

# THE EFFECT OF NICOTINIC ACID AMIDE ON EXPERIMENTAL TUBERCULOSIS OF WHITE MICE

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## INTRODUCTION

**I**N SCREENING some two to three thousand chemicals against the tubercle bacillus on the chorioallantoic membrane of the chick embryo, we<sup>1</sup> found that 15 per cent of the compounds were inhibitory. Later all of these active chemicals were screened according to a technique that utilizes white mice. This technique was introduced by two Russian workers, Shpanier and Chertkova,<sup>2</sup> in 1944 and by Youmans and McCarter<sup>3</sup> in this country in 1945.

In the Russian report the mice were infected by an intravenous injection of 0.5 mg. of a 2-week-old culture of the human type of tubercle bacillus, H<sub>37</sub>Rv. They were treated with intramuscular injections of the chemical suspended in oil, receiving ten such injections in fifteen days. Evaluation of the results was made by culturing a suspension of the parenchymal organs on egg medium and after one month examining for macroscopic growth of the tubercle bacillus.

In this country Youmans and McCarter<sup>3</sup> developed the technique of producing experimental tuberculosis in mice and established a readily reproducible test. Thus they contributed a convenient in vivo method of screening a large number of compounds. They injected intravenously 0.1 mg. of a T.B. suspension made from a 3-week-old culture. Youmans<sup>4,5</sup> used this technique with considerable success in demonstrating the activity of streptomycin and later the activity of *para*-amino-salicylic acid.

## EXPERIMENTAL

Swiss mice\* weighing approximately 17 to 20 grams were used in our experiments. These were infected intravenously with 0.25 mg. of a T.B. suspension (H<sub>37</sub>Rv) made from a 14- to 18-day-old culture. Animals so infected and left untreated usually died in three to three and one-half weeks. Though there was no apparent loss of weight in the first one to two weeks, the animals lost weight in the latter stage of infection, and at the time of death they usually weighed 15 to 18 grams. For the most part, oral treatment of infected animals was begun on the day after inoculation. The compounds were administered in the diet. The diet consisted of ground Rockland mouse pellets.

*Nature of Infection.*—Though the bacteria were given intravenously, there was no generalized miliary tuberculosis. Instead the disease seemed to be centered mainly in the lungs; the other organs such as the liver, spleen, and

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Received for publication, June 26, 1948.

\*Obtained from Tumblebrook Farm, Brant Lake, N. Y.

