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BRIEF COMMUNICATION

The Immunological Behaviour of Mature C57BL/6J Mice Thymectomized at Birth*

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Thymectomy of neonatal mice has frequently been shown to abrogate immunological responses, to prolong the rejection time of allogeneic skin grafts, and to cause wasting disease (Miller, Marshall and White, 1962). This study deals with the failure of C57BL/6J mice, thymectomized on the day of birth, to exhibit any of these features; it was prompted by the observation that such mice, when 9 weeks old, had titres of heterohaemagglutinins which did not differ significantly from those of controls.

Nine weeks after birth thymectomized and control mice were injected with the capsular polysaccharide of type III *Diplococcus pneumoniae* (SIII), an H preparation of *Salmonella typhimurium*, and bovine serum albumin (BSA) in incomplete adjuvant; they were bled 3 weeks later. With the first two antigens, but not with BSA, no differences were observed in the titres of experimental and control animals (Table 1). All successfully thymectomized animals failed to form antibodies to BSA, whereas all but one control formed normal amounts of these antibodies. Characterization of the antibodies in the ultracentrifuge and by 2-mercaptoethanol treatment showed that antibodies to BSA were 7S and that antigen binding capacity titres were unchanged by mercaptoethanol (3·5 *vs.* 3·6), whereas agglutinins against erythrocytes were 19S and removed by mercaptoethanol (1 :256 *vs.* 0). Such a clear distinction was not observed with the other antibodies: activity appeared in several fractions and was reduced by about 50 per cent on mercaptoethanol treatment.

At 15 weeks the mice received a full thickness skin graft from A/J donors. Grafts were scored visually and some mice killed 10 and some 16 days after grafting. The grafts were removed, sectioned, stained and scored microscopically. No difference was observed between the two groups (Table 1). At 12 weeks only about 50 per cent of the thymectomized mice had a lymphopenia ($62 \pm 12{\cdot}5$ *vs.* $73 \pm 9{\cdot}8$) and it was not nearly so marked as that observed in other strains (Miller *et al.*, 1962; Parrott, 1962).

When the experiment was terminated between the sixteenth and eighteenth weeks, in addition to two mice which died before being exhaustively studied (510-4 in apparent good health and 510-5 grossly underweight), four thymectomized and one control had died. Two thymectomized mice died during grafting (511-2, 515-3) and the other two (515-1, 511-3) a few days later. Three of these were underweight but otherwise had no signs of wasting disease. However, the lymphocyte counts of two of these animals were

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TABLE 1

IMMUNOLOGICAL REACTIONS OF THYMECTOMIZED AND CONTROL C57BL/6J MICE

Mouse No.	*Titres against:**				*Skin graft rejection grafts removed at:†*		*Thymic tissue*	
	Sheep erythrocytes	*Salm. H*	*S-III*	*BSA*	10 *days*	16 *days*	*Gross*	*Microscopic‡*
THYMECTOMIZED								
508-1	16	0	0	0		10	Neg.	0
508-2	64	160	0	0		0	Neg.	0
509-1	32	80	16	0		10	Neg.	1
509-2	8	80	32	0		0	Neg.	1
510-3	256	80	16	0		0	Neg.	2
510-4	128	—	—	—			Not obs.	
510-5	16	—	—	—			Not obs.	
510-6	512	80	16	0·16		0	Thymus	3
511-1	16	0	32	0		0	Neg.	2
511-2	0	160	16	0			Neg.	0
511-3	0	80	64	0	50 (day 6)		Not obs.	
512-4	64	80	32	0	50		Neg.	1
512-5	8	40	32	0	90		Neg.	0
512-6	8	160	128	0	50		Neg.	0
512-7	128	80	4	0	25		Neg.	0
512-8	16	160	68	0		0	Neg.	0
515-1	64	80	4	0			Neg.	0
515-3	32	0	64	0			Neg.	0
			6					
Mean §	25·4 ±4·3	64 ±26	20·1 ±3·7	0·01 ±0·04				
UNOPERATED								
510-1	512	160	32	1·0	0			
510-2	512	40	32	0·67	50			
512-1	16	80	32	0·27	0			
512-2	8	160	16	0	10			
512-3	8	160	16	0·3	10			
515-2	16	640	16	0·67		0		
536-1	8	0	16	0·43	50 (day 6)			
536-2	16	320	64	0·67		0		
536-3	8	160	16	0·56		0		
536-4	16	160	64	—				
Mean	24·3 ±5·3	122 ±30	26 ±1·7	0·5 ±0·29				
Significance	$0·2<P<0·3$	$0·1<P<0·2$	$0·1<P<0·2$	$P>0·01$				

