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Infection of Mice with Tubercle Bacilli Grown in Tween-Albumin Liquid Medium.

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Tubercle bacilli, growing diffusely in liquid media containing a water dispersible ester of oleic acid (Tween 80), retain unaltered many of their morphological and biological properties.[1,2] The present paper describes the virulence of these cultures for mice of different genetic backgrounds inoculated under various experimental conditions. The cultures used in the infection tests to be reported were grown for 7-10 days in a medium containing 0.05% Tween 80 and 0.2% bovine albumin (serum Fraction V).[2] Macroscopically, these cultures appear homogeneous, but in reality consist of microscopical clumps. Their density corresponds to approximately 0.20 mg/cc in terms of dry weight of bacilli.

Mice, 3 to 6 weeks old, of the Rockefeller Institute strain inoculated intravenously with 0.01 cc of whole culture begin to lose weight during the second week after infection and die in 3 to 4 weeks with a disease primarily pulmonary. The pulmonary lesions consist of discrete and confluent nodules varying in size, pearly gray in color and firm in consistency. Stained microscopic sections show innumerable tubercle bacilli in these areas. Heart muscle is often infiltrated with very many small lesions. When the inoculum is reduced 10-fold to 0.001 cc, only an occasional animal dies after the 4th week of infection, although all animals sacrificed at this time show pulmonary lesions.

Much larger amounts of culture (0.5-1.0 cc) are required to produce death within 3 to 4 weeks when the infective dose is introduced by the intraperitoneal route. This minimal lethal dose can be reduced appreciably by adding fresh egg yolk to the bacterial suspension.

Fresh egg yolk is diluted with an equal part of 0.85% saline. One volume of culture is emulsified with one volume of the diluted egg yolk suspension immediately prior to infection. The data presented in Table I illustrate the enhancement of infection by the addition of fresh egg yolk to the culture.

The infection tests just described were carried out with the classical H37Rv culture obtained through the courtesy of Mr. William Steenken of the Trudeau laboratory. Similar results have been obtained with other human and bovine cultures of tubercle bacilli recently isolated from pathological materials. On the other hand, saprophytic acid-fast bacilli and variants derived from pathogenic strains but known to be devoid of pathogenicity for guinea pigs and chick embryos, fail to establish a progressive disease in mice even when injected in large amounts. Table II illustrates the results of inoculating mice (Rockefeller Institute strain) by the intravenous route with 2 different variants of the H37 culture of human tubercle bacilli, H37 (virulent) and H37Ra (avirulent). Even with the addition of egg yolk, 0.2 cc of H37Ra culture fails to establish a progressive infection or to produce grossly visible lesions.

Fifteen different strains of mice have been compared for susceptibility to tuberculous infection by the intravenous and intraperitoneal routes. The progress of the disease was determined by weight changes of the animals at weekly intervals, length of survival, numbe and extent of macroscopic pulmonary lesions, and enlargement of the spleen. Whatever the mode of infection, and whatever the criteria used for evaluating the severity of the disease, the differences observed are consistent and permit the recognition of marked

1 Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

2 Dubos, R. J., Davis, B. D., Middlebrook, G., and Pierce, C., *Am. Rev. Tuberc.*, 1946, **54**, 204.

TABLE I.
Effect of Egg Yolk upon Infection of Mice with Tubercle Bacilli (H37) via Intraperitoneal Route.

Culture H37	Egg yolk suspension	Wt. at weekly intervals after infection				No. dead/ Total inoculated
		Initial	1 wk	2 wk	3 wk	
cc	cc	g	g	g	g	
0.25	0	20.0	20.2	22.6	24.2	0/6
0.25	0.25	20.5	20.7	18.0	Dead	6/6

TABLE II.
Comparison of H37 (Virulent) and H37Ra (Avirulent) Strains Inoculated Via Intravenous Route.

Culture		Egg yolk suspension	Wt at weekly intervals after inoculation					No. dead/ Total inoculated
			Initial	1 wk	2 wk	3 wk	4 wk	
	cc	cc	g	g	g	g	g	
H37	0.01	0	18.3	21.3	22.2	19.7	19.2	5/6
H37Ra	0.2	0	18.3	21.6	24.2	25.9	26.7	0/6
H37Ra	0.2	0.1	18.3	20.9	23.3	24.6	25.3	0/6

differences in susceptibility among the different strains of mice. In general, albino mice derived from the so-called Swiss strain are somewhat more resistant than the white mice of the Rockefeller Institute strain. On the contrary, a number of other strains such as C57 black, dba, and wild mice *(Mus musculis domesticus)* are markedly more susceptible. The C_3H strain is intermediate between the latter group and the Rockefeller Institute strain.

Albino Swiss mice die within 3 weeks following intravenous injection of 0.05 cc of H37 culture; they survive and exhibit only limited pulmonary lesions when infected with smaller doses. On the other hand, 0.003 cc of culture is sufficient to cause death of dba mice within 3 weeks; moreover, animals of this strain, sacrificed 4-5 weeks after intravenous injection of 0.00003 (corresponding to 0.000005 mg dry weight becilli), show extensive pulmonary lesions often involving whole lobes.

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Alpha Naphthylthiourea (ANTU) in Dogs: Electrophoretic and Cholesterol Studies on Blood Plasma and Pleural Effusion.*

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Richter[1] recently described a new rat poison, alpha naphthylthiourea (ANTU), which exerts its toxic effect within a few hours by increasing the permeability of the pulmonary and pleural capillaries. In the dog and rat, the pulmonary edema and pleural effusion cause death. An analysis of the electrophoretic patterns of the serum and effusion fluid in animals poisoned with ANTU provides an approach to the problem of diffusion of proteins across damaged capillary walls.

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

[1] Richter, C. P., *J. Am. Med. Assn.*, 1945, **129**, 927.