TABLE I. TREATMENT OF EXPERIMENTAL TUBERCULOSIS OF WHITE MICE WITH DERIVATIVES OF NICOTINIC ACID

| CHEMICAL NUMBER | CHEMICAL NAME                               | PER CENT CHEMICAL IN DIET | AVERAGE BEGINNING WEIGHTS (GM.) | AVERAGE WEIGHTS (3 WK.) | PER CENT SURVIVAL | GROSS APPEARANCE OF EXCISED LUNG† | AMOUNT OF DISEASE SHOWN IN LUNG SECTION‡ | ACTIVITY OF TREATMENT CHEMICAL§ |
|-----------------|---|---------------------------|---------------------------------|-------------------------|-------------------|-----------------------------------|--|---------------------------------|
|                 | None (uninfected mice)                      | None                      | 19.4                            | 22.6                    | 100               | 0                                 | 0  |                                 |
| A217N           | Streptomycin*                               | None                      | 19.8                            | 20.1                    | 100               | 0                                 | ±-1                                      | Active                          |
| 606L            | Nicotinic acid amide                        | 0.75                      | 18.4                            | 21.0                    | 90                | 1                                 | 1  | Active                          |
|                 |   | 0.5                       | 18.4                            | 22.6                    | 100               | 1                                 | 2  | V. Sl. Active                   |
| 525L            | N-Nicotinyl-3-aminopyridine                 | 0.124                     | 19.1                            | 17.9                    | 70                | 2-3                               | 2-3                                      | V. Sl. Active                   |
| 526L            | N-(2-Thiazolyl)nicotinamide                 | 1.5                       | 19.0                            | 16.2                    | 60                | 2-3                               | 2  | V. Sl. Active                   |
| 528L            | N-(2-Pyrimidyl)nicotinamide                 | 0.25                      | 18.8                            | 16.1                    | 40                | 2                                 | 2-3                                      | V. Sl. Active                   |
| 738L            | N-Isopropylnicotinamide                     | 0.25                      | 19.8                            | 19.0                    | 10                | 2-3                               | 2  | V. Sl. Active                   |
| 732L            | N-Barythnicotinamide                        | 0.25                      | 19.0                            | 19.0                    | 10                | 2-3                               | 2-3                                      | V. Sl. Active                   |
| 644L            | N-γ-Piperidyl-propyl-nicotinamide           | 0.25                      | 19.0                            | 19.5                    | 50                | 2                                 | 2-3                                      | V. Sl. Active                   |
| 668L            | N-Nicotinyl derivative of inupure Mercaptan | 0.25                      | 19.5                            | 17.0                    | 0                 | 3                                 | 3  | V. Sl. Active                   |
| 637L            | N-Nicotinyl-1-aminoanthraquinone            | 0.25                      | 20.8                            | 19.3                    | 100               | 2                                 | 3  | None                            |
| 638L            | N-Cyclohexylnicotinamide                    | 0.25                      | 19.3                            | 17.6                    | 10                | 4                                 | 4  | None                            |
| 640L            | N-Dodecylnicotinamide                       | 0.25†                     | 19.6                            | 17.0                    | 20                | 4                                 | 4  | None                            |
| 642L            | 5-(Nicotinylamino)-2-methylcoumarone        | 0.25                      | 19.0                            | 19.0                    | 20                | 4                                 | 4  | None                            |
| 650L            | Nicotinylidicyandiamide                     | 0.25                      | 20.1                            | 19.0                    | 50                | 4                                 | 4  | None                            |
| 641L            | N-Nicotinylbenzylamine                      | 0.25                      | 20.1                            | 15.0                    | 0                 | 4                                 | 4  | None                            |
| 645L            | 4-(Nicotinylamino)-salicylic acid           | 0.25                      | 20.5                            | 20.2                    | 40                | 4                                 | 4  | None                            |
| 527L            | N-Nicotinyl-2-aminopyridine                 | 1.5†                      | 19.0                            | 17.0                    | 10                | 4                                 | 4  | None                            |
| 649L            | N-Nicotinyl-2-amino-5-azoisole              | 0.25                      | 20.2                            | 20.3                    | 50                | 4                                 | 4  | None                            |
| 652L            | 2-(Nicotinylamino)phenol                    | 0.25                      | 17.7                            | 20.5                    | 10                | 4                                 | 4  | None                            |
| 653L            | 3-(Nicotinylamino)phenol                    | 0.25                      | 17.8                            | 19.7                    | 10                | 4                                 | 4  | None                            |
| 654L            | 4-(Nicotinylamino)phenol                    | 0.25                      | 17.8                            | 20.2                    | 10                | 4                                 | 4  | None                            |
| 662L            | d,1-α-Nicotinylaminobutyric acid            | 0.25                      | 20.6                            | 17.1                    | 10                | 4                                 | 4  | None                            |
| 729L            | p-(Nicotinylamino)benzoic acid              | 0.25                      | 20.0                            | 20.3                    | 40                | 4                                 | 4  | None                            |
| 725L            | 2-(N-Nicotinylamino)-5-carbethoxythiazole   | 0.25                      | 18.5                            | 15.6                    | 0                 | 4                                 | 4  | None                            |
| 739L            | N-Methoxypropylnicotinamide                 | 0.25                      | 18.5                            | 16.4                    | 10                | 4                                 | 4  | None                            |
| 661L            | p-(Nicotinylamino)acetamide                 | 0.25                      | 20.4                            | 18                      | 20                | 4                                 | 4  | None                            |
| 747L            | 6-Chloronicotinamide                        | 0.25                      | 18.5                            | 13.5                    | 10                | 4                                 | 4  | None                            |
| 765L            |   |                           |                                 |                         |                   |                                   |  |                                 |
| or 770L         | 6-Aminonicotinamide                         | 0.11                      | 19.4                            | 16.1                    | 10                | 4                                 | 4  | None                            |
| 768L            | 6-Butoxynicotinamide                        | 0.25                      | 20.1                            | 16.5                    | 0                 | 4                                 | 4  | None                            |
| 724L            | 2-Aminonicotinamide                         | 0.25                      | 19.8                            | 16.5                    | 0                 | 4                                 | 4  | None                            |
| 749L            | Ethyl-nicotinate                            | 0.25                      | 19.0                            | 18.5                    | 10                | 4                                 | 4  | None                            |

All treatment chemicals were included in the diet at the maximum tolerated concentration. \*Instead of being administered in the diet, streptomycin was given subcutaneously, 1 mg. four times daily.

