Effect of Adrenalectomy on Tuberculin Reaction in Mice.* (20842)

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Both delayed-type bacterial allergy and acquired immunity are features of established infections in most mammalian species. The relation between these processes is difficult to assess because of complex problems which arise in setting up adequate experimental models for their study. The natural tuberculin anergy of the mouse(1), a species which may show both high and low degrees of immunity to experimental tuberculosis(2), is an exception of some interest. Tuberculin anergy in the sensitized host, although unusual, also occurs, for example during cortisone(3) and ACTH(4) treatment and in the course of some disease states(5). Since small mammals like the mouse have proportionally larger adrenals than those of heavier animals(6), it seemed possible that adrenalectomy might reverse the tuberculin anergy in mice. The present report describes the results of this procedure on mice immunized with either heat-killed tubercle bacilli in mineral oil or with active BCG infections.

Materials and methods. Tubercle bacilli. A culture of H37Rv grown on mass culture media(7) was killed at 115° for 20 minutes, washed by centrifugation from 3 changes of distilled water, and lyophilized. Suspensions of these dried organisms 10 mg/ml were prepared in light mineral oil. Turbid 8-day cultures of strains of BCG Tice and BCG Phipps maintained in Dubos liquid media(7) were used to infect mice. *Mice.* Swiss albino mice 6 weeks old weighing 20 to 30 g were divided into groups as indicated in Table I. Animals were skin tested with second strength PPD (5 γ in 0.1 ml)(8) and with brucellergen(9) 0.1 ml. Intradermal injections were done by first passing a No. 27 needle through the skin to the subcutaneous space and then maneuvering the tip of the needle into the corium from below. In this way it was possible to be certain that an intracutaneous injection was given. These skin tests produced an immediate bleb which was rapidly absorbed with the first 24 hours, leaving a non-specific 2-3 mm slightly indurated area which was gone by 30 hours.

Experimental. In the first experiment (Table I) 25 mice were skin tested and found to have negative reactions at 24 and 48 hours. Animals then received subcutaneous injections of 0.1 ml of H37Rv in mineral oil at weekly intervals for 4 weeks. The mice remained healthy and showed a moderate weight gain. After one month skin tests were repeated and found to be negative at 24 and at 48 hours. The group was divided into 3 sections as indicated in Table I. Under ether anesthesia bilateral adrenalectomy was done through a midline dorso-lumbar skin and bilateral costovertebral muscle-splitting incision. Sham operations consisted of exploration of the renal fossae and identification of the adrenals without their removal. Adrenalectomized mice were maintained postoperatively on 1% NaCl in their drinking water. In the second experiment (Table I) animals were skin tested with 10 mg O.T. in 0.1 ml of 0.85% saline 48 hours before inoculation with 0.05 ml of one or the other BCG cultures into the tail vein. These skin tests were negative at 24 and 48 hours. After operation the skin test was repeated (Table I). Autopsies on animals infected with BCG revealed many acid fast organisms in impression smears of the enlarged livers and spleens.

Results. Of the 35 mice in this study immunized with living or dead tubercle bacilli one showed a significant area of erythema and induration to tuberculin at 24 hours. This was an animal immunized but not operated upon. One sham operation animal showed a significant tuberculin reaction to a larger amount of tuberculin at 48 hours. In 3 instances slight necrosis occurred at skin test sites, in each case in animals which also showed necrosis and ulceration at the site of

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TABLE I. Skin Tests in mm of Mice in Exp. 1 and 2.

Exp.	Group	No. animals	Survived operation	Tested 48 hr postop. PPD, 5 γ 24 hr	Tested 48 hr postop. PPD, 5 γ 48 hr	Tested 96 hr postop. OT, 10 mg 24 hr	Tested 96 hr postop. OT, 10 mg 48 hr
1†	Adrenalectomy	11	6	Neg.	Neg.	Neg.	Neg.
				4 × 4 mm	4 × 4 mm	3 × 3 mm	3 × 3N* mm
				Neg.	Neg.	"	2 × 2N*
				"	"	Neg.	Neg.
				"	"	3 × 3	" *
				"	"	Died	
	Sham operation	6	5	"	" *	3 × 3	Neg.
				"	" *	4 × 4	8 × 8N*
				"	"	Neg.	2 × 2
				"	"	"	Neg.
				"	"	"	"
	Control	8	8	"	"	"	"
				"	"	2 × 2	"
				10 × 10	10 × 10*	Neg.	"
				5 Neg.	5 Neg.	5 "	5 "
2	Adrenalectomy			OT, 10 mg			
	Phipps	13	10	Neg.	3 × 3 mm		
				9 "	9 Neg.		
	Tice	12	6	3 × 3 mm	1 "		
				"	"		
				4 Neg.	4 "		
	Sham operation						
	Phipps	6	5	5 "	5 "		
	Tice	4	3	1 "	1 "		
				2 × 2	1 "		
				"	Died		

