centrifugations a purified protein giving sharp boundaries with $s_{20} = 77 \times 10^{-13}$, $s_{20} = 117 \times 10^{-13}$ and sometimes $s_{20} = 54 \times 10^{-13}$ was obtained from the juice of healthy pea plants, *Pisum sativum* L. var. *arvense* Poir. It gave the usual protein color tests, contained a pentose, and was probably a nucleo-protein. Both the broad bean and pea plant proteins were pigmented and of limited stability. Solutions of the former were dark green; after repeated sedimentations in the cold the pigment became insoluble leaving a colorless opalescent suspension of inhomogeneous material. Solutions of the pea protein were light green in color and, after standing for several days in the refrigerator, they too became inhomogeneous and no longer sedimented with sharp boundaries.

Similar ultracentrifugal procedures have not isolated homogeneous macromolecules from the juice of healthy tobacco plants. If such proteins exist, they are either highly unstable or are present only in minute amounts.

9806

Toxicity of Nicotinic Acid.

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Nicotinic acid (I), chemically pyridine-β-carboxylic acid, was prepared as early as 1867 by Huber[1] from the oxidation of nicotine (II). During the early investigation of vitamin B, nicotinic acid was isolated from rice polishings,[2, 3, 4] and suspected to have an antineuritic action in pigeons, although subsequent tests definitely excluded this possibility.[5] A renewal of interest was aroused by the publication of Elvehjem, Madden, Strong, and Woolley,[6] who showed that nicotinic acid or its amide isolated from the liver extract cures canine blacktongue of the Goldberger type.[7] Their

1 Huber, C., *Liebigs Ann. Chem.*, 1867, **141**, 271; *Ber. deut. chem. Gesellsch.*, 1870, **3**, 849.

2 Funk, C., *J. Physiol.*, 1911-12, **43**, 395; 1912-13, **45**, 489; 1913, **46**, 173.

3 Suzuki, U., Shimamura, T., and Odake, S., *Biochem. Z.*, 1912, **43**, 89.

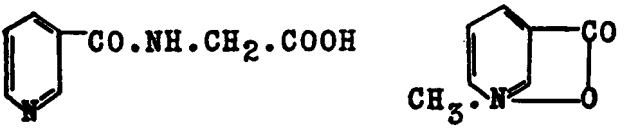
4 Drummond, J. C., and Funk, C., *Biochem. J.*, 1914, **8**, 598.

5 Funk, C., *J. A. M. A.*, 1937, **109**, 2086.

6 Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767; *J. B. C.*, 1938, **123**, 137.

7 Smith, D. T., *J. A. M. A.*, 1937, **109**, 2086.

(I) (II)



(III) (IV)

work has been confirmed by Street and Cowgill.[8] Favorable clinical results in the treatment of human pellagra with nicotinic acid have already been obtained by Spies, Cooper, and Blankenhorn,[9] Fouts, Helmer, Lepkovsky, and Jukes,[10] Smith, Ruffin, and Smith,[11] and Margolis, Margolis, and Smith.[12]

Pharmacologically, Hunt and Renshaw[13] failed to observe any effect upon the autonomic nervous system with nicotinic acid and its derivatives. In dogs Ackermann[14] and Komori and Sendju[15] reported that nicotinic acid is excreted in urine partly as nicotinyl glycine (III) and trigonelline (IV), and partly unchanged. It is also apparent from these publications that nicotinic acid has a relatively low toxicity.

In view of the present interest in nicotinic acid (I) for the management of pellagra, and its suggestive relationship, although chemically remote, to nicotine (II), it appears desirable to have some precise information concerning its toxicity in animals. The sample of nicotinic acid we investigated was entirely colorless and melted at 236-237°C. (corrected). The solutions employed for injection were neutralized with adequate amounts of sodium carbonate—a

8 Street, H. R., and Cowgill, G. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 547.

9 Spies, T. D., Cooper, C., and Blankenhorn, M. A., *J. Am. Med. Assn.*, 1938, **110**, 622 and 766.

10 Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 405.

11 Smith, D. T., Ruffin, J. M., and Smith, S. G., *J. A. M. A.*, 1937, **109**, 2054.

12 Margolis, L. H., Margolis, G., and Smith, S. G., cited by Smith *et al.*

13 Hunt, R., and Renshaw, R. R., *J. Pharmacol. and Exp. Therap.*, 1929, **35**, 75; 1929, **37**, 177.

14 Ackermann, D., *Z. f. Biol.*, 1912, **59**, 17.

15 Komori, Y., and Sendju, Y., *J. Biochem.* (Japan), 1926, **6**, 163.

1% solution having a pH of 7.4, one of 10% that of 7.8, and one of 20% that of 8.1. Minimal lethal doses were determined in white mice, white rats, and guinea pigs by intravenous injection with nicotine and nicotinic acid, respectively. The body weight of mice varied from 15 to 20 gm., that of rats 80 to 110 gm., and that of guinea pigs from 250 to 280 gm. As shown in Table I, nicotine is approximately 5625 times as toxic as nicotinic acid in mice, 3500 times as toxic in rats, and 778 times as toxic in guinea pigs. With lethal or near-lethal doses of both nicotine and nicotinic acid, all animals developed clonic convulsions, and either died promptly or recovered without apparent after effects.

TABLE I.
Acute Toxicity of Nicotine and Nicotinic Acid.

