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DEVELOPMENT OF ANTI-TISSUE ANTIBODIES IN RATS

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SUMMARY

The sera of 'normal' rats have been found to contain anti-tissue antibodies from shortly after birth. The antibody titres rose rapidly over the first 3 weeks of life reaching adult levels by the time the rats were 3–6 weeks old. Neonatal rats were rendered tolerant by exposure to bovine serum albumin (BSA). In contrast, higher levels of anti-tissue antibody were found in the sera of neonatal rats exposed to tissue antigens released by experimental liver damage than in the sera of littermate controls. It is suggested that the particulate nature of the tissue antigens may be associated with their ability to provoke an immune response in neonatal rats and that this in turn may be a factor hindering the induction of tolerance to these antigens.

INTRODUCTION

Liver damage, induced by carbon tetrachloride, has been shown to stimulate the production of anti-tissue antibodies in rats (Weir, 1963, 1966; Weidermann, Rheinhardt & Denk, 1966; Weir & Suckling, 1968). This finding led us to investigate the possibility that the tissue antigens involved in this reaction may be released under 'normal' physiological conditions and evoke an immune response. Anti-tissue autoantibodies of the IgM type were detected in 'normal' rat sera by complement fixation and passive haemagglutination (Weir *et al.*, 1966) and by the selective uptake of immunoglobulins on mitochondrial preparations of rat liver exposed to normal rat sera (Elson & Weir, 1967; Elson, 1968). This finding suggests that rats do not become tolerant to their own sub-cellular components which are the antigens reactive with the anti-tissue autoantibody (Pinckard & Weir, 1966). This report presents evidence which supports this contention by showing that rats can react to sub-cellular tissue antigens during the neonatal period when tolerance to other antigens can readily be induced.

MATERIALS AND METHODS

Animals

Albino rats of both sexes originally derived from the Wistar strain were used. They were bled by cardiac puncture under light ether anaesthesia.

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Serological tests

The 'four drop' complement fixation test was performed in MRC pattern plates using 1·25 MHD guinea-pig complement as described by Weir (1967). The Perspex plates containing the test reagents were kept on ice until the antigen was added. The tests were incubated at 37°C for 30 min. Sensitized sheep erthrocytes were added and after a further 40-min incubation the tests were read. The end point was taken as the last tube containing unlysed cells.

The ammonium sulphate precipitation test of Farr (Minden & Farr, 1967) was used to calculate the antigen binding capacity of sera with 0·02 μg N [^{131}I]bovine serum albumin (I*BSA) prepared by the chloramine T method of Hunter & Greenwood (1962).

Preparation of tissue antigens

Tissue antigens were prepared in 0·25 M-sucrose at 4°C as described by Pinckard & Weir (1966). Rat livers were cut up into small pieces and homogenized in a Potter–Elvejhem glass homogenizer with a fitted Teflon pestle rotating at 1200 rev/min. A 'mitochondrial' fraction (F_3) was prepared from whole liver homogenate by differential centrifugation. F_3 was taken as the fraction sedimenting between 9×10^4 and $1{\cdot}5 \times 10^5$ *g* min.

Injection schedules

Litters of various ages were injected subcutaneously with 'Analar' carbon tetrachloride (CCl_4). Each rat received 0·005 ml CCl_4 diluted in 0·045 or 0·05 ml liquid paraffin/10 g body weight. Four days after injection they were bled. The sera were collected and tested in the complement fixation test against F_3.

Neonatal rats from the same colony were given a single intraperitoneal injection of either 5 mg alum-precipitated BSA, 0·5 mg alum-precipitated BSA or alum alone. They were challenged at 5 weeks of age with 5 mg alum-precipitated BSA and their sera taken 12 days later. The antigen binding capacities of these sera against I*BSA were determined by the ammonium sulphate precipitation test.

RESULTS

Over a period of 2 years 'normal' adult rats were bled and their sera tested, without prior heating to inactivate complement, against whole liver homogenate. Fig. 1 shows that of 105 sera, 103 gave positive reactions in the complement fixation test with titres ranging from 1:8 to 1:512, one was negative and one anti-complementary. It can be seen that the titres appear to follow a normal distribution.