* Animals having ulceration at sites of H37Rv immunization at time skin test read
N Necrosis of 1-2 mm in center of tuberculin reaction.
† Brucellergen 0.1 ml given I.D. at 48 hr and 96 hr to surviving animals caused no reactions.
No Brucellergen tests done in Exp. 2.

the H37Rv injections. Other reactions reported were interpreted to be the result of the trauma of the procedure.

Discussion. In the present study relative tuberculin anergy in mice has been observed. Since adrenal size bears a linear logarithmic relation to body mass, it was suspected that mouse anergy might be the result of relative increased adrenal activity normal for this species and comparable to that which can be induced in guinea pigs(4) and man(3) with cortisone or ACTH therapy. Were this so, adrenalectomy in the mouse should reactivate latent tuberculin allergy. This has not been found to be the case. Although it is difficult to be certain of complete removal of all adrenal tissues unless microscopic studies are done, it is likely that regeneration of adrenal rests would not be factors within the first few days after adrenalectomy. It was within this interval that the several skin testing procedures, including one with whole dead tubercle bacilli, were negative.

Mouse leucocyte responses, as well as dermal reactions, to tuberculin differ from those of man and of guinea pig. Peripheral blood and splenic lymphocytes from tuberculous mice are lysed by tuberculin; polymorphonuclear leucocytes are not injured by tuberculin(10). In man(11) and the guinea pig(10) possessing tuberculin hypersensitivity both lymphocytes and neutrophiles may be lysed *in vitro* by tuberculin. It is possible that the failure of tuberculin to injure mouse polymorphonuclear cells in the sensitized host is related to the anergy which this species shows to tuberculin.

Summary. 1. Albino Swiss mice immunized with heat-killed H37Rv tubercle bacilli in mineral oil or with active BCG infections are

relatively anergic to PPD and O.T. used for skin tests. 2. Bilateral adrenalectomy does not reverse mouse tuberculin anergy.

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Propagation of Infectious Canine Hepatitis Virus in Tissue Culture. (20843)

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The successful propagation of poliomyelitis virus in tissue culture, recently reviewed by Weller(1), has led several investigators to apply the roller tube method to the study of other viruses. Thus, members of the Coxsackie group were also found capable of multiplication in monkey tissue cultures by Riordan and others(2), who observed a cytopathogenic effect similar to that obtained with poliomyelitis virus. Similarly, using a stable strain of human epithelial cell, Syverton and Scherer(3) reported the serial growth of poliomyelitis virus with cytopathogenic effects, as well as that of a variety of other viral agents.

The purpose of this communication is to describe the successful use of the roller tube method for the propagation of infectious canine hepatitis virus in dog kidney tissue cultures.

Materials and methods. Virus. The virus employed in this study had been used for the experimental infection of puppies, as reported in previous publications(4,5), and its origin described(5). *Tissue culture method.* Roller tube cultures of monkey, rabbit and dog kidney and of whole chick embryo in 1 ml of synthetic medium No. 199(6) containing trypsin inhibitor, were incubated at 37°C at 8 to 10 rph. The kidney tissues were grown for from 4 to 5 days and the chick embryo tissue for 2 to 3 days. The medium was renewed before infection, and no further changes were made thereafter. The inoculum consisted of 0.1 ml of 10% dog liver suspension, or infected tissue culture fluid. Infected and control tubes were observed daily for not more than 7 days. When cytopathogenic effects occurred, the infected tissue fragments and fluid were removed by scraping and suction to a Tenbroek grinder. The culture suspension thus obtained was available for either subculture or for storage in the dry ice chest. *Qualitative neutralization test.* This was done by mixing equal volumes of undiluted harvest fluids and undiluted fox immune serum previously inactivated at 56°C for 30 minutes. Controls were similarly made by mixing equal volumes of harvest fluid with either inactivated normal dog serum or inactivated ICH fox immune serum. After further incubation for one hour at 37°C, each mixture was injected in 0.1 ml amounts into groups of 3 or 4 dog kidney culture tubes. These were observed daily until complete cytopathogenic effect had taken place in the normal dog serum control tubes.

ICH fox immune serum. The standard serum was prepared in foxes as follows: seven