Compound	Species	Dose, mg. per kg.	No. of Animals used	No. died	Minimal lethal dose, mg. per kg.
Nicotine	Mice	0.4	1	0	0.8
		0.5	1	0	
		0.6	6	0	
		0.7	7	0	
		0.8	10	7	
		0.9	5	5	
	Rats	0.5	1	0	1.0
		0.7	5	0	
		0.8	5	2	
		0.9	5	2	
		1.0	5	3	
		1.1	5	5	
		1.2	3	3	
	Guinea Pigs	3.0	4	1	4.5
		4.0	3	0	
		4.5	4	3	
		5.0	3	3	
Nicotinic Acid	Mice	2500.0	3	0	4500.0
		3000.0	3	0	
		4000.0	3	0	
		4500.0	3	3	
	Rats	3000.0	5	2	3500.0
		3500.0	5	3	
	Guinea Pigs	3000.0	3	0	3500.0
		3500.0	5	3	

A group of 5 mice, injected daily by the tail vein, each with 500 mg. of nicotinic acid per kg. of body weight for 4 weeks except Saturdays and Sundays, survived with an increase in body weight.

Two adult dogs, weighing 13.8 and 15 kg., respectively, each received daily *per os* 2 gm. of nicotinic acid dispensed in capsules. The smaller animal began to excrete bloody feces on the 11th day, de-

veloped a series of convulsions on the 12th day, became increasingly irritable and bit the attendants. Since it was suspected of having rabies, medication was stopped on the 13th day. The dog was found dead on the 19th day, with a total loss of 4.2 kg. of body weight, not from rabies but apparently from poisoning due to nicotinic acid. The larger dog received a total amount of 40 gm. of nicotinic acid in the course of 20 days, gradually lost appetite until it refused to eat, and began to have occasional convulsions after the second week of experimentation. On the 20th day, the convulsions, tonic in character, became increasingly frequent, each series lasting from 1 to 4 minutes. The animal was unable to stand up during interconvulsive periods. Bloody feces were expelled. The condition was so critical that it was considered advisable to sacrifice the animal in order to avoid postmortem changes. The total loss of body weight in this animal was 3.6 kg. Autopsies were performed by Dr. Paul N. Harris. In the smaller dog, there were fatty metamorphosis of the liver and intussuception of small intestines which apparently existed for some time but was not sufficient to account for the death. In the larger dog, there were 6 erosions, 2 to 5 mm. in diameter, in the lesser curvature of the stomach which was filled with some bloody fluid; slight hyperemia of both small intestines and colon; petechiæ in the mucosa of the colon; a small amount of blood in colon contents; and fatty metamorphosis of the liver. Perhaps a more significant finding was the fact that in both dogs a few ganglion cells of the cortex, hippocampus and basal ganglia were shrunk and deeply stained. If this indicated an injury caused by excessive doses of nicotinic acid, it might be then that the convulsions observed before death were merely its manifestations. Further investigation will be necessary before this point can be settled.

Four additional dogs were given daily for a period of 8 weeks, except Saturdays and Sundays, smaller doses by mouth. They were 1, 0.5, 0.2, and 0.06 gm., respectively. At the end of the 8th week, all 4 animals appeared in good health and had gained in weight. A trace of albumin was found twice in the urine of the dog on the 1 gm. dose and in that of the dog on the 0.5 gm. dose. A trace of sugar was also detected once in the urine of both animals.

Unlike nicotine, nicotinic acid has no action upon the autonomic ganglia as already proved by Hunt and Renshaw.[13] A concentration of 1:5,000 of the acid did not elicit any response from the isolated rabbit's intestine. Similarly, a solution of 1:2,000 was inert to the isolated guinea pig's uterus. An intracutaneous injection of 0.1 cc.

of a 1% solution of nicotinic acid was followed by no hyperemia or ulceration. The substance has therefore little irritating action. Since nicotinic acid is often prepared from nicotine,[16] caution should certainly be exercised to eliminate any possibility of contamination by the alkaloid. Simple animal tests will detect such an impurity.

Summary. Nicotinic acid is at least several hundred times less toxic in mice, rats, and guinea pigs than nicotine. Nicotinic acid is devoid of action upon the autonomic ganglia. Nevertheless, repeated administration of large doses, 2 gm. daily, in dogs has resulted in poisoning and deaths.

9807

Renal Excretion of Exogenous Creatinine in the Aglomerular Toadfish, *Opsanus tau.*

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We have presented elsewhere a quantitative analysis of the relationship between plasma concentration and urine flow, and the tubular excretion of phenol red by the aglomerular fishes, *Lophius piscatorius* and *Opsanus tau.*[1] It was shown, in each of these species, that above 6.0 mg. % plasma phenol red there was no significant increase in the rate of excretion and that this maximal rate was not influenced by the extent or direction of the diffusion gradient. The present study represents an attempt at a similar analysis of the tubular excretion of exogenous creatinine by *Opsanus.* Marshall and Grafflin[2] have shown in this species that the fraction of injected creatinine which is excreted in an 18-hour period decreases progressively with increasing amounts of injected creatinine; furthermore, the fraction of a small injection excreted in a given period of time increases with increasing urine flow.

The present experiments may be divided into 2 groups. In the first group one urine collection period, 10 to 12 hours in duration and beginning 8 to 12 hours after the injection of creatinine, was made after single doses of 50 to 1500 mg. per kg. Under these con-

[16] McElvain, S. M., *Organic Synthesis,* 1925, **4**, 49.

[1] Shannon, J. A., *J. Cell. and Comp. Physiol.,* 1938, in press.

[2] Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.,* 1932, **1**, 161.