in at least some of the cells. Although the cells gave a fractional resealed volume of only 40%, no obvious differences in morphology could be observed to separate the cells into 2 populations, and all cells showed some surface irregularities[12].

Resumen. El contenido de cationes en una preparación de eritrocitos resellados, usado como criterio de resellado, dió un volumen parcial de 40%. La morfologia de la superficie de dicha preparación ha sido investigada mediante el microscopio electrónico de barrido. Todas las células presentaban irregularidades en su superficie. La distribución del volumen de células reselladas medida en el contador electrónico de células es diferente de la de celulas intactas, pero las diferencias morfológicas observadas no eran suficientes para distinguir entre células reselladas y permeables.

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Suppressed Multiplication of Pulmonary Tubercle Bacilli in Bacillary Trypsin Extract-Immunized Mice

Previous evidence that bacillus-free, water-soluble extracts of trypsin-digested tubercle bacilli will immunize mice and guinea-pigs specifically against experimental tuberculosis[1-3]. has been indirect. Herein we present direct confirmation of this.

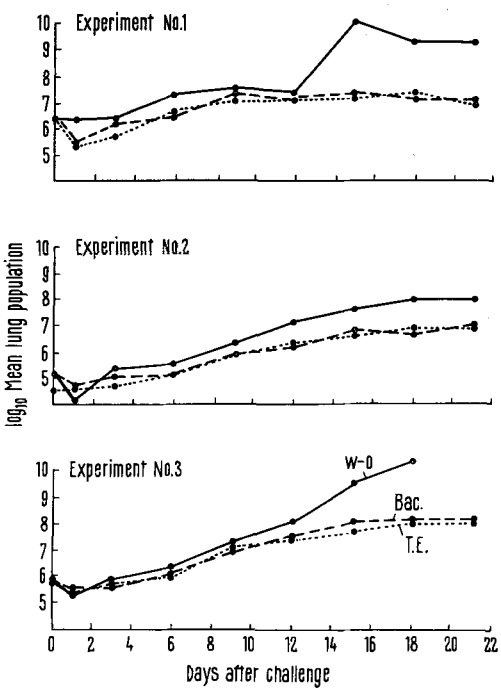
Materials and methods. Preparation of our immunogen has been detailed previously[1,4]. CF-1 female mice used in groups of 30 were injected s.c. on days 0 and 7 with 0.1 ml volumes of water-in-oil emulsion[5], containing either no antigen (nonimmune control group) or containing 0.25 mg quantities of trypsin extract (test group) or acetone-killed bacilli (immune control group; cf. ref. [5]. On day 35 each mouse was infected i.v. with 0.2 ml of a second subculture on Middlebrook 7H9 medium of human-type tubercle bacilli, maintained in stock culture on Kirschner medium. Lungs were removed from 3 mice of each group at various intervals after infection, homogenized aseptically in 10 ml of 7H9 medium, and plated by the drop technique[6] in serial 10-fold dilutions in duplicate on Middlebrook 7H10 medium. Colonies were counted after 4 weeks' incubation at 37 °C and results are reported here in mean total culturable tubercle bacilli per set of lungs.

Results. Our data are summarized in the Figure. Challenge infections for these 3 experiments read 45 (on the Klett-Sumerson colorimeter), 12, and 45, respectively. H37Rv tubercle bacilli were used for the first 2 experiments and Erdman for the third, constituting severe, moderate, and very severe challenges, respectively.

As seen, immunization with either trypsin extract or acetone-killed bacilli significantly suppressed pulmonary tubercle bacillus multiplication. This bacteriostatic immunity was more obvious against heavy than against moderate challenge, and it did not appear until after the 12th day of infection.

