Public Health Significance of Tubercle Bacilli Resistant to Isoniazid

DANIEL WIDELOCK, Ph.D., F.A.P.H.A.; LENORE R. PEIZER, F.A.P.H.A.; and SARAH KLEIN

The propensity of the tubercle bacillus to acquire drug resistance remains at least a potential threat to public health control measures. Hence, the laboratory research reported here may have as much of interest to the epidemiologist and others in the tuberculosis control field as to the laboratory workers to whom it was addressed.

Conflicting evidence has accumulated in the literature concerning the significance of isoniazid-resistant tubercle bacilli. Our concern with regard to these organisms was increased following the establishment by the New York City Department of Health of a policy of treating the unhospitalized patient with isoniazid.[1, 2] We had to determine the extent of the risk involved in the spreading of these resistant organisms by the unhospitalized patients to their contacts. We did not understand the role played by the isoniazid-resistant tubercle bacilli in the maintenance and spread of the active lesions in the individual patient. In other words, are tubercle bacilli resistant to isoniazid, virulent or avirulent?

The available evidence is contradictory. In vitro experiments, conducted in our laboratory, clearly demonstrated that pure cultures of resistant tubercle bacilli may regain their sensitivity, as well as their virulence for guinea pigs, if they are grown for several generations in a drug-free medium.[3]

The host employed in the various studies with regard to the virulence of isoniazid-resistant tubercle bacilli appears to influence the conclusions of the investigators. That resistant tubercle bacilli are virulent was the opinion of a number of individuals[4-7] when the mouse was employed as the laboratory animal. However, at least one report[8] indicated that isoniazid-resistant tubercle bacilli did not cause progressive disease in mice.

There seems to be a definite loss of virulence of the isoniazid-resistant tubercle bacilli if the guinea pig is the host.[7, 9-11] However, it is worthy of note that the resistant organisms do multiply in the guinea pig, though no progressive disease is usually produced, and that these organisms are probably eventually eliminated. In a previous paper[11] we noted that guinea pigs inoculated with the INH-resistant organisms became tuberculin-positive within four to 16 weeks and that local abscesses were noted in at least some of the guinea pigs. These local abscesses contained tubercle bacilli which retained the same degree (100 μg/ml.) of resistance to isoniazid as was present in

Dr. Widelock is assistant director; Miss Peizer, senior bacteriologist; and Miss Klein, bacteriologist, City Department of Health, Bureau of Laboratories, New York, N. Y.

This paper was presented before the Laboratory Section of the American Public Health Association at the Eighty-second Annual Meeting in Buffalo, N. Y., October 12, 1954.

the original inoculum. However, with the exception of guinea pigs which developed an enteritis and died, no progressive tuberculosis developed. Moreover, the tuberculin-positive guinea pigs eventually (in about 30 weeks) reverted to tuberculin-negative. What is worthy of note was the fact that the resistant organisms in the guinea pigs which developed a concomitant disease (enteritis) multiplied and produced tuberculous lesions in the lungs, spleen, and liver. Subcultures from these tuberculous lesions produced cultures of tubercle bacilli resistant to 100 μg of INH/ml. of media which was equivalent to the degree of resistance present in the original inoculum. We must therefore not ignore the thought that though resistant tubercle bacilli have seemingly lost their virulence for guinea pigs, that these organisms will multiply and produce tuberculous lesions under the proper conditions, such as concomitant enteritis.

It was previously determined that [12] if resistance to isoniazid develops in a patient, these resistant organisms will continue to multiply and the bacteriologic picture will worsen. In general, it was noted [1] that some correlation existed between the development of resistant organisms in patients and nonimprovement by both x-ray and bacteriologic standards when the patient was continued under isoniazid therapy.

The New York City Department of Health is interested in determining the extent of the spreading of these isoniazid-resistant organisms in the community by the unhospitalized patient undergoing isoniazid therapy. This will be the subject of a future report. In view of the laboratory observation that the resistant tubercle bacilli will produce lesions in guinea pigs with a concomitant disease, and because the sputa of patients with resistant organisms will usually also contain varying proportions of sensitive tubercle bacilli, we attempted to determine the role of the resistant organism: (1) when mixed with virulent tubercle bacilli and inoculated into normal animals, and (2) when injected into guinea pigs which previously had been inoculated with virulent tubercle bacilli.

