TABLE 1

OCCLUSION RESPONSE AND EXTRACTABLE COPPER AND ZINC IN LOUISIANA-MISSISSIPPI SOIL MATERIALS*

Occlusion response	Per cent of Materials Examined	Extractable Cu, ppm		Extractable Zn, ppm	
		Range	Average	Range	Average
a. Both Cu and Zn occluded in presence of each other	15	0–150	19	11–150	59
b. Cu occluded in presence of Zn; Zn not occluded in presence or in absence of Cu	40†	0–7	1	1–63	13
c. Neither Cu nor Zn occluded singly or in presence of each other	45	0–1	0.2	0–24	4

* The occlusion test consists of the following operations: (*a*) 50 g of air-dry soil shaken in a 200-u-stoppered bottle with 100 ml of a solution containing 20 ppm Cu^{++} and 20 ppm Zn^{++} in 0.01 *N* HCl and allowed to settle; (*b*) supernatant liquid is withdrawn, filtered, if necessary, and tested for Cu^{++} and Zn^{++} by the dithizone method. pH of the system is maintained between 5 and 7.5 by HCl or NH_4OH. The appropriate blanks are run concurrently. With soils occluding both Cu^{++} and Zn^{++}, the blanks are scarcely required. Such soils, in fact, can be used to remove traces of the two metals from distilled water and reagents, including ammonia.

† One-fifth of these materials showed a small or a doubtful capacity to occlude Zn.

is shown in Table 1, and is as follows. The greater the amounts of HCl-extractable Cu and Zn in the soil, the more likely is the soil to occlude additional small amounts of Cu and Zn. This crude generalization suggests merely presence or absence of certain mechanisms responsible for the reaction. Quantitative studies of a "Cu-Zn capacity" or some such possible characterization of our materials are deferred. Empirically, however, the test has already shown some value in the field, as may be illustrated by a summary of Table 1.

In this table, materials believed to be representative of the area studied are grouped in three categories: (*a*) Occluders of both Cu and Zn, singly and in the presence of each other. These materials include freshly deposited Mississippi River sediments, some midden soils near Sicily Island, Louisiana, buried middens in the delta south of New Orleans, deeper horizons of natural levees at Mauvais Bois, Point au Chien, and Carlysle, La. Their texture ranges from silty sand to clay and their calcium carbonate content is highly variable. (*b*) Occluders of Cu but not of Zn. This prominent group contains mature loess profiles, to 100 in. depth or so, near Natchez, Miss., leached and unleached loess near Doloroso, Miss., some artificial levee horizons (in place for 75 years or so), and some topsoil horizons from Poverty Mound, in the Arkansas River area. Their texture and $CaCO_3$ range are like those in the preceding group. (*c*) Occluders of neither Cu nor Zn. This most numerous group includes soil profiles on Red River deposits, degraded loess, "brown loam" soils on the older Pleistocene Mississippi River terrace, mature soils on the Prairie terrace (late Pleistocene), the lower Atchafayalla backswamp clay, materials from Teche I channel, most of the Poverty Mound Traverse, leached clays near Sicily Island, La., profiles near Marksville, La., both on made ground and natural buried soil, and senile soils on early Pleistocene and pre-Pleistocene materials throughout the area.

It may be possible to make use of the occlusion test in the identification of sediments in areas where geomorphologic-geologic data alone are insufficient for the purpose, especially in the Recent geologic areas in the delta. This possibility, if sustained, may be realized right in the field. All equipment and reagents for the test are easily portable and the test itself requires only a few minutes. It is my hope to ascertain further validity of the test in the coming season, all other things being favorable.

A tentative view of the significance of the observations here stated may be as follows. The Cu-Zn occluding mechanisms are very common in Recent materials of Mississippi River origin but not in the Red River sediments and, perhaps, not in the Arkansas River sediments. These mechanisms may deteriorate when the sediments containing them are exposed to subaerial pedogenesis. The zinc-occluding mechanisms tend to deteriorate and disappear, in the Recent, far more rapidly than the copper-occluding mechanisms. Loessification of sediments tends either to conserve or to produce the copper-occluding mechanisms.

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Some Observations on the Pathogenicity of Isoniazid-Resistant Variants of Tubercle Bacilli[1]

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It has been demonstrated (*1*) that the incidence of variants of tubercle bacilli resistant to isoniazid (INH) is even higher than is the case with streptomycin—a most disappointing observation in terms of what one could predict at that time concerning its usefulness in the treatment of tuberculosis. Since then we have had the opportunity of investigating more intensively the properties of these INH-resistant mutants both in the experimental laboratory and in the clinic.

The Vallée strain (bovine) and the H37Rv strain (human) were exposed to isoniazid on the oleic acid

[1] Presented before the Colorado Trudeau Society April 18, 1953.