†Treatment concentration is toxic.

‡No gross lesions; no disease in the lungs. 4. Maximum amount of monocytic exudate. 4. Maximum amount of pathology observed. Microscopically, caseation and numerous acid fast bacilli. 3. 75 per cent of the maximum amount of pathology observed. 4. Maximum amount of pathology observed. 5. Maximum amount of pathology observed. 6. Maximum amount of pathology observed. 7. Maximum amount of pathology observed. 8. Maximum amount of pathology observed. 9. Maximum amount of pathology observed. 10. Maximum amount of pathology observed. 11. Maximum amount of pathology observed. 12. Maximum amount of pathology observed. 13. Maximum amount of pathology observed. 14. Maximum amount of pathology observed. 15. Maximum amount of pathology observed. 16. Maximum amount of pathology observed. 17. Maximum amount of pathology observed. 18. Maximum amount of pathology observed. 19. Maximum amount of pathology observed. 20. Maximum amount of pathology observed. 21. Maximum amount of pathology observed. 22. Maximum amount of pathology observed. 23. Maximum amount of pathology observed. 24. Maximum amount of pathology observed. 25. Maximum amount of pathology observed. 26. Maximum amount of pathology observed. 27. Maximum amount of pathology observed. 28. Maximum amount of pathology observed. 29. Maximum amount of pathology observed. 30. Maximum amount of pathology observed. 31. Maximum amount of pathology observed. 32. Maximum amount of pathology observed. 33. Maximum amount of pathology observed. 34. Maximum amount of pathology observed. 35. Maximum amount of pathology observed. 36. Maximum amount of pathology observed. 37. Maximum amount of pathology observed. 38. Maximum amount of pathology observed. 39. Maximum amount of pathology observed. 40. Maximum amount of pathology observed. 41. Maximum amount of pathology observed. 42. Maximum amount of pathology observed. 43. Maximum amount of pathology observed. 44. Maximum amount of pathology observed. 45. Maximum amount of pathology observed. 46. Maximum amount of pathology observed. 47. Maximum amount of pathology observed. 48. Maximum amount of pathology observed. 49. Maximum amount of pathology observed. 50. Maximum amount of pathology observed. 51. Maximum amount of pathology observed. 52. Maximum amount of pathology observed. 53. Maximum amount of pathology observed. 54. Maximum amount of pathology observed. 55. Maximum amount of pathology observed. 56. Maximum amount of pathology observed. 57. Maximum amount of pathology observed. 58. Maximum amount of pathology observed. 59. Maximum amount of pathology observed. 60. Maximum amount of pathology observed. 61. Maximum amount of pathology observed. 62. Maximum amount of pathology observed. 63. Maximum amount of pathology observed. 64. Maximum amount of pathology observed. 65. Maximum amount of pathology observed. 66. Maximum amount of pathology observed. 67. Maximum amount of pathology observed. 68. Maximum amount of pathology observed. 69. Maximum amount of pathology observed. 70. Maximum amount of pathology observed. 71. Maximum amount of pathology observed. 72. Maximum amount of pathology observed. 73. Maximum amount of pathology observed. 74. Maximum amount of pathology observed. 75. Maximum amount of pathology observed. 76. Maximum amount of pathology observed. 77. Maximum amount of pathology observed. 78. Maximum amount of pathology observed. 79. Maximum amount of pathology observed. 80. Maximum amount of pathology observed. 81. Maximum amount of pathology observed. 82. Maximum amount of pathology observed. 83. Maximum amount of pathology observed. 84. Maximum amount of pathology observed. 85. Maximum amount of pathology observed. 86. Maximum amount of pathology observed. 87. Maximum amount of pathology observed. 88. Maximum amount of pathology observed. 89. Maximum amount of pathology observed. 90. Maximum amount of pathology observed. 91. Maximum amount of pathology observed. 92. Maximum amount of pathology observed. 93. Maximum amount of pathology observed. 94. Maximum amount of pathology observed. 95. Maximum amount of pathology observed. 96. Maximum amount of pathology observed. 97. Maximum amount of pathology observed. 98. Maximum amount of pathology observed. 99. Maximum amount of pathology observed. 100. Maximum amount of pathology observed.