The sera of sixteen neonatal rats, taken during the 1st week of life, were tested in the complement fixation test against whole liver homogenate. Ten of these sera were positive with titres ranging from 1:2 to 1:16. It was difficult to obtain sera from rats during the 1st week of life without considerably reducing their chances of survival. Thus, rats were bled weekly from 1 week after birth and their sera collected and stored at −25°C until required for testing. In order to reduce any variation in titre attributable to changes in the conditions of testing the sera were tested simultaneously. The results (Fig. 2) show that the complement fixation titres rose rapidly over the first 3 weeks of life reaching adult levels by the time the rats were 3–6 weeks old.

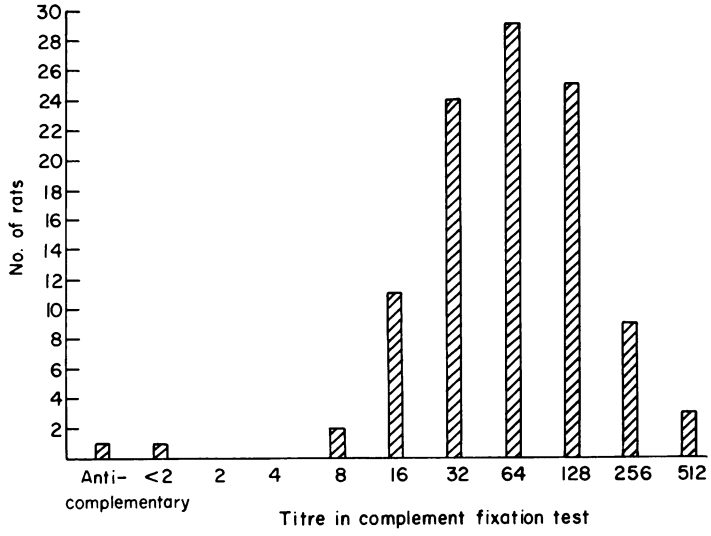

FIG. 1. Distribution of titres of complement fixing anti-liver antibody in 105 normal adult rat sera.

The ability of neonatal rats to respond to their own tissue antigens was tested by comparing the complement fixing anti-mitochondrial (F_3) activity of sera from neonatal rats injected with CCl_4 with those of littermate controls. The results are recorded in Table 1. It can be seen by inspection that the eight groups of rats injected with CCl_4 have, on average, higher titres of anti-F_3 antibody than littermate controls. This in itself shows that there is a significant ($P<0{\cdot}01$) difference between the experimental and control groups.

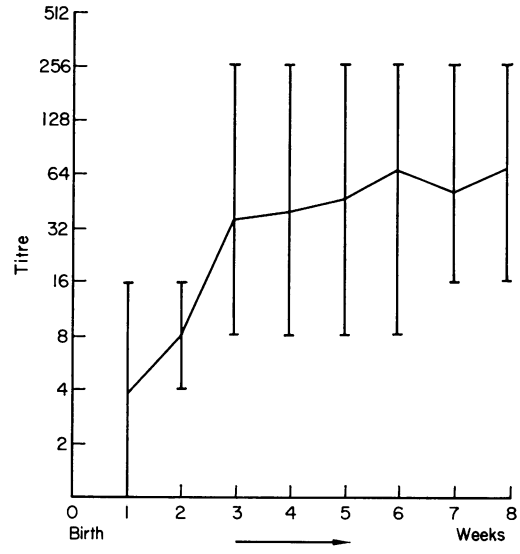

FIG. 2. Complement fixation titres of sera taken at intervals after birth of twelve normal rats. The line is drawn through the mean titre at each interval.

The effect of exposing neonatal rats from the same colony to BSA was tested as a check on the ability of the colony to develop tolerance. The sera of eighteen of twenty rats pretreated neonatally with 5 mg alum-precipitated BSA bound essentially no BSA (<0·01 μg N I*BSA). Similarly, the sera of eight out of fourteen rats pretreated neonatally with 0·5 mg alum-precipitated BSA bound less than 0·01 μg N I*BSA. The antigen binding capacities of the sera from rats pretreated with alum alone ranged from 0·12 to 0·41 μg N I*BSA.

