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Toxicity of 1-(3,4-Dihydroxyphenyl)-2-Isopropylaminoethanol Hydrochloride (Isuprel).

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Konzett,[1] Lands, Nash, McCarthy, Granger and Dertinger,[2] and Siegmund, Granger and Lands[3] have described the pharmacologic actions of 1-(3, 4-dihydroxyphenyl)-2-isopropylaminoethanol HCl (Isuprel*). Stolzenberger-Seidel,[4] Dautrebande *et al.*,[5] and Segal and Beakey[6] have shown this substance to be an effective bronchodilator in bronchial asthma. This communication reports a series of toxicity studies carried out in this laboratory in an effort to evaluate better this new therapeutic agent.

Acute Toxicity. Albino mice, weighing 15-21 g, raised in our laboratory, were used for the determination of acute toxicity. The test animals were housed in the colony room for the duration of the experiment at a constant temperature of 76°F. Isuprel was injected intraperitoneally and the resultant deaths taking place during the 72 hours following injection were recorded. Epinephrine, arterenol, and racemic epinephrine, similarly administered, were injected into a comparable number of mice in order to compare the relative toxicity of these substances with that of Isuprel. The data were calculated by the method of Miller and Tainter,[7] with the results shown in Table I.

Isuprel was found to have an LD_{50} of 494 mg/kg when injected intraperitoneally, as compared with 4.6 mg/kg for epinephrine, 15.6 mg/kg for arterenol, and 7.8 mg/kg for racemic epinephrine. Arterenol appears to be 38 times and epinephrine 107 times more toxic than Isuprel. Doses as large as 720 mg/kg of Isuprel did not cause death in some instances. Acute 24 hour intravenous toxicities were determined in albino mice. These data also show the relatively low toxicity of Isuprel. Racemic epinephrine was found to be 16 times and arterenol 7.8 times more toxic than Isuprel.

Albino rats taken from our own colony and housed under the same environmental conditions as the colony animals were given Isuprel by subcutaneous injection or orally by intubation. Results obtained are included in Table I. Rats were injected subcutaneously with a dose as large as 100 mg/kg without the appearance of toxic effects. Richards[8] has reported the LD_{50} of epinephrine to be 5.3 mg/kg when administered subcutaneously in rats, while West[9] has reported a value of 12 mg/kg for the same compound. Arterenol, with an LD_{50} of less than 2.0 mg/kg, is even more toxic when administered in this manner (Schulte *et al.*).[10] Isuprel, administered by subcutaneous injection, was found much less toxic than these structurally similar compounds.

When given by intubation to rats, Isuprel

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3 Siegmund, O. H., Granger, H. R., and Lands, A. M., *J. Pharm. and Exp. Therap.*, 1947, **90**, 254.

* Isuprel is the registered trade mark of Winthrop-Stearns, Inc.

4 Stoltzenberger-Seidel, M., *Klin. Wochenschr.*, 1940, **51**, 1306.

5 Dautrebande, L., Philippot, E., Charlier, R., Dumoulin, E., and Nogarede, F., *Arch. internat. Pharmacodyn. Therap.*, 1942, **68**, 117.

6 Segal, M. S., and Beakey, J. F., *Bull. New Eng. Med. Cen.*, 1947, IX, 62.

7 Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.

8 Richards, R. K., *J. Pharm. and Exp. Therap.*, 1943, **79**, 111.

9 West, G. B., *Nature*, 1945, **155**, 20.

10 Schulte, J. W., Reif, E. C., Bacher, J. A., Lawrence, W. S., and Tainter, M. L., *J. Pharm. and Exp. Therap.*, 1941, **71**, 62.

TABLE I.
Acute Toxicity of Isuprel and Related Compounds.

