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.

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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

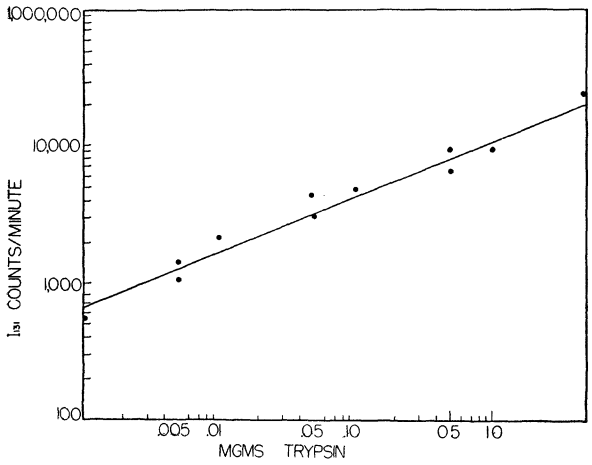

FIG. 1.

formation of unbound radioiodine that, though not necessarily in inorganic form, is no longer attached to the whole protein and may be separated from the latter by selective precipitation of the albumin. The radioactivity contained in the supernatant should then be proportional to the proteolytic activity of the enzyme.

Radioactive iodinated albumin is dialyzed against cold running water for 72 hr to free it of any unbound inorganic I^{131}. The proteolytic solution is then added to the labeled albumin. After 20 min, 1 cc of β-naphthalene sulfonic acid and 1 cc of human serum albumin as a carrier are added to precipitate the proteins. The mixture is centrifuged and the supernatant plated and counted with a thin end-window Geiger-Müller tube.

The results using varying concentrations of trypsin (Tryptar-Armour) in 1 cc of solution are shown in Fig. 1.

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Various Absorption Coefficients for 23.5-Mev X-Rays

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In choosing the material for a window through which one can view patients receiving betatron x-ray therapy, it was necessary to measure the half-value layers of glass,[1] lead glass, water, and zinc bromide.[2] It was found necessary to have a protective window-thickness providing about 8–9 half-value layers of absorbing material to protect the betatron personnel in the control room satisfactorily.

TABLE 1

23.5 MEV X-RAYS

Material	Half-value layer	Absorption coefficient
Water	29.6 cm	0.023 cm^{-1}
Glass	11.9 cm	0.058 cm^{-1}
Zinc bromide	9.4 cm	0.074 cm^{-1}
Lead glass	3.6 cm	0.19 cm^{-1}
Lead	1.4 cm	0.48 cm^{-1}

K. R. Ferguson (*1*) and others have discussed various liquid and solid absorbers for window constructions that can be used for high energy radiation protection. In order to select the best material in respect to lowest space consumption and cost as well as chemical stability, the following absorption coefficients were measured with a 23.5-Mev x-ray beam.

The absorption coefficients of glass, lead glass, and

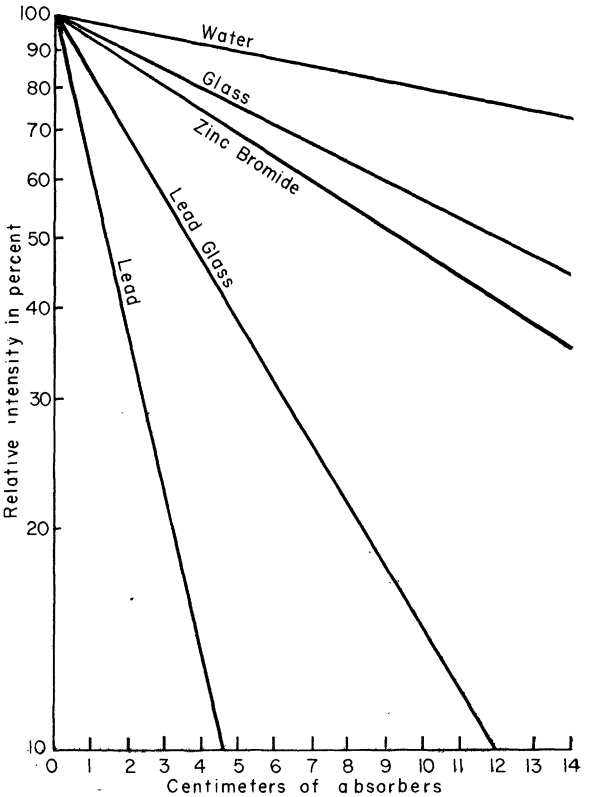

FIG. 1. Absorption qualities of various materials for protective windows against 23.5 Mev x-rays are shown. The lead curve is given for comparison.

[1] Samples of plate glass and lead plate glass were furnished by the Pittsburgh Plate Glass Company.

[2] Zinc bromide solution containing hydroxylamine hydrochloride to prevent coloring due to oxidation products was supplied by the Dow Chemical Company.