A Spectrophotometric Determination of Isonicotinic Acid Hydrazide

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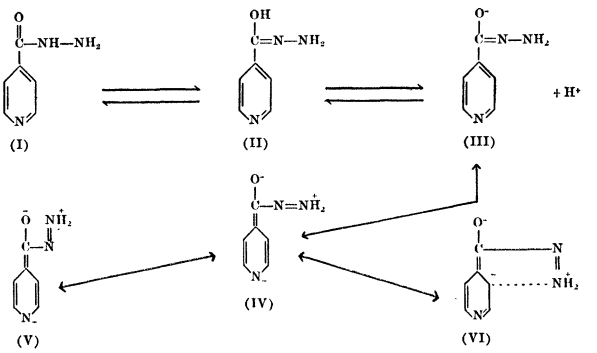
Experiments in this laboratory on the metabolic activities of certain antituberculous drugs necessitated the development of a rapid, sensitive method for the determination of isonicotinic acid hydrazide (INAH) in aqueous solution. Several methods for the determination of INAH and its derivatives have appeared in the recent literature. These methods involve either condensation of isonicotinic acid with CNBr (*1*), condensation of hydrazine with p-dimethylaminobenzaldehyde (*2*) or glutaconic aldehyde (*3*), reduction of ferricyanide by hydrazine (*4*), or measurement of the 265-mμ peak of an isoamyl alcohol extract of plasma (*5*). None of these methods is suitable for the rapid determination of INAH at the level of 1 μg or less per milliliter.

The method described here is based on *p*H-engendered changes in the absorption spectrum of INAH solutions (Fig. 1) In strongly alkaline solutions, a new peak emerges having an extinction coefficient at 298 mμ of 4.8×10^6 cm²/mole (*6*). This peak disappears, reversibly, in acid solution.

Sufficient NaOH is added to an aqueous solution of INAH to bring the final concentration of hydroxide to 0.1*N*. The optical density at 298 mμ of the solution is measured in a spectrophotometer. The lower limit of the method is about 0.5 μg of INAH per milliliter with an over-all sensitivity of about 0.1 μg/ml. The linearity of the concentration curve is shown in Fig. 2.

The effect of *p*H on the absorption at 298 mμ is shown in Fig. 3; the pK_a of the hydrazide is approximately 11. The change in absorption spectrum of INAH in alkaline solution may be associated with the quinoid forms (IV–VI), which are postulated as arising from the enolate ion (III). That the structural



modification causing this change in the absorption spectrum is associated with both an unsubstituted hydrazide group and a free ring nitrogen is demonstrated by the fact that none of the following related compounds (*7*) shows the absorption peak at 298 mμ in alkaline solution: nicotinamide, isonicotinamide, isonicotinic acid, 1-isonicotonyl-2-isopropyl hydrazine, 1-isonicotonyl-2-allyl-2-isopropyl hydrazine dihydrochloride, and N^1-methyl-isonicotinic acid hydrazide (*8, 9*). Substitution of the primary nitrogen may result in steric hindrance preventing the formation of V or VI; substitution of the ring nitrogen could prevent the formation of V. Either of these factors could thus prevent the spectral change associated with free INAH.