Drug	Albino species	Route	No. animals	LD_{10}	Toxicity mg/kg $LD_{50} \pm$ S.E.	LD_{90}
Racemic Isuprel	Mice	Intrap.	273	377	494 ± 13.9	650
		Intrav.*	30	57.7	77 ± 7.0	99
	Rats	Subcut.	22	>100		
		Oral	20		>2000	
Epinephrine	Mice	Intrap.	80	2.15	4.6 ± 0.55	9.9
		Intrav.*	30	1.99	3.14 ± 0.23	4.8
	Rats	Subcut.[8]	96		5.3	
		Subcut.[9]			12	
Racemic Arterenol	Mice	Intrap.	72	4.6	15.6 ± 3.8	53.5
		Intrav.*	80	3.0	9.8 ± 1.9	27.4
	Rats	Subcut.[10]	10	<2.0	>2.0	
Racemic Epinephrine	Mice	Intrap.	80	2.92	7.8 ± 1.3	21
		Intrav.*	30	2.5	4.8 ± 1.08	9.3
	Rats	Subcut.	14	>15	<20	

* Values for 24-hour LD_{50}.

was found to be tolerated by some animals in doses as large as 2000 mg/kg. Toxic symptoms observed were salivation and nervousness, followed by depression. Four of 10 animals given 1800 mg/kg died within 24 hours after drug administration.

Acute oral and intramuscular toxicity of Isuprel was determined in 16 healthy dogs of both sexes chosen from animals recently procured. The results obtained are presented in Table II. When a dose of 10 mg/kg was given by intubation, toxic symptoms began to appear. Two of the 3 animals at this dose level showed no symptoms other than depression and some apprehension, although the heart rate was increased as much as 164 beats/min. 15 minutes after the drug administration. The third animal vomited frequently and appeared restless for 2½ hours after receiving Isuprel. One death was observed at 15 mg/kg. Gross examination revealed an acute respiratory condition, but it seems improbable that this resulted directly from drug administration. Animals given doses of 20 mg/kg vomited frequently during the period 15 to 60 minutes after medication. This was followed by a period of depression lasting for more than 2 hours, with a return to normal in less than 24 hours. Of the 3 animals given 50 mg/kg, 2 showed less severe toxic symptoms than were observed on lower doses. The third animal showed, in addition to vomiting, restlessness and depression, a marked cardiac arrhythmia and profuse salivation. In 24 hours the dog appeared normal, but 4 days later was found dead in the cage. Pathological examination revealed petechial hemorrhages of the endocardium, extensive hemorrhages of the lungs with necrosis, acute bronchopneumonia and pulmonary abscess. Autolysis of the gastric mucosa was also noted.

Subcutaneous administration of 2-5 mg/kg caused salivation and restlessness followed by depression. After subcutaneous doses of 10-20 mg/kg, the symptoms were similar to those observed in dogs after oral doses of 20-50 mg/kg. Less than 2 hours after medication 1 animal given 15 mg/kg died. Pathological examination showed ecchymotic interstitial hemorrhages of the pancreas and congestion of the spleen and kidneys. The animal given 20 mg/kg was found dead 24 hours after drug administration. Gross examination revealed the same general findings as those described above.

A group of 18 rabbits weighing from 1.3-2.3 kg was given Isuprel by intravenous injection in doses of 35-60 mg/kg. Animals

TABLE II.
Acute Toxicity of Isuprel Hydrochloride in Dogs.