TABLE 1

PATHOGENICITY OF TUBERCLE BACILLI IN SPUTUM CONCENTRATES FROM PATIENTS TREATED WITH INH FOR AT LEAST 2 MONTHS

No. of Cases	Culture results		Pathogenicity for normal guinea pigs
	Primary isolation	Subcultures from animals	
4	*S* INH $<$ 1 μg/ml OA+; ATS+	*S* INH $<$ 1 μg/ml	Typical generalized visceral tuberculosis
9	*R* INH $>$ 1 μg/ml OA+; ATS+	*R* INH $>$ 10 μg/ml (2 cases) *R* INH $>$ 1 μg/ml (5 cases) Partially sensitive to 1 μg INH/ml (2 cases)	Local abscesses; enlarged lymph nodes; smears positive for AFB
5	*R* INH (not known) OA−; ATS+	Negative cultures from abscesses and spleens	Local abscesses; enlarged lymph nodes; smears positive for AFB
3	*R* INH (not known) OA−; ATS±	Negative cultures from abscesses and spleens	Local abscesses; normal to slightly enlarged lymph nodes; smears positive for AFB

Note. *S*, sensitive; *R*, resistant; OA, unmodified oleic acid albumin agar medium; ATS, American Trudeau Society egg yolk potato medium; +, growth; −, no growth; ±, growth rare or absent; AFB, acid-fast bacilli.

albumin solid medium (OA medium), and variants, each resistant to 10 μg of INH/ml of medium, were isolated and subcultured through three passages in medium containing 10 μg of INH/ml. Then these organisms were tested for their pathogenicity by injection into each of two normal guinea pigs intravenously in a dose of 1 cc of undiluted Tween-albumin culture. The parent isoniazid-sensitive strains of H37Rv and Vallée were also used at the same time to infect each of two guinea pigs with the same numbers of living bacterial cells. The two guinea pigs injected with the isoniazid-sensitive Vallée strain died, as expected, at 12 and 19 days after infection. The companion animals infected with the strain resistant to more than 10 μg of INH/ml survived for 33 and 43 days respectively; the animal surviving for 33 days died of an unknown cause and with only minimal evidence of active tuberculosis; very few acid-fast rods were visible in a stained smear of its lungs. The animal which died on the 43rd day after infection probably died of tuberculosis, although there is no doubt that this isoniazid-resistant bovine strain had suffered striking loss of pathogenicity. The two guinea pigs injected with the INH-sensitive H37Rv strain died on 19 and 25 days respectively, of extensive tuberculosis. Yet, their companions infected with the INH-resistant H37Rv strain are still living now at 60 days. Thus, the INH-resistant Vallée strain was at least partially attenuated compared with its parent INH-sensitive strain, whereas the INH-resistant H37Rv strain is revealed to be markedly attenuated, if not completely avirulent, when compared with the parent INH-sensitive H37Rv strain. These experiments indicated that these strains of tubercle bacilli may become partially or completely attenuated for the guinea pig when they become resistant to 10 μg of INH/ml of OA or Tween-albumin liquid medium, under experimental laboratory conditions.

Studies have subsequently been made on tubercle bacilli freshly isolated from patients treated for at least 2 months with INH alone or INH plus streptomycin and other chemotherapeutic agents.

Eleven different strains resistant to at least 1 μg of INH/ml of OA medium were isolated from as many patients. These strains were first isolated on medium containing 1 μg of INH/ml and then subcultured only once or twice in Tween-albumin liquid medium without INH and used to infect guinea pigs; two guinea pigs were injected intravenously with 1 cc of fully grown undiluted Tween-albumin culture. Subcultures were made from the lungs and spleens of animals that died in less than 60 days after injection, and on all animals that were still surviving and were, therefore, sacrificed at 60 days. The organisms so isolated were tested for their resistance to 1 and 10 μg of INH/ml, and, in some cases, to higher concentrations of INH. Three of these strains proved to consist exclusively of populations completely resistant to 10 μg of INH/ml of OA medium; and the guinea pigs infected with these strains survived for 60 days. At necropsy no evidence of tuberculosis was discovered in these guinea pigs. Seven of these strains consisted of populations resistant to 1 μg, but partially or completely sensitive to 10 μg, of INH/ml; all guinea pigs infected with these strains died within 60 days after challenge. One strain proved to be sensitive to 1 μg of INH/ml; and the guinea pigs infected with this strain died at 17 and 27 days of generalized tuberculosis. The results of this preliminary survey have led us to the conclusion that resistance of human type tubercle bacilli to 1 μg of INH/ml of medium is not always associated with a significant degree of attenuation, but that, on the other hand, resistance to 10 μg or more of INH/ml of OA medium may be accompanied by marked loss of pathogenicity for normal guinea pigs.

