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Effect of Pinealectomy on Immune Reactions in the Rat

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Summary. Non-inbred Wistar rats were pinealectomized at birth or in adult life, and tested for classical immune responses. Neonatal pinealectomy did not exert any notable effect on immune capacity of rats. On the other hand, rats pinealectomized at 6 weeks of age showed partial and transient impairment of immune potential. Arthus reactivity to bovine serum albumin was diminished and oedema of the skin less pronounced, and delayed reactions were reduced and of short duration. Slight delay in homograft rejection was noted. The incidence of allergic encephalomyelitis was diminished, and no clinical signs were observed. The cellular make-up of lymphoid tissues of pinealectomized adult rats was normal.

INTRODUCTION

The extensive work done on the epiphysis cerebri indicates that this organ is most probably an endocrine gland in a neuro-hormonal system (Thiéblot, 1965), which may play a role in the function of various tissues. It is well known that serotonin, as well as other biogenic amines, is exceptionally concentrated in the pineal body, and this mediator is considered to be important for the symptomatology of inflammatory reactions (Garattini and Valzelli, 1965) and the expression of some immune responses (Austen and Humphrey, 1963). It was considered, therefore, that it might be of interest to correlate the function of the pineal body and immune reactions in the rat.

MATERIALS AND METHODS

Pinealectomy

Wistar rats were used in the experiment. Neonatal pinealectomy was performed within 24 hours of birth (Andersen and Wolf, 1934), deep hypothermia being used as the anaesthetic. Sham-pinealectomized neonates were used as controls. Removal of the pineal organ from 6-week-old rats was performed according to the method of Hoffman and Reiter (1965). In sham-operated animals, the forceps were introduced in the vicinity of the pineal without injuring the gland or neighbouring vascular and brain tissue.

Immunization and testing of rats with bovine serum albumin (BSA)

Animals of various groups were sensitized to crystalline BSA (Pentex, Kankakee, Illinois) at the age of 8 weeks as described previously (Janković, Waksman and Arnason, 1962). All rats were skin-tested at 10, 20 and 30 days with 30 μg of BSA injected intradermally in the depilated flank. Arthus reactivity was read at 3 hours, and delayed reaction

at 24 hours. The booster consisted of 2 mg of BSA in 1 ml of saline, administered intraperitoneally 31 days after the first injection. Arthus reactivity and delayed response were provoked 5 and 15 days following the booster.

Antibody determination

Rats immunized with BSA were bled 10, 20 and 30 days after the first injection, and 5 and 15 days after the booster. Serum was heated at 56° for 20 minutes and absorbed with formalinized sheep erythrocytes. The mercaptoethanol-resistant and mercaptoethanol-sensitive anti-BSA antibodies were detected by a modified passive haemagglutination technique, using formalinized sheep red blood cells.

Induction of experimental allergic encephalomyelitis

The induction of disease with allogeneic spinal cord in adjuvant, and clinical and histological evaluation of encephalomyelitis were described in an earlier paper (Arnason, Janković, Waksman and Wennersten, 1962).

Skin homograft rejection

One full-thickness skin graft about 1·5 × 1·5 cm in size was removed from inbred Lewis rats and transplanted to a