Dose, mg/kg	Time after drug, min.	Results: Change in heart rate, beats/min.	Results: Other observations
Subcut.			
2	5	+116	Salivating, nervous
	15	+100	Restless
	55	+122	Depressed
	210	+136	Vomited
	300	+108	Behavior normal
5	5	+ 86	Panting, salivating
	10	+ 82	Restless
	15	+ 78	Depressed
	55	+114	Depressed
	305	+128	Depressed
10	5		Vomited twice
	10	+174	Panting
	30	+150	Arrhythmia, panting, depressed
	105	+148	Arrhythmia, panting, depressed
	310	+190	Alert, panting
15	5	+154	Restless, panting, salivating
	10	+122	Arrhythmia, panting, salivating
	20	+100	Arrhythmia, depressed, diarrhea
	40	+116	Arrhythmia, profuse salivation (frothing), panting heavily
	70	+148	Salivating, panting, depressed
	100		Dead
20	10	+126	Panting, depressed
	15	+104	Arrhythmia, depressed
	45	+ 84	Arrhythmia, restless, apprehensive
	75	+108	Arrhythmia, restless, apprehensive
	150	+136	Depressed, breath short, almost inaudible gasps
	180	+152	Panting, apprehensive, slight salivation
	300	+ 92	Quiet
	(24 hr)		Dead
Oral			
10	5	+144	Depressed, apprehensive
	15	+164	Depressed, apprehensive
	65	+140	Depressed, apprehensive
	95	+100	Depressed, apprehensive
	(24 hr)	0	Apparently normal
15	5	+ 75	Restless
	15	+100	Restless, whining
	30	+100	Depressed
	60	+ 84	Very depressed
	(4 days)		Dead
20	5	+108	Skin hot and pink
	10	+142	Panting, coughing
	15	+112	Vomited twice, panting, apprehensive
	25		Vomited several times, panting heavily
	35	+128	Vomited twice, panting
	60	+136	Lying down, quiet, panting
	100	+140	Lying down, quiet
	(24 hr)	+ 16	Apparently normal
50	5	+ 84	Nervous, vomited
	10	+108	Restless
	20	+136	Vomited, restless
	40	+164	Marked cardiac arrhythmia, profuse salivation
	70	+140	Nervous, panting heavily
	115	+ 82	Lying down, alert, panting
	425	+ 70	Slightly depressed
	(24 hr)	0	Apparently normal
	(4 days)		Dead (1/2)

given 35-45 mg/kg showed general asthenia immediately following injection but seemed normal within 5-15 minutes. One of the animals given 35 mg/kg appeared normal at 60 minutes but at 65 minutes convulsed and died. At 30 minutes after medication both animals given 40 mg/kg and 1 of the 2 at 45 mg/kg convulsed and died. At onset of convulsions pallor was noted in the unpigmented area around the mouth and the scleral vessels of the eye became less distinct. At 50 mg/kg all animals showed general asthenia with some respiratory difficulty immediately following injection. Three of the 4 rabbits appeared normal 5 minutes later, but convulsed suddenly 1-1½ hours after injection. Of this group, only 1 animal recovered. The fourth rabbit suffered clonic spasms with opisthotonos within 5 minutes after injection followed by death in 7 minutes. Three of the 6 animals given 55 mg/kg died within 3 minutes after medication, with respiratory difficulty, clonic spasms and opisthotonos as prominent manifestations. Two animals appeared to recover from these symptoms after 10 minutes but were found dead at 8 and 48 hours, respectively, after medication. One animal recovered completely 15 minutes after injection. Both rabbits given 60 mg/kg showed the above symptoms with death at 10 minutes and 1½ hours, respectively.

Subacute Toxicity. A group of 20 rats weighing from 75-136 g and equally divided between the sexes were given 100 mg/kg of Isuprel by intubation daily for 15 days. Throughout the experiment all rats grew at a normal rate and showed no toxic symptoms. The animals were sacrificed at the end of the experiment for gross and histopathological examination. No significant abnormalities were found.

Eleven female rats were given subcutaneous injections of 100 mg/kg of Isuprel daily for 5 days. Six of this group were weanling rats weighing 48-60 g, and the remaining 5 were adults weighing 193-225 g. A similar group of rats was injected with distilled water as controls and 3 days after the final medication all rats were sacrificed. Although a gross examination of both experimental and control rats showed enlarged uteri with prominent blood vessels, vaginal smears did not indicate estrus. It was therefore concluded that this condition must be normal for these rats.