Discussion. These experiments indicate that the lungs of trypsin extract-immunized mice suppress bacillary multiplication just as has been reported for the lungs of BCG-immunized mice[7,8], including even the puzzling but well-confirmed[7], commonly described delay in the appearance of bacteriostasis. Reasons for this 12-day delay are unclear but may relate to the use of Tween-80 in challenge bacillus culture medium. It is absent when Tween is not used[9-11] and thus may not be associated with time required for multiplication of challenge bacilli to a postulated level sufficient to evoke local immunity[12]. The delay also is avoided when Tween-cultured bacilli are used for challenge if Tween-grown immunizing bacilli are delivered directly to mouse lungs by aerosol or i.v. injection[13]. Perhaps Tween-associated tubercle bacilli have an immunogenic specificity of their own. This could explain why Ribi et al.[14] found that in contrast to Ribi's 'oil disruption product' and to BCG, Youmans' 'particulate fraction'[15] immunized mice poorly against either i.v. or airborne challenge; because in these experiments both immunizing and challenging bacilli and the oil disruption product were at one time or another in their use exposed to Tween, whereas the particulate fraction was not.

[1] A. J. Crowle, Tubercle *42*, 479 (1961).
[2] A. J. Crowle, Tubercle *44*, 241 (1963).
[3] A. J. Crowle and F. Teramura, Tubercle *45*, 40 (1964).
[4] A. J. Crowle, Z. ImmunForsch. exp. Ther., in press.
[5] A. J. Crowle, Tubercle *42*, 470 (1961).
[6] F. Fenner, S. P. Martin and C. H. Pierce, Ann. N.Y. Acad. Sci. *52*, 751 (1949).
[7] F. M. Levy, G. Conge, H. Mauss, S. Lindenmann and Y. Lucel, Rev. Immunol. Thér. antimicrob. *30*, 275 (1966).
[8] F. M. Levy, G. A. Conge, J. F. Pasquier, H. Mauss, R. J. Dubos and R. W. Schaedler, Am. Rev. resp. Dis. *84*, 28 (1961).
[9] J. L. Sever and G. P. Youmans, Am. Rev. Tuberc. pulm. Dis. *76*, 616 (1957).
[10] The Sever-Youmans paper indicates that Dubos et al.[11] found a similar stasis of tubercle bacillus population in the lungs of BCG-immunized mice challenged with Tween-grown tubercle bacilli also beginning with the first sampling. But this first sampling was made 2 weeks after infection, when the bacillary population already had plateaued, even in the nonimmunized mice (Dubos' Table III). An experiment reported by Fenner et al.[6], and showing stasis of the population beginning at 5 min after infection, must be excluded from our discussion because the same was also seen in unimmunized control mice.
[11] R. J. Dubos, C. H. Pierce and W. B. Schaefer, J. Exp. Med. *97*, 207 (1953).
[12] G. B. Mackaness, Am. Rev. resp. Dis. *97*, 337 (1968).
[13] C. L. Larson and W. C. Wicht, Am. Rev. resp. Dis. *85*, 833 (1962).
[14] E. Ribi, C. L. Larson, W. Wicht, R. List and G. Goode, Proc. Soc. expl Biol. Med. *118*, 926 (1965).
[15] A. S. Youmans and G. P. Youmans, J. Bact. *87*, 278 (1964).



Graphic summaries of increases in the lung populations of challenging tubercle bacilli in nonimmune (w-o), immune (Bac.), and trypsin extract-immunized (T.E.) mice. Data points for experiments 2 and 3 are mean values from triple sets of lungs, but some of those for experiment 1 are for less because contamination interfered with readings on several sample plates.

An added, but related, complication of using minute challenge infections may account for findings[13,16] that mice vaccinated s.c. with BCG appear to have no protection against intrapulmonary multiplication of the challenge bacilli (grown with Tween). We might have seen the same result in our second experiment had we used a somewhat lower challenge infection. Hence, unless tuberculoimmunity is induced by a Tween-associated immunogen applied directly to the lungs, or unless challenge with Tween-grown bacilli suffices to cause progressive bacillary multiplication in unimmunized mice beyond 2 weeks, an acquired immunity actually present may not be detected by Tween-grown challenge bacilli[17].

Zusammenfassung: Extrakte aus Tuberkelbazillen, die mit Trypsin verdaut werden, erzeugen in Mäusen eine Tbc-Immunität, die jener durch BCG entspricht.

