4

[a] ns = no specimen.

Table V—Tissue Distribution of ^{3}H-Labeled Serum of King Snake (*L. getulus*) in the Rat (Expressed in Terms of nc./g. Tissue of One Animal at Each Time Interval)

Organs	Hours				
	0.5	1	2	4	8
Blood	5.0	5.0	9.1	9.3	4.6
Brain	3.2	3.7	3.3	3.0	2.4
Bone	137.8	258.0	620.5	636.9	684.6
Heart	1.8	2.3	2.5	1.7	1.6
Intestines	39.6	81.3	695.8	262.6	175.2
Kidneys	11.6	59.2	58.4	20.2	20.4
Liver	172.1	611.7	574.9	339.4	259.3
Lungs	3.5	4.3	4.2	3.7	0.4
Muscle	80.9	70.2	75.6	92.0	53.8
Spleen	3.3	6.9	36.8	32.7	14.1
Stomach	120.3	102.0	110.6	61.4	23.6
Urine	ns[a]	ns	13.9	24.3	46.0
Feces	ns	ns	ns	386.4	556.7
Abdominal washings	1325.0	660.4	91.1	50.7	10.3

[a] ns = no specimen.

SUMMARY

1. ^{3}H-Labeled venoms of *C. atrox*, *C. adamanteus*, and *A. piscivorus* and ^{3}H-king snake serum were administered intraperitoneally to albino rats to study the tissue distribution and excretion patterns of these materials.

2. The elimination pattern of these materials was quite consistent with predominantly fecal excretion. The average excretion for these snake venoms during the 80-hr. period was 42–48%, and it was 49% for the king snake serum.

3. Tissue distribution indicated that the majority of the administered venoms was picked up by the liver and excreted in the intestines *via* the bile secretion.

4. King snake serum showed the highest concentration in the liver, which coincided with the tissue distribution pattern of the snake venoms; this fact indicated that these substances were concentrated and metabolized in the liver.

5. Exchange of tritium between these materials and the neuroamines was negligible.

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