Chronic Toxicity. Six dogs (3 male and 3 female), selected from animals recently procured, were dewormed and immunized in the usual manner. Isuprel was administered orally in a daily dose of 5 mg/kg for 6 to 17 weeks. At intervals of a week, chemical analyses for blood sugar, chloride, non-protein nitrogen and serum protein, and a determination of the formed elements of the blood were made. Throughout the experiment the blood picture remained normal. All animals appeared healthy and gained weight in the course of the test. Three and a half weeks after the beginning of drug administration 1 of the females delivered a litter of 5 normal pups. Gross and histopathological examination of the vital organs of all test animals revealed no significant abnormalities.

Isuprel was incorporated into a standard rat diet to make 0.5% by weight and this mixture was given as the only source of food to 30 weanling rats (15 male, 15 female) until the smallest animal weighed 146 g (6 weeks). A control group of 20 rats, litter mates of the above, was given the standard diet without Isuprel. There was no difference in the rate of growth between the experimental and control groups. Gross and histopathological examination disclosed no significant abnormalities.

Summary. Isuprel has a low acute toxicity in mice. Epinephrine is about 24 (intravenous) to 107 (intraperitoneal) times more toxic than this substance. Intravenous injection of Isuprel in rabbits was usually characterized by delayed deaths (30-90 minutes), the onset of terminal symptoms being rapid and unpredictable. Some deaths were observed at all doses tried (35-60 mg/kg). Subcutaneous injection into rats of 100 mg/kg once daily for 5 days caused no deaths.

Isuprel orally administered to dogs in doses of 10 mg/kg caused tachycardia, nervousness followed by depression and, with larger doses, salivation and vomiting. Death was observed with a dose as small as 15 mg/kg although

some animals survived oral doses of 50 mg/kg. Rats tolerated oral doses of 100 mg/kg daily for 15 days. Oral administration to dogs of 5 mg/kg daily for 6 to 17 weeks was well tolerated. Gross and histopathological examination of the vital organs of the medicated rats and dogs revealed no significant abnormalities. Isuprel did not interfere with growth when incorporated into a standard diet to make 0.5% by weight and given as the only source of food to weanling rats.

From the above data it is concluded that Isuprel has a comparatively low toxicity.

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Radiation-Induced Hemorrhagic Disease in Chickens.*

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Several articles describing the hemorrhagic disease in animals and man following radiation have recently appeared.[1-3] In these subjects hemorrhage is one of the predominant features in the postradiation syndrome. Allen and Jacobson[1] concluded that, contrary to the prevalent idea, thrombocytopenia plays only a secondary role in producing hemorrhage following radiation in dogs. They felt that the most significant change was the presence in the blood of an increased amount of free heparin. Liebow[2] and Cronkite[3] mentioned the presence of increased numbers of mast cells in tissues of victims of the atomic bombings, a finding of possible significance in view of the suggested relationship between mast cells and heparin production.

The blood changes and hemorrhagic disease we observed in chickens following continuous internal radiation from P^{32} administered subcutaneously were significantly different from those reported above in animals. In comparing the results of our work with the previous work, 3 factors should be kept in mind. First, the radiation differed in two respects from the external radiation of the above experiments. Throughout our experiment, radiation by P^{32} was continuous in contrast with the brief interval of exposure above; while widespread throughout the body, the P^{32} did not give the uniformity of body radiation which results from external total body gamma radiation. Second, the platelets or thrombocytes of the chicken are nucleated cells therefore differing in origin and character from the non-nucleated mammalian forms. Third, compared to the mammals studied, the chicken is relatively resistant to radiation.[4]

Our course of P^{32} injections killed 25% of the 20 experimental chickens and made those surviving at the end of the 17-day observation period moribund. The course of the blood counts and the coagulation times as measured by the capillary tube method using venous blood are recorded on the accompanying graph. The surviving birds were killed after 17 days of radiation, and clot retraction and blood chemistry determinations were made. Gross and microscopic autopsy studies were also done.

Observations. RBC counts decreased stead-

* Work done under contract from Office of Naval Research.

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