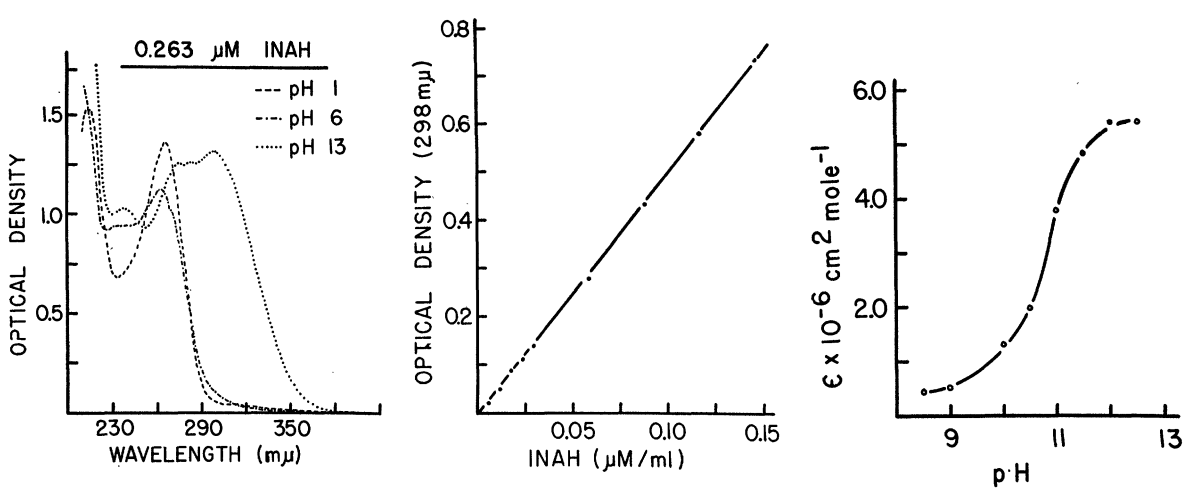


Fig. 1 (left). Effect of the solution *p*H on the absorption spectrum of INAH. Figure is a composite obtained by transcription of three tracings obtained from the Carey recording spectrophotometer. Fig. 2 (center). Concentration curve for the spectrophotometric determination of INAH. Samples were read in 1.0-ml quartz cuvettes in the Beckman spectrophotometer. Fig. 3 (right). Effect of *p*H on the absorption at 298 mμ of solutions of INAH. Solutions containing 0.263 μM INAH per milliliter were adjusted to the indicated *p*H, and the optical density at 298 mμ was measured.

References and Notes

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6. The Carey recording spectrophotometer used in this study was made available through the generosity of A. L. Wilds, Department of Chemistry, University of Wisconsin.
7. All pyridine derivatives used in this study were generously supplied by Hoffmann-LaRoche, Inc., Nutley, N.J.
8. This compound, in alkaline solution, exhibits the yellow color having an absorption peak at 370 to 380 mμ characteristic of N^1-substituted pyridine compounds (*9*).
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Effect of Topically Applied Stannous Chlorofluoride on the Dental Caries Experience in Children

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At the present time approximately 20 million Americans drink water with a fluorine concentration of approximately 1 ppm or above (*1*). This is nearly one-eighth of the entire population of the United States, but it is probable that at no time will the number ever be more than 50 to 60 percent of the total population. This results from the fact that approximately one-half of the population is served by rural water supplies that are too small to be safely fluoridized. Thus, from a preventive dentistry viewpoint, other effective methods of fluoride therapy must be instituted that will compare favorably with communal fluoridation. The topical application of a concentrated solution of sodium fluoride has been introduced for this purpose, and through its judicious use a reduction in dental caries by 30 to 40 percent in children has been reported (*2*), while a reduction of 50 to 60 percent in new cavities is experienced in 6-yr-old children whose teeth calcified during the period that they ingested fluoridated water (*3*).

Conservative estimates of the annual backlog of dental services are placed at more than 1 billion units per year (*4*), with the cost of dental health care per family in the United States at more than $200 a year (*1*). This comprises 15.6 percent of the nation's entire health bill. It would seem that search should be made for a more effective topical agent.

In an attempt to find a more beneficial compound for use as a topical agent, stannous chlorofluoride (*5*) was applied to the erupted teeth of approximately 800 children, ages 6 to 15 yr, in Gas City, Ind. One-half of the children were treated with a 1-percent aqueous unbuffered solution of sodium fluoride, and approximately the same number received a 4-percent stannous chlorofluoride solution. Both fluorides were applied to the teeth by cotton applicators in a manner similar to that described by Knutson (*6*). This consisted of a thorough dental prophylaxis followed immediately by the first topical fluoride application. Within a period not exceeding 10 days, three additional topical treatments were given, although only the first was preceded by a prophylaxis. The treatment consisted of keeping all surfaces of the teeth moist throughout each 4-min treatment series.

Howell examined all the children in a Mobile Dental Trailer. Excellent light, compressed air, and new dental explorers and mirrors were used. The concentrations of the fluorides used in this study were chosen so that the fluorine levels would be similar. Since an 8-percent stannous chlorofluoride solution was considered unpleasant for use by the children, a conventional 2-percent solution of sodium fluoride could not be used and still maintain equal fluorine concentrations in both solutions. All the solutions were prepared fresh each morning and noon from oxygen-free water (*7*). Approximately 1 yr after the initial fluoride applications, the children were reexamined by the same dentist. At no time did the examiner have any knowledge of which group he was examining or of the previous caries history of the children.

The results of the topical fluoride treatments are shown in Table 1. Only the children who were examined and treated at both the initial and 12-mo periods were included in computing the data. The analysis was confined to the dental caries experience in the erupted permanent teeth at the time of the first examination. The incidence of new caries in previously noncarious teeth treated with stannous chlorofluoride was reduced by about 85 percent more than the reduction in the children who received the sodium fluoride treatment. Through the use of this new fluoride, approximately the same degree of protection was obtained on surfaces in teeth that were noncarious at

Table 1. Comparison between topically applied stannous chlorofluoride and sodium fluoride on the dental caries reduction in children.

Group	No. of children	Initial examination		12 mo later		Reduction (%)	
		No. of noncarious teeth	Decayed, missing, or filled surfaces	Newly decayed, missing, or filled teeth*	Newly decayed, missing, or filled surfaces*	Teeth	Surfaces
NaF	397	5569	1869	245	281		
SnClF	394	5407	1846	32	46	86.9	83.5

* Compared with initial noncarious teeth or surfaces.