§None. No gross appearance of pathology. Microscopically, 1. Clear lung.

lymph nodes were but slightly involved. The infection in the lungs at the time of death was extensive, varying from discrete white nodular patches to extreme consolidation of whole lobes. Microscopically these nodular patches showed varying stages of consolidation, caseation, and monocytic and lymphocytic infiltration to a mere proliferation of tissue. Numerous clumps of acid-fast bacilli were observed throughout the tubercles.

**Chemotherapy.**—The apparent tuberculostatic activity of pyridine carboxylic acid on the chorioallantoic membrane of the chick embryo led us to repeat all of the active compounds in infected mice. For the most part, these compounds were fed in the diet. Of this group nicotinic acid and its amide seemed to be the most active compounds. Consequently, Kushner and co-workers<sup>6</sup> synthesized a series of thirty derivatives of nicotinamide either in the form of substituents in the acid amide group or as nuclear substitutions. These were tested in mice by the technique already described. The results are given in Table I, but the chemical synthesis and properties of these compounds are published elsewhere.<sup>6</sup> Nicotinamide was the most active chemical tested. All the changes introduced into the nicotinic acid amide molecule either increased the toxicity or reduced the activity or both.

The failure to increase the activity or even to retain the activity of nicotinic acid amide by any slight alteration in the molecule led us to suspect that we might be dealing with the specific activity of a vitamin. Calculating from our results with mice, on a weight basis the therapeutic dose in human beings would be about 100 to 125 grams—a dose which could not be tolerated by the human subject. So other vitamins were tried at 0.1 per cent concentration in the diet with or without a smaller amount of nicotinic acid amide. Of these, only riboflavin seemed to have a slight effect in securing the desired end of reducing the total treatment dose of the nicotinic acid amid. See Table II.

In addition, possible naturally occurring precursors of nicotinic acid amide, such as tryptophane and 3-hydroxy-anthranilic acid, were attempted as chemotherapy, but all yielded negative results.

**Resistance of the Tubercle Bacilli to Nicotinic Acid Amide.**—One of the possible disadvantages of streptomycin in the treatment of human tuberculosis is that the organisms become resistant to streptomycin in the course of time. Similar resistance studies were made in regard to the possibility of the organisms' becoming resistant to the nicotinic acid amide. The organisms were recovered from the mouse previously treated with nicotinic acid amide and cultured on egg medium. The bacterial growth was resuspended and used for infecting the test mice. Five mouse passages showed little evidence of resistance of the tubercle bacilli, but later passages showed some evidence of resistance.

However, when the suspension of the lung excised from the mouse that had been treated with a combination of nicotinic acid amide and streptomycin was placed on egg medium, such a sparse growth appeared after a prolonged period of incubation that we believe a very much more reduced number of viable organisms existed in the lungs than when streptomycin was administered alone.

TABLE II. EFFECT OF TREATING EXPERIMENTAL TUBERCULOSIS OF WHITE MICE WITH NICOTINIC ACID AMIDE IN COMBINATION WITH DIFFERENT VITAMINS