* All antibody titres except those to BSA are expressed as reciprocals of that serum dilution at which the end point was observed. BSA results are expressed as μ of I*131 BSA bound per 1 ml. of undiluted serum determined at the dilution of serum which binds 33 per cent of 0·02 μ BSA nitrogen added. Serum dilutions for the four titrations started at 1 : 2, 1 : 10, 1 : 4, and 1 : 20 respectively.

† The mice were killed at either 10 or 16 days after grafting, the graft bed removed, fixed, sectioned and stained and then scored microscopically on the basis of epithelial survival. 10 = 10 per cent survival, 90 = 90 per cent survival, etc.

‡ Thymuses were graded microscopically on the basis of the amount of thymic tissue present. 2 = thymic fragments; 1 = tiny thymic fragments; 0 = no evidence of thymic tissue.

§ Means and standard deviations were calculated on the basis of the dilution number (1, 2, 3, etc.) and then converted to actual titres.

lower, and their neutrophil counts higher, than those of any other animal. The remainder of the thymectomized mice appeared in splendid condition: a subjective view confirmed objectively by their weights.

At the termination of the experiment, the neck region was examined grossly and a large section removed, step-sectioned and examined microscopically. Although there was gross

evidence of thymic tissue in only one thymectomized mouse (510-6), microscopically thymic fragments were noted in several animals. Yet, the immunological and haematological picture of these mice did not differ from those in which there was no microscopic evidence of thymic tissue.

After completion of this work my attention was drawn to similar findings of Humphrey, Parrott and East (1964) who used C57BL × C3H/Bi mice. The difference between the above results and those of most other workers may be due to differences in the maturity at birth of various mouse strains. The C57BL/6J may represent the extreme end of a spectrum, approaching at birth the immunological maturity of the rabbit (Porter, 1960) and indeed, the inability of C57BL/6J mice to form anti-BSA and yet possessing a normal homograft reaction, parallels the results in the rabbit (Porter, 1960; Good, Dalmasso, Martinez, Archer, Pierce and Papermaster, 1962). Abrogation of the immune response to BSA, but not to other antigens, may be related to the relative ease with which unresponsiveness to BSA (Dietrich and Weigle, 1963) as opposed to other antigens is induced in C57BL/6J neonates. If tolerance depends on a critical antigen to cell ratio, then thymectomy may reduce the number of cells to a level at which a dose of antigen which elicits immunity in the intact animal will confer tolerance on the thymectomized. It may be possible by thymectomy and judicious antigen dose to interfere specifically with some, but not all, immune responses. Or again, it may be that as in the chicken (Szenberg and Warner, 1962) the thymus may not be the only source of immunologically competent cells or their precursors so that thymectomy removes cells putatively engaged in antibody production to BSA, but does not extirpate cells concerned in the other immune responses studied. Ectopic thymic tissue has been observed in some mouse strains (T. B. Dunn, 1964, personal communication): the C57BL/6J strain was not examined. Yet another possibility is that there is a slow exodus during ontogeny rather than an explosive eruption of immunologically competent cells from the thymus and that only some immune responses will be abrogated by extirpation at birth. Both latter hypotheses favour the clonal selection theory of immunogenesis and weaken the theory of the totopotential immunological capacity of a cell.

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