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Susceptibility of the White Mouse to Tuberculosis.

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The opinion that the mouse is practically immune or at least highly resistant to tuberculous infections seems to be quite generally expressed. Kettle[1] states that the tubercle bacillus is practically non-pathogenic to the mouse in the sense that it does not cause the necrotic or other lesions seen in susceptible animals and does not necessarily kill the animal. Trommsdorff[2] observed that mice were much more susceptible to bovine strains than to human strains when injected intravenously and suggested the possibility of using mice for type differentiation. His work was confirmed by Peters[3] and Binder.[4] Lange[5] used 3 human strains and 2 bovine strains of virulent tubercle bacilli for intravenous injections and observed no marked differences in the susceptibility of mice to the two types.

This report calls attention to the possibility of using white mice for certain types of work in the field of tuberculosis. The relatively low susceptibility of the mouse enhances its value in experiments where chronicity of the disease is desired. The rarity of spontaneous infection and the consistent results from intravenous administration of suitable doses suggest the possibility of a usefulness in virulence tests and possibly for experiments in immunity.

Recently isolated human and bovine strains and a virulent avian strain* were used for intraperitoneal and intravenous injections in full grown white mice. The expectation that mice would be resistant to infection led us to use unnecessarily large doses in the earlier experiments. Two or 3 weeks old cultures were emulsified by shaking with glass beads and the concentrations were made such that the desired dose was contained in 0.5 cc. of saline. In many cases the concentration was reduced by allowing the coarse clumps of bacilli

1 Kettle, E. H., *J. Path. and Bact.*, 1932, **35**, 395.

2 Trommsdorff, R., *Arb. a. d. Kais. Gesundheitsamt*, 1909, **32**, 568.

3 Peters, E., *Zentralbl. f. Bakteriol., Parasitenkrankh. u. Infektionskrankh.*, Abt. I, Orig., 1912, **62**, 1.

4 Binder, cited by Lange.

5 Lange, B., *Z. f. Hygiene u. Infektionskrankh.*, 1922, **98**, 229.

* Obtained from Dr. A. J. Vorwald of the University of Chicago. It was originally isolated by Dr. Van Es of the Department of Pathology and Hygiene, University of Nebraska.

to settle out so that the actual dose given was considerably less than that indicated in the table.

In the animals infected with human or bovine strains the tendency for macroscopic lesions to appear in the lungs is striking. Those mice showing advanced pulmonary tuberculosis usually exhibited multiple tuberculous lesions in the liver. Only a few had macroscopic tubercles in the spleen although many showed splenic enlargement. Involvement of the liver and spleen was more frequent with the use of bovine than with human strains.

TABLE I.

	Intraperitoneal			Intravenous	
Human Strains of Tubercle Bacillus					
Dose (moist wt. in mg.)	1.0	0.1-0.25	0.025	0.25	0.025
No. mice studied	40	13	8	6	8
Total No. with macroscopic lesions	90%	69%	25%	100%	87%
No. with macroscopic lesions in lungs	77%	69%	12%	100%	87%
No. with lesions in liver or spleen	22%	15%	12%	17%	12%
Aver. survival period (days)	46	79	120	24	59
Bovine Strain of Tubercle Bacillus					
Dose (moist wt. in mg.)	1.0			0.25	
No. mice studied	16			6	
Total No. with macroscopic lesions	75%			100%	
No. with macroscopic lesions in lungs	69%			83%	
No. with lesions in liver or spleen	44%			66%	
Aver. survival period (days)	45			18	

In the table the results of intraperitoneal and intravenous injections are compared and while the second group is small the data are sufficient to indicate that for a given dose the intravenous inoculations are more effective. The average period of survival is almost precisely the same in mice given 1 mg. of bovine bacilli as in those receiving the same dose of human type. The gross and microscopic appearances of the lesions resulting from the use of the 2 types were indistinguishable. Beginning as discrete, disseminated tubercles, these became confluent and progressed until they occupied one-half to two-thirds of the lung tissue at the death of the animal. Tuberculous pneumonia was not uncommon. Caseating tubercles were unusual in the liver and spleen but caseous nodules in the peritoneum were common in the mice inoculated intraperitoneally.

Seventy-eight mice which received various doses of avian tubercle bacilli through the tail vein, were etherized at different intervals up to 46 weeks. None died as a result of tuberculous infection and the smaller doses resulted in no gross lesions but epithelioid tubercles and lymphocytic infiltration were found in the microscopic sections of liver and spleen in most cases. When large doses (1 mg.) were used, characteristic lesions appeared almost invariably. The liver and spleen were enlarged but the lungs were usually free from gross lesions. Caseation never occurred in the avian infections.

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State of Calcium in Protein-containing Fluids.*

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We have investigated the nature of the relationship between calcium and protein in blood serum and other protein-containing fluids, using the frog-heart method, previously described,[1] for direct estimation of calcium ion concentrations, and have accumulated evidence concerning the hypothesis[2] that there is a substance, like citrate, and other than protein, which binds calcium in serum and other fluids of the body. When purified electrolyte-free serum proteins (beef) are dissolved in solutions of approximately the electrolyte concentration of the serum, calcium combines with the protein in a manner analogous to that previously described for citrate.[3] The combination represents an equilibrium between protein, total calcium, and calcium ions, and this equilibrium may as a first approximation be described by the mass law equation

$$\frac{[Ca^{++}] \times [Prot^{=}]}{[CaProt]} = K \qquad (1)$$

in which

Ca^{++} = calcium ion concentration—mols. per kg. water.
† $Prot^{=}$ = base combining capacity of protein present at pH of solution, in equivalents $\times$ 0.5, less equivalents combined with calcium, per kg. of water
CaProt = calcium combined with protein expressed as mols of Ca per kg. water.
K = constant.

In logarithmic form this equation is written as $pCa^{++} + pProt^{=} - pCaProt = pK_{CaProt}$ in which p indicates the negative logarithm.

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[1] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1344.

[2] Greenwald, I., *J. Biol. Chem.*, 1926, **67**, 1.

[3] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1136.

† The assumption, implied by the equation, that the base combining equivalents of protein behave as though they were divalent ions is purely empirical, in order to fit the data at hand.