TABLE 1. Complement fixation titres of sera from neonatal rats injected with CCl_4 (T) and littermate controls (C)

Litter	Age when injected (days)	Reciprocal titres in complement fixation test						
1	3	T	48	96	24	48	192	
		C	24	24	48	24		
2	3	T	24	24	24	6	24	
		C	12	12	24	12		
3	4	T	24	48	24	12		
		C	12	24	3			
4	5	T	192	96	96	96	24	
		C	48	<3	24	24	12	
5	6	T	48	48	24			
		C	12	12	6			
6	7	T	48	24	48	96	192	48
		C	48	48	24	12	48	24
7	8	T	24	48	24	24	48	
		C	24	12	12			
8	9	T	48	12	48			
		C	24	6				

DISCUSSION

These results show that complement fixing anti-tissue antibodies appear in the sera of 'normal' rats shortly after birth. Kidd & Friedewald (1942) and Hook *et al.* (1966) failed to detect complement fixing anti-tissue antibodies until 49 days after birth in rabbits. However, these workers heated the rabbit sera at 56°C for 30 min, prior to testing and it had been shown that this procedure markedly reduces the complement fixing activity of rat anti-sera without affecting its capacity to bind antigen (Weir & Elson, 1968).

In view of the work of Sterzl *et al.* (1965), in which antibodies reactive with a wide range of antigenic material were not detected in the sera of precolostral germ-free piglets fed on non-antigenic diets, it seems likely that the anti-tissue autoantibodies found in normal rat sera arise as a result of antigenic stimulation. The results reported here demonstrate that neonatal rats do produce anti-tissue antibodies in response to sub-cellular tissue antigens released by experimental liver damage. This implies that the anti-tissue autoantibodies in 'normal' rat sera (Fig. 1) arise as a result of stimulation by tissue antigens and not as a

result of stimulation by cross-reacting antigens. It is well established that tolerance can be subverted by appropriate cross-reacting antigens (Weigle, 1961; Paul, Siskind & Benacerаf, 1967; Rose & Cinader, 1967) and that this subversion can be prevented by injecting the original tolerance inducing antigen together with the cross-reacting antigen (Weigle, 1964; Humphrey, 1964; Weigle *et al.*, 1967). For example, Weigle (1964) induced anti-bovine serum albumin antibodies in rabbits tolerant to BSA by immunization with chemically altered BSA. In contrast he found no anti-BSA antibodies in rabbits rendered tolerant to BSA and challenged simultaneously with chemically altered BSA and BSA. In the same way if it is argued that rats are tolerant to the tissue antigens and that the anti-tissue antibodies found in normal rat sera are induced by cross-reacting or altered antigens then the release of tissue antigens during normal cell turnover would strengthen tolerance rather than result in the production of anti-tissue antibodies. However, the results do not support this possibility showing that the levels of anti-tissue antibody rise over the first few weeks in neonatal rats and when they are exposed to tissue antigen released by experimental liver damage.

Weir & Pinckard (1967) showed that rats subjected to an intense injection schedule of liver antigens from birth to 5 weeks were not rendered tolerant as judged by their ability to respond to subsequent challenge with liver antigen. In this work rats given a single injection of BSA neonatally were shown to be unresponsive to subsequent challenge with BSA. This shows that tolerance to a simple protein antigen could readily be induced in the colony of rats used. In contrast, neonatal rats were found to produce anti-tissue antibodies in response to stimulation by the corresponding tissue antigens. These observations force us to the conclusion that tolerance to these antigens does not exist. In other species it has been shown that the capacity to produce IgM antibodies in response to stimulation by certain antigens develops perinatally whereas the capacity to produce IgG antibodies is delayed (Uhr, Dancis & Franklin, 1962; Bellanti *et al.*, 1963; Smith & Eitzman, 1964). Particulate bacterial antigens which are poor tolerogens have been found to stimulate high IgM responses in rats (Ada, Nossal & Austin, 1965). The anti-tissue antibodies described here are of the IgM type and the tissue antigens with which they react are sedimentable (Weir *et al.*, 1966; Elson, 1968). This suggests that the particulate nature of the antigens may be associated with their ability to provoke an immune response in neonatal rats which in turn may be related to the failure of these antigens to induce tolerance.

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