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The comparative toxicity for mice of five commonly used nicotine preparations[1]

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Summary

Pure nicotine, (–) nicotine, nicotine H+ tartrate, nicotine sulfate and total alkaloids extracted from cigarette smoke (more than 90 % nicotine), all of which have been used in much physiological and behavioral research, were compared as to their LD_{50} in mice. Significant differences in toxicity were noted among several of the preparations, indicating possible differences in potency at supposedly identical dosage levels, in regard to the literature, and also helping to clarify some other discrepancies which exist in experiments where these substances have been used.

Despite the utilization of different nicotine preparations for research at various laboratories, there have been no published reports in which several of these compounds have been compared in the same experiment. With the exception of one brief abstract [1], this holds true even for the basic determination of LD_{50} (lethal dose) in mice. In this study, we have measured the toxic effects of five commonly used nicotine preparations for mice. In addition to investigating the comparative potency of these compounds, we were particularly interested in comparing (–)nicotine with recently supplied nicotine purissimum. These two preparations should normally be identical, since pure nicotine is also laevorotatory, or (–). It has been our impression, however, that the nicotine purissimum obtained here in recent months has not been as effective in rat behavior experiments as earlier samples received from the same source.

600 male albino mice, all from one supplier and weighing between 25–30 g, were employed, each animal being used only once. All preparations were intraperitoneally injected, in logarithmically calculated doses, into groups of ten mice each, with all drugs being administered on any given day of injection. A toxicity estimation was thus obtained for each drug ranging from 0 to 100 % mortality, and the LD_{50} was determined from these values by applying a Probit analysis [2]. As in previous studies [3], it was observed that either the mice died within a few minutes after injection (during convulsions), or they survived.

The LD_{50} for mice of intraperitoneally injected L- (pure) nicotine is about 10 mg/kg [4]. Although the results of this present experiment are in relative conformity to this figure, occasional differences in the literature are seen, since such factors as age, number of animals per cage and environmental temperature, to name but a few, probably play a role in the toxicity of nicotine, as they do with amphetamine. With the latter drug, for example, the strain of mouse used has been also shown to exert a considerable influence on the LD_{50} [5]. The important consideration in comparing various preparations, therefore, is that the tests be conducted at the same time and place, with like animals, and under the same environmental conditions. This having been done here, it can be seen from the results (Fig. 1) that the nicotine H^+ tartrate and total smoke alkaloid preparations were significantly more toxic than those of nicotine purissimum and nicotine SO_4. The exact LD_{50} values for each preparation follow, with the respective pH given for each in parenthesis: nicotine purissimum 10.8 mg/kg (pH 9.3), (–)nicotine 9.5 mg/kg (pH 9.4), nicotine SO_4 10.4 mg/kg (pH 6.7), nicotine H^+ tartrate 9.15 mg/kg (pH 3.2) and total smoke alkaloids 8.7 mg/kg (pH 3.3). All LD_{50} values are expressed in terms of the nicotine base.

The pH of nicotine solutions has long been known to play an important role in their effect on living organisms, with many researchers having demonstrated that nicotine is better absorbed, by various routes of administration, when in alkaline solution. It has also been shown, however, that the

[1] Based on a presentation at the scientific meeting of the Swiss Society for Preventive Medicine, Geneva, June 22, 1972.

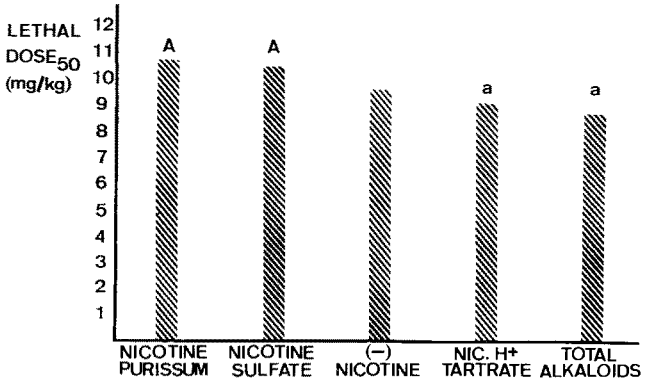

Fig. 1 The comparative toxicity for mice of five nicotine preparations.
A vs. a = $p < .05$

irritating properties of nicotine solutions, possibly irrespective of pH, can also increase nicotine absorption, probably through vasodilation and other physiological mechanisms [6]. In that study it was demonstrated that intraperitoneal injections of cigarette smoke solutions were substantially more toxic in mice than injections of nicotine acetate of equal nicotine concentration, and that the toxicity of smoke solutions was unlikely to be due to any other ingredient than nicotine. In this present experiment (disregarding the values for nicotine purissimum, whose recent reduction in potency seems to have been confirmed), it can be seen that although (−)nicotine (pH 9.4) was slightly more toxic than nicotine SO_4 (pH 6.7), both the nicotine H^+ tartrate (pH 3.2) and total alkaloids (pH 3.3) were significantly more toxic than the nicotine SO_4. This result encourages speculation that the irritant nature of such strongly acid solutions may result in increased nicotine absorption when injected intraperitoneally. In this regard it should be mentioned that although a complete comparison has not yet been made, buffered (pH 6.0) nicotine H^+ tartrate solution, injected into groups of ten mice each at two dosage levels (10.0 and 9.5 mg/kg), appeared to be only slightly less toxic than this compound at pH 3.2.

Another comparison was made in this study which is worthy of note at this time. A 6.67–10.0 mg/kg dose range for nicotine H^+ tartrate was repeated, with five groups of ten mice each. All injections, however, were preceded by 30 min with a subcutaneous injection of 12 mg/kg mecamylamine HCl, expressed as base. This dose is ½ of the minimal lethal dose for this substance (intraperitoneal), as calculated in this present study, and a much smaller fraction of the lethal dose when injected subcutaneously [7]. As expected, mecamylamine was 90–100 % effective in "blocking" the lethal and convulsive actions of nicotine at all dosage levels of the latter. In the two mice which died, however, and a few of the others as well, symptoms were observed which were identical to those seen with high doses of mecamylamine alone. These were initial hyperactivity, followed by depression and huddling, during which the mice displayed jerky movements and shaking when trying to walk. Death was preceded by difficult breathing in an upright position, and occurred as late as

15 minutes after injection, with the mouse remaining in the same position. In contrast, the injection of a comparably high dose of nicotine alone was normally followed in quick succession by cramping, normal activity, aimless wandering, convulsions while lying on one side, respiratory gasping and death or, if the mouse survived, depression and remaining very still after resuming an upright position. Although it has been previously determined that both the lethal effects of nicotine in mice and the "blocking" action of mecamylamine on these effects takes place centrally [3], the peculiar "mecamylamine-like" symptoms seen here, upon combining lethal doses of nicotine with definitely sub-lethal doses of mecamylamine, demonstrate that the interaction of these two substances in the brain is far more complicated than any simple blocking action of one against the other.

In addition to further investigation of the nicotine-mecamylamine interaction described above, another interesting project to be undertaken as a follow-up to this present study is to determine the exact role which factors such as the pH and "irritating effect" of various nicotine preparations by different injection routes, as well as different environmental conditions, play in the absorption (and toxicity) of nicotine.

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