| VITAMIN  | PER CENT NICOTINIC ACID AMIDE IN DIET | PER CENT ADDITIONAL VITAMIN IN DIET | AVERAGE BEGINNING WEIGHTS (GM.) | AVERAGE WEIGHTS (3 WK.) | PER CENT SURVIVAL (10 MICE PER GROUP) | GROSS APPEARANCE OF LUNG AT AUTOPSY† | AMOUNT OF DISEASE IN LUNG SECTION† | ACTIVITY‡     |
|--|---------------------------------------|-------------------------------------|---------------------------------|-------------------------|---------------------------------------|--------------------------------------|------------------------------------|---------------|
| None   |                                       |                                     | 17.6                            | 24.1                    | 100                                   | 0                                    |                                    |               |
| Streptomycin*                                    | None                                  | None                                | 17.8                            | 23.2                    | 100                                   | 0                                    | ±1                                 | Active        |
| Streptomycin* plus nicotinic acid amide          | 0.75                                  | None                                | 19.2                            | 21.6                    | 100                                   | 0                                    | ±1                                 | Active        |
| Nicotinic acid amide                             | 0.25                                  | None                                | 18.4                            | 20.9                    | 80                                    | 2                                    | 2-3                                | V. Sl. Active |
| Nicotinic acid amide                             | 0.5                                   | None                                | 18.4                            | 22.6                    | 100                                   | 1                                    | 2                                  | Sl. Active    |
| Riboflavin                                       | None                                  | 1                                   | 19.0                            | 18.8                    | 0                                     | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus riboflavin             | 0.25                                  | 0.1                                 | 20.2                            | 20.9                    | 50                                    | 2                                    | 2-3                                | V. Sl. Active |
| Nicotinic acid amide plus riboflavin             | 0.25                                  | 0.5                                 | 19.2                            | 21.2                    | 100                                   | 0                                    | 1-2                                | Active        |
| Nicotinic acid amide plus calcium pantothenate   | 0.1                                   | 0.1                                 | 19.7                            | 17.9                    | 0                                     | 3-4                                  | 4                                  |               |
| Nicotinic acid amide plus calcium pantothenate   | 0.25                                  | 0.5                                 | 19.0                            | 20.8                    | 30                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus para-aminobenzoic acid | 0.1                                   | 0.1                                 | 19.7                            | 18.7                    | 0                                     | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus para-aminobenzoic acid | 0.25                                  | 0.1                                 | 19.7                            | 18.9                    | 30                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus inositol               | 0.1                                   | 0.1                                 | 18.8                            | 17.9                    | 20                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus inositol               | 0.25                                  | 0.1                                 | 18.8                            | 17.0                    | 10                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus choline chloride       | 0.1                                   | 0.1                                 | 18.9                            | 16.0                    | 20                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus choline chloride       | 0.25                                  | 0.1                                 | 18.8                            | 17.6                    | 30                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus ascorbic acid          | 0.1                                   | 0.1                                 | 20.4                            | 20.3                    | 30                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus ascorbic acid          | 0.25                                  | 0.1                                 | 20.4                            | 20.5                    | 80                                    | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus mixed tocopherol       | 0.25                                  | 0.1                                 | 18.4                            | 16.4                    | 0                                     | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus vitamins A and D       | 0.25                                  | A: 450 u./day<br>D: 108 u./day      | 18.6                            | 16.2                    | 0                                     | 4                                    | 4                                  | None          |
| Nicotinic acid amide plus vitamins A and D       | 0.1                                   | A: 450 u./day<br>D: 108 u./day      | 18.9                            | 15.2                    | 0                                     | 4                                    | 4                                  | None          |
| None (untreated tuberculous mice)                |                                       |                                     | 19.2                            | 15.6                    | 0                                     | 4                                    | 4                                  |               |

†All treatment chemicals were included in the diet at the maximum tolerated concentration.

\*See footnote 2, Table I.

†See footnote 3, Table I.

‡See footnote 4, Table I.

## SUMMARY

The oral administration of 0.5 to 0.75 per cent of nicotinic acid amide in the diet will markedly suppress the spread of tuberculosis in experimentally infected mice.

This amount of nicotinic acid amide in the diet was apparently roughly equivalent in activity to injections of 1 mg. of streptomycin four times daily over the same test period.

We wish to express our appreciation to Miss Florence Anderson, Miss Barbara Gosford, Mr. Samuel Smith, and Miss Hester Snider for their technical assistance.

## REFERENCES

1. McKenzie, D., Vincent, N., Stadler, M., James, E., Kinley, E., Rogers, Y., and SubbaRow, Y.: Unpublished data.
2. Shpanier, F. L., and Chertkova, E. I.: Chemotherapeutic Properties of Some Amides of the  $\alpha$ -furan Carbonic Acid in Tuberculosis, Problems of Tuberculosis No. 4, p. 9, 1944.
3. Youmans, G. P., and McCarter, J. C.: A Preliminary Note on the Effect of Streptomycin on Experimental Tuberculosis of White Mice, Quart. Bull., Northwestern Univ. M. School 19: 210, 1945.
4. Youmans, G. P.: The Effect of Streptomycin in Vitro on M. Tuberculosis var. Hominis, Quart. Bull. Northwestern Univ. M. School 19: 207, 1945.
5. Youmans, G. P.: The Effect of Para-amino-salicylic Acid in Vitro and in Vivo on Virulent Human Type Tubercle Bacilli, Quart. Bull. Northwestern Univ. M. School 20: 420, 1946.
6. Kushner, S., et al.: Experimental Chemotherapy of Tuberculosis. I. Substituted Nicotinamide. In press.