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Comparative Toxicity of Nicotine Alkaloid and Nicotine Salts.

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The idea is generally accepted by most pharmacologists that the physiological activity and toxicity of powerful alkaloids are practically the same as that of their salts, provided allowance is made for the slight increase in molecular weight of the salts due to the acid radicles, and for the difference in solubility between the alkaloids and their salts. The chief exception to the rule, in a limited way, is cocaine. The addition of sodium bicarbonate to cocaine solution increases its efficiency as a local anesthetic when applied directly to nerve trunks, and this is claimed to be due to the greater penetration of the alkaloid for the nerve tissue. Such a difference is not noted after hypodermic or intradermal injections.[1, 2, 3]

In connection with a comparative pharmacological and toxicological study of β-pyridyl-α(N-methyl)-pyrrolidine, or nicotine, and a new series of related chemical derivatives of pyrrolidine, the writers noted a marked difference in the physiological activity of the alkaloid itself and its salts. Previous writers have noted a difference in toxicity between nicotine and its salts but their work dealt mostly with the effects of these chemicals as insecticides. Del Quercio,[4] reporting some experiments on silkworms sprayed with aqueous solutions of nicotine and its salts, states that acidulated nicotine was less active than the alkaline substance itself. Moore and Graham,[5] comparing the effects of nicotine sulphate with and without sodium carbonate on certain aphids, found that the sulphate was less active than nicotine solution containing sodium carbonate. Moore and Row[6] found nicotine itself more toxic for the frog than nicotine hydrochloride. De Ong[7] showed that free nicotine administered orally to chickens was more toxic than nicotine sulphate; and Langley and Dickinson noted that nicotine solution was more

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[1] Gros, *Arch. f. exp. Pathol. u. Pharmakol.*, 1910, **62**, 380.

[2] Laewen, *Münch. med. Wchnschrft.*, 1910, **57**, 2044.

[3] Sollmann, *J. Am. Med. Assn.*, 1918, **70**, 216.

[4] Del Guercio, *Nuove Relaz. R. Staz. Ent. Agr. Firenze*, 1900, **1**, 124.

[5] Moore and Graham, *J. Agr. Research*, 1917, **10**, 47.

[6] Moore and Row, *J. Physiol.*, 1897, **22**, 273.

[7] De Ong, *J. Econ. Ent.*, 1923, **16**, 486.

active for ganglion cells and nerve fibers than nicotine sulphate.[8] Richardson and Shepard found that the toxicity of nicotine for mosquito larvae varied to some extent with the hydrogen ion concentration.[9]

This difference in activity between the alkaloid and its salts was explained either by the variation in the hydrogen ion concentration of the solutions or by the volatility of nicotine itself and its consequent rapid diffusion through the bodies of the insects. These explanations may hold good for experiments on insects and other small organisms, but it was deemed desirable to determine definitely whether a difference between the nicotine alkaloid and its salts may be demonstrated in large animals and also in living plants.

A nicotine of the highest chemical purity was prepared by one of the authors, and solutions of its various salts were so made as to contain exactly the same amount of nicotine by weight, irrespective of anions. The following 5 salts were studied: hydrochloride, acetate, salicylate, sulphate and tartrate. Phytopharmacological studies were made of the toxicity of the various compounds for the seedlings of *Lupinus albus* suspended in dilute solutions of the drugs; and the zoöpharmacological activity of the compounds was studied on goldfish, *Carassius aureus;* minnows, *Gambusia;* larvae of tadpoles, *Rana sylvatica,* 4 weeks old, and *Rana catesbiana,* 1 year old; leeches, *Hirudo medicinalis;* small crayfish, *Cambarus;* frogs, *Rana clamata;* white mice, white rats, canaries and on cats. The results are exhibited in Tables I and II.

Without exception there was a marked difference in toxicity between the nicotine alkaloid and its salts for both animals and living plants. Obviously, this difference in toxicity cannot be explained on the basis of volatility of the nicotine alkaloid as has been suggested in case of sprays for insecticidal purposes, nor by variations in the hydrogen ion concentration of the solutions used, especially when the drugs were injected in such animals as rats and mice intramuscularly or intraperitoneally, and intravenously in cats. The concentrations in which the seedlings of *Lupinus albus* were suspended were made by dissolving the drugs in Shive solution,[10] containing magnesium sulphate, diacid potassium phosphate and calcium nitrate. Studies on the hydrogen ion concentration of such solutions of nicotine and its salts in this plant physiological medium showed very little difference in their reactions. In Table II it will be noted

[8] Langley and Dickinson, *Roy. Soc. (London) Proc.,* 1889, Ser. B 46, 423.

[9] Richardson and Shepard, *J. Agr. Research,* 1930, **41**, 337.