In addition to these observations, carried out under relatively controlled conditions, we have made the following observations with primary sputum concentrates

from patients who had previously been treated with INH for at least 2 months. Twenty-one sputum concentrates, all of which were positive on smear for acid-fast rods, were inoculated onto American Trudeau Society egg medium, onto OA medium, and into guinea pigs, two guinea pigs for each specimen. These injections were made into the groin by the classical method. Table 1 summarizes the results obtained from the study of these 21 sputum concentrates. It will be noted that 4 of these strains proved to be pathogenic, growing on both the OA and the ATS media and causing extensive visceral tuberculosis within 2 months after infection; subcultivation and primary testing for sensitivity to INH proved them to be invariably sensitive to 10 μg of INH, and completely or partially sensitive to 1 μg of INH. The remaining 17 strains caused little or no tuberculosis to develop in the guinea pigs within the 2-month period before sacrifice. The tuberculosis which did develop consisted of local abscesses at the sites of injection and occasional necrotic lymph nodes draining these sites. Subcultivation from these lesions revealed either no cultivatible bacterial cells or populations consisting predominantly of tubercle bacilli resistant to more than 1 μg of INH. Of some interest is the fact that all these local lesions at the sites of inoculation contained acid-fast rods demonstrable on direct smear.

Of special significance for our present studies is our unexpected observation that 8 of these nonpathogenic strains of tubercle bacilli failed to grow on the unmodified OA medium, although 5 of them gave good growth on the ATS medium. The 3 remaining strains, which failed to grow on either OA medium or the ATS medium, and which did not produce progressive disease in guinea pigs are of particular interest to us. All 3 of these patients persistently cough up acid-fast rods which are invariably present on direct smear and present in enormous numbers in sputum concentrates. There is little doubt in our minds that these acid-fast rods must derive from a multiplying population in their respiratory tracts—in all likelihood in one or more of the cavities which are visible by x-ray in these patients. Cultures of tubercle bacilli were readily isolated on egg medium from these patients before INH therapy was initiated. It seems possible that these observations are related, at least in part, to Fisher's observation (*2*) that a variant of the H37Rv strain, resistant to more than 10 μg of INH/ml of medium, has growth requirements different from the parent INH-sensitive H37Rv strain.

We wish to emphasize that these data refer only to pathogenicity of tubercle bacilli for normal guinea pigs, because, as yet, we have no direct, conclusive evidence that these INH-resistant strains of tubercle bacilli are equally nonpathogenic for normal human tissue. Indeed, it is already clear that these organisms can proliferate in open cavities in human lungs. Thus, it would appear that with the development of resistance to the antimicrobial effects of INH, tubercle bacilli acquire an inability to initiate multiplication in normal, non-necrotic areas of guinea pig tissue.

As is well known, tubercle bacilli multiply in lung cavities in association with many autolyzing leucocytes. Therefore, it is tempting to postulate that most strains of tubercle bacilli which are resistant to more than 10 μg of INH/ml of artificial medium have a special growth requirement for a substance (or substances) which, on the one hand, is present but bound and unavailable in normal tissue, but, on the other hand, free and available to these fastidious strains in necrotic tissue. This substance (or substances) is evidently present in moderate but not always sufficient amount in egg yolk media, and is present in much smaller quantities in the OA medium.

We would like to point out and warn that the population of tubercle bacilli which appears in the sputum of an INH-treated patient, may, and often does, consist of mixed populations of organisms with various degrees of resistance to INH and, thus, of mixed populations with varying degrees of pathogenicity. Also, reversion of strains from INH-resistance and nonpathogenicity for normal tissue to INH-sensitivity and pathogenicity has already occurred in our laboratory on repeated subcultivation in medium deficient in the special growth factor(s) to which we have already referred. The observations reported here have many implications for the future with respect to the diagnosis, treatment, and epidemiologic control of tuberculosis. These are beyond the scope of this paper.

References

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Radioactive Measurement of Proteolytic Activity

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Proteolytic enzymes play a vital role in many physiological and pathological phenomena of the body. Not only are they essential for normal every day economy, but abnormalities in their concentration have been believed to be the cause of many pathologic states. Among these are: pancreatitis (*1, 2*), obstetrical complications (*3*), bleeding dyscrasias (*4*), venous thrombosis (*5*), and cancer (*6*).

The measurement of this proteolytic activity in body fluids has been quite difficult. Most methods have not been direct since they measure antiproteolytic factors, nor have they lent themselves to simple quantitative measurement (*2, 7, 8*).

A procedure for direct quantitative estimation of proteolytic activity has been devised. The principle of the method depends upon the digestion of I^{131}-labeled albumin by a proteolytic enzyme. This will result in