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[16] D. W. SMITH, E. WIEGEHAUS, R. NAVALKAR and A. A. GROVER, J. Bact. *91*, 718 (1966).

[17] The work reported in this paper was supported by United States Public Health Service grant No. AI 07784.

The Effect of Pinealectomy and Environmental Lighting on the Gonadal, Thyroid, and Total Body Weight of Female Long-Evans Rats

The mammalian pineal gland has been assigned various possible roles in normal physiology. Dominant among present theories are those which ascribe to this organ a possible hormonal influence upon the gonads[1-6]. The pineal contains large amounts of serotonin and this amine undergoes a circadian rhythm[5,7-10]. Serotonin is converted into melatonin (*N*-acetyl-5-methoxytryptamine) by the enzyme hydroxyindole-*o*-methyl transferase (HIOMT)[2,5,11]. This enzyme is thought to be localized exclusively in the pineal gland in mammals. Activity of this enzyme is thought to be circadian and light influenced, increased in dark and decreased in light. Evidence for this phenomenon has been quite contradictory[2,3,12-14].

Most current theories on pineal control of the gonads have stemmed from the hypothesis that this influence is mediated via pineal melatonin which supposedly acts to inhibit the gonads; melatonin levels are thought to be controlled by environmental lighting through HIOMT activity[2,12,15]. Evidence for this hypothesis has been indirect and is derived from reports that: (1) rats kept in continual light have hypertrophied ovaries while rats kept in total dark have atrophied ovaries[3,4]; (2) melatonin injections cause ovarian atrophy in the rat[16,17] and, (3) ovaries hypertrophy following pinealectomy[1,18].

Because of the pineal-gonadal hypothesis and the various reported interactions between gonads, environmental lighting, and the pineal gland, it was deemed significant to examine gonadal weight in relation to pinealectomy when coupled with variations in environmental lighting conditions. Since it has been reported that pineal melatonin may act to inhibit the secretion of thyroxin[19], the

[1] J. I. KITAY, Endocrinology *54*, 114 (1954).
[2] J. AXELROD, R. J. WURTMAN and S. H. SNYDER, J. biol. Chem. *240*, 949 (1965).
[3] R. J. WURTMAN and J. AXELROD, Scient. Am. *123*, 50 (1965).
[4] R. J. WURTMAN and J. AXELROD, Science *141*, 277 (1963).
[5] W. B. QUAY, Pharmacol. Rev. *17*, 321 (1965).
[6] W. B. QUAY, Progr. Brain Res. *8*, 61 (1964).
[7] W. B. QUAY, Gen. comp. Endocr. *1*, 3 (1963).
[8] W. B. QUAY and A. HALVEY, Physiol. Zool. *35*, 1 (1962).
[9] W. B. QUAY, Z. Zellforsch. *60*, 479 (1963).
[10] W. B. QUAY, Brain Res. *3*, 277 (1967).
[11] S. GERATTINI and L. VALZELLI, *Serotonin* (Elsevier, N.Y. 1965), p. 42.
[12] W. B. QUAY, Physiologist *10*, 286 (1967).
[13] W. B. QUAY, Proc. Soc. expl Biol. Med. *115*, 710 (1964).
[14] W. B. QUAY, Z. Zellforsch. *60*, 479 (1963).
[15] R. COHEN, R. WURTMAN and S. SNYDER, Ann. intern. Med. *61*, 1144 (1964).
[16] W. MCISAACS, R. TABORSKY and G. FARRELL, Science *145*, 63 (1964).
[17] J. IFFT, Endocrinology *71*, 181 (1962).
[18] R. WURTMAN, W. ROTH, M. ALTSCHULE and J. WURTMAN, Acta Endocr. *36*, 617 (1961).
[19] T. ISHIBASHI, D. HAHN, L. SIRVESTAVA, P. KUMARESAN and C. TURNER, Proc. Soc. expl Biol. Med. *122*, 644 (1966).