[10] Shive, *Physiol. Researches,* 1915, **1**, 327.

TABLE I.

Toxicity of Nicotine and its Salts for Animals.

Animals	Nicotine Alkaloid Concentration	Dead	Nicotine Salts	Concentration	Dead
Carassius aureus	1:5,000	3 min.	N. hydrochloride	1:5,000	12 min.
			N. acetate	1:5,000	18 min.
			N. salicylate	1:5,000	11 min.
	1:10,000	4 min.	N. sulphate	1:5,000	13 min.
			N. tartrate	1:5,000	4½+ hr.
Gambusia	1:10,000	2 min.	N. hydrochloride	1:5,000	4 min.
Larvæ of *Rana sylvatica*, 4 weeks old			N. hydrochloride	1:10,000	2+ hr.
			N. acetate	1:10,000	2½+ hr.
			N. salicylate	1:10,000	18 hr.
			N. salicylate	1:5,000	2½ hr.
			N. sulphate	1:10,000	2½+ hr.
	1:10,000	9 min.	N. tartrate	1:10,000	18 hr.
Larvæ of *Rana catesbiana*, 1 year old			N. hydrochloride	1:5,000	65 min.
			N. salicylate	1:5,000	29 min.
	1:5,000	3 min.	N. sulphate	1:5,000	49 min.
Hirudo medicinalis	1:10,000	6 min.	N. hydrochloride	1:5,000	12 min.
Cambarus	1:10,000	53 min.	" "	1:5,000	113 min.
Rana clamata	0.5 mg. per 30 gm.; paralysis and characteristic posture in 25 min.		" "	0.5 mg. per 30 gm., little or no effect in 25 min.	
White Mice	0.1 mg. per 10 gm.; dead in 3 min.		" "	0.1 mg. per 10 gm.; dead in 6 min.	
	0.1 mg. per 20 gm.; dead in 4 min.		" "	0.1 mg. per 20 gm.; dead in 10+ min.	
White Rats	M.l.d., 27 mg. per kilo		" "	recovery after 27 mg. per kilo	
Canary	0.05 mg.; immediate convulsions		" "	0.05 mg., little or no effect	
Cats	Intravenous injection under ether, 1:2,000 solution gives m.l.d., 2 mg. per kilo		" "	intravenous injection under ether of 1:2,000 solution at same rate gives m.l.d., 2.6 mg. per kilo	

TABLE II.

Toxicity of Nicotine and Its Salts for Lupinus Albus.

Nicotine	Index of Growth in Concentration 1:5,000 %	Index of Growth in Concentration 1:2,000 %
Alkaloid	52	34
Acetate	69	50
Hydrochloride	81	50
Salicylate	26	10
Sulphate	90	56
Tartrate	85	80

that the salicylate of nicotine was much more toxic for plant tissues than other salts. This agrees with the findings of Macht and Livingston on the great toxicity of benzoates for living plants,[11] which observation was later found to hold good also for salicylates. The marked difference between the alkaloid nicotine and its salts is especially interesting when compared with the relative effects of certain other alkaloids and their salts, which were used as controls. We have made comparative pharmacological studies with solutions of atropine and atropine sulphate, cocaine and cocaine hydrochloride and strychnine (alcoholic solutions) and strychnine sulphate and found that those alkaloids did not differ substantially in toxicity from their salts.

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The Critical Period for Control of Sex in Moina.

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Banta and Brown[1] have shown that with crowding under favorable food conditions the critical period for sex control with *Moina macrocopa* is approximately 4 hours before the eggs are laid into the brood chamber. Stuart, Cooper and Miller,[2] while unable to affect control of the sex of the same Cladoceran species later than the fourth hour before egg laying, concluded that sex could be influenced by conditions prevailing previous to the fourth hour before egg laying. The following experiment was designed to show that the critical period for sex control is dependent to some extent upon the environmental conditions under which the animals are reared, *i. e.*, upon environmental conditions present earlier than, and interrupted before, the time of the critical period as described by Banta and Brown for crowding under good food conditions.

In 4 series of bottles, A, B, C and D, each series containing from 15 to 20 bottles, were placed 75 ml. of varying dilutions of manure infusion and 10 young *Moina macrocopa* females. The infusion in series A and B was diluted 12.5 times, in series C, 25 times, and in

[11] Macht and Livingston, *J. Gen. Physiol.*, 1922, **4**, 573.

[1] Banta, A. M., and Brown, L. A., *Proc. Nat. Acad. Sci.*, 1929, **10**, 71.

[2] Stuart, C. A., Cooper, H. J., and Miller, Rose, *Roux. Arch.*, Abt. D, 1932, **126**, 325.