It was hoped that we would thus obtain information which might be of use in interpreting our findings with regard to the possible spreading of resistant organisms by the unhospitalized patient.

Method

Experiment A—A single strain, No. 859, of tubercle bacilli virulent for mice and guinea pigs and sensitive to 0.1 μg INH/ml. of media, was grown on INH-containing Proskauer-Beck medium until the organisms became resistant to 100 μg INH/ml. The 100 μg/ml.-resistant organisms were then transplanted three times on media containing 100 μg/ml. The third transplant became the source of the resistant organisms used in the experiment requiring mixtures of resistant and sensitive organisms. The sensitive organism was the original strain, No. 859, which was isolated from a patient in our clinic.

The inoculum was prepared by suspending the growth of either the resistant or sensitive organisms in a phosphate buffer, shaking the suspensions with glass beads for one hour, and then adjusting the turbidity to equal the No. 1 tube of the MacFarland nephelometer (3×10^8 organisms). Mixtures of the resistant and sensitive suspension were then prepared in proportions as indicated in the protocol (Table 1).

Mice were inoculated intravenously with 0.1 ml. of the inoculum; guinea pigs with 0.25 ml. into the inguinal area.

Experiment B—Suspensions of 10 virulent and sensitive strains of tubercle bacilli isolated from 10 different clinic patients were each diluted to equal the No. 1 tube of the MacFarland nephelo-

Table 1—Guinea Pigs and Mice Injected with Mixtures of Varying Proportions of INH-Sensitive and INH-Resistant (100 ug/ml.) Tubercle Bacilli. Lesions Produced Were Cultured and the Isolated Tubercle Bacilli Tested for Sensitivity to Isoniazid

Inoculum Mixture	Guinea Pigs *			Mice		Sensitivity to μg/ml. Isoniazid of Tubercle Bacilli Isolated from Lesions	
						Guinea Pig † μg/ml.	Mouse † μg/ml.
	No. of animals	Tuberculin test following injection	Death in weeks with tuberculous lesions	No. of animals	Death in days with tuberculous lesions	Control 0.1 1.0 5.0 10.0 25.0 50.0 100.0	Control 0.1 1.0 5.0 25.0 50.0 100.0
100% sensitive	5	Positive	10, 13, 16 (2 died of enteritis)	10	16, 20, 21, 22, 26 26, 42, 46, 49, 56	4+ 0 0 0 0 0 0 0	4+ 0 0 0 0 0 0
100% resistant	5	Positive	None after 5 months	10	None after 5 months	..	..
50% resistant + 50% sensitive	5	Positive	5, 6, 7, 11, 16	10	24, 24, 24, 24, 26, 28, 38, 42 (2 alive after 5 months)	4+ 0 0 0 0 0 0 0	4+ 1+ 1+ 1+ 1+ 1+ 1+
90% resistant + 10% sensitive	5	Positive	5, 7, 7, 13, 14	10	29, 29, 29, 29, 36, 79, 108 (3 survived after 5 months)	4+ 20 0 0 0 0 0 0	4+ 2+ 2+ 2+ 2+ 2+ 2+
98% resistant + 2% sensitive	5	Positive	13, 13, 16 (sacrificed 17, 17—lesions in liver, spleen, and inguinal nodes)	10	56, 115 (8 alive after 5 months)	4+ 0 0 0 0 0 0 0	4+ 1+ 1+ 1+ 1+ 1+ 1+

* Guinea pigs were tuberculin-negative before injections.
† Control tube does not contain any INH. Culture growth reported as 4+, 3+, 2+, 1+, <1, 0.

meter. The virulent and sensitive organisms were injected into different guinea pigs (0.25 ml. subinguinal).

Twenty-five days later (seven days after becoming tuberculin-positive) the guinea pigs were reinjected with various strains of tubercle bacilli, each resistant to 100 μg INH/ml. media. Normal guinea pigs were inoculated at the same time with the same resistant organisms.

In both Experiments A and B, after autopsying the animals, the lesions were cultured, and the tubercle bacilli isolated were tested for sensitivity to isoniazid.

Results

Experiment A

The purpose of this experiment was to determine if resistant organisms would produce tuberculous lesions in guinea pigs and mice if the inoculum also contained sensitive organisms. The salient points indicated in Table 1 were that:

1. All guinea pigs became tuberculin-positive.

2. All tuberculous lesions of guinea pigs consisted of only INH-sensitive

tubercle bacilli. No resistant organisms were in any of the lesions, even when the original inoculum was a mixture of 98 per cent INH-resistant and only 2 per cent INH-sensitive tubercle bacilli.

3. In mice, some resistant organisms took part in the formation of the tuberculous lesions, though the sensitive organisms were by far more predominant, even when the inoculum consisted of 98 per cent resistant and 2 per cent sensitive tubercle bacilli.

Experiment B

In this experiment the guinea pigs were first injected with 10 different sensitive strains of tubercle bacilli and one week after the animals became tuberculin-positive (25 days after original injection) were reinjected with 10 resistant strains. By this means we attempted to determine if the resistant organisms would establish themselves in the animal which had previously been exposed to the sensitive bacilli.

No resistant organisms were isolated from tuberculous lesions of the autopsied guinea pigs. All normal guinea pigs became tuberculin-positive following the injection by the resistant organisms.

Discussion

Our primary concern with regard to the isoniazid-resistant tubercle bacilli is the determination of their importance in the epidemiology of the disease, as well as their relationship to the active lesion in a patient.

The results of the experiments described in this report accentuate the doubt concerning the virulence of the isoniazid-resistant organisms. When comparatively few (2 per cent) of the inoculum were the isoniazid-sensitive organisms, and 98 per cent consisted of the resistant organism, the latter played a minor, if any, part in the formation of the tubercular lesions. All the organisms in guinea pig lesions and the bulk of the organisms in the mouse lesions were isoniazid-sensitive. Resistant organisms could not be found in the tissues even when the guinea pigs were first given isoniazid-sensitive organisms and were injected with the resistant strains after the animals were converted to tuberculin-positive.

Although the results point toward a loss of virulence for guinea pigs and to a lesser degree for mice, we cannot, as yet, draw definite conclusions with regard to the human host. It must be remembered that the resistant organisms did produce lesions in guinea pigs suffering from a concomitant disease (enteritis); that these organisms do revert to isoniazid-sensitive in vitro if grown in drug-free media; and that patients as a rule worsen bacteriologically once these organisms appear.

It is possible, of course, that these resistant organisms play no part in the maintenance and spread of tubercular lesions in a patient. If that is true then the bacteriologic relationship to non-improving patients should be a consistant increase or, at least maintenance of the isoniazid-sensitive organisms, even when isoniazid-resistant organisms are being produced. In other words, the sensitive organisms (or perhaps partially resistant ones) are producing the lesions and these organisms are not eliminated by the drug. They multiply as sensitive organisms and maintain the active disease, and at the same time resistant organisms are produced which play no part in the formation of new lesions.

To prove this would require a correlation between clinical findings and a quantitative study of resistant and sensitive tubercule bacilli in sputum of patients. We are at present undertaking this experiment by means of the quantitative catalase test,[13] which has proved of value in rapidly identifying the percentage of resistant and sensitive tubercle bacilli in a culture.[14] We hope by

this means to study and report the relationship between the clinical findings on the one hand and the resistant-sensitive correlation on the other.

Summary and Conclusion

1. Guinea pigs and mice were injected with mixtures of isoniazid-resistant and isoniazid-sensitive tubercle bacilli. The inoculum consisted of 100 per cent sensitive organisms; 50 per cent sensitive and 50 per cent resistant; 90 per cent resistant and 10 per cent sensitive; 98 per cent resistant and 2 per cent sensitive. After autopsy of the animals, the lesions were cultured and the tubercle bacilli tested for isoniazid sensitivity.

2. Guinea pigs were injected with sensitive strains of tubercle bacilli; 25 days later (seven days after reverting to tuberculin-positive) they were reinjected with isoniazid-resistant tubercle bacilli. After autopsy the tubercle bacilli isolated were tested for sensitivity to isoniazid.

3. The results indicate that the resistant tubercle bacilli play little, if any part, in the formation of tubercles in the guinea pigs or mice under the conditions of the experiments described.

4. The possible significance of the isoniazid-resistant organisms is discussed.

REFERENCES

1. Robins, A. B.; Abeles, H.; Chaves, A. D.; Arønsohn, H. H.; Breuer, J.; Widelock, D.; and Peizer, L. The Unhospitalized Tuberculosis Patient. Am. Rev. Tuberc. 69:26 (Jan.), 1954.
2. Widelock, D., and Robins, A. B. Laboratory Aspects of Tuberculosis in the Ambulatory Patient. A.J.P.H. 44:794 (June), 1954.
3. Unpublished data.
4. Goulding, R.; King, M. B.; Knox, R.; and Robson, J. Relation Between in vitro and in vivo Resistance to Isoniazid. Lancet 2:69 (July), 1952.
5. Karlson, A. G., and Ikemi, Y. Effect of Isonicotinic Acid Hydrazide (Isoniazid) on Survival Time of Mice Infected with Tubercle Bacilli Resistant to the Drug in vitro. Proc. Staff Meet. Mayo Clin. 27:373 (Sept.), 1952.
6. Bloch, H.; Widelock, D.; and Peizer, L. R. Susceptibility to Isoniazid and Pathogenicity of Tubercle Bacilli. Am. Rev. Tuberc. 68:734 (Nov.), 1953.
7. Morse, W. C.; Weiser, O. L.; Kuhns, D. M.; Fusillo, M.; Dail, M. C.; and Evans, J. R. Study of the Virulence of Isoniazid-Resistant Tubercle Bacilli in Guinea Pigs and Mice. Am. Rev. Tuberc. 69:464 (Mar.), 1954.
8. Grunberg, E. Minutes of the Meeting of the American Association for the Advancement of Science, St. Louis, 1952.
9. Steenken, W., and Wolinsky, E. Virulence of Tubercle Bacilli Recovered from Patients with Isoniazid. Am. Rev. Tuberc. 68:554 (Oct.), 1953.
10. Peizer, L. R.; Widelock, D.; and Klein, S. Virulence in Guinea Pigs of Isoniazid-Resistant Cultures Isolated from Clinical Specimens. Ibid. 68:290 (Mar.), 1953.
11. Peizer, L. R.; Minken, A.; and Widelock, D. A Further Study of Virulence in Guinea Pigs of INH-Resistant Tubercle Bacilli Isolated from Clinical Material. Ibid. (To be published.)
12. Widelock, D.; Peizer, L. R.; and Dangler, C. Laboratory Diagnostic Procedures Employed on Sputum Specimens from Ambulatory Tuberculous Patients Treated with Isoniazid. Ibid. 2:349 (Aug.), 1954.
13. Peizer, L. R., and Widelock, D. A Colorimeter Quantitative Test for Measuring Catalase Activity of Cultures of M. tuberculosis. (To be Published.)
14. ————. The Relationship of Degree of Catalase Activity to Isoniazid Sensitivity and Guinea Pig Virulence of Primary Cultures of M. tuberculosis. (To be published.)

VA Clinical Psychologist Examinations

The U. S. Civil Service Commission announces an examination to fill clinical psychologist positions, including a few speech pathologist positions, in Veterans Administration hospitals and offices throughout the country. Annual salaries range from $5,940 to $10,800.

No written test is required. Applicants will be rated on the basis of evaluation of their personal and professional qualifications.

Applications, being accepted until further notice, must be filed with the Board of Civil Service Examiners, Veterans Administration, Washington 25, D. C. Further information and application forms may be obtained from post offices or from the U. S. Civil Service Commission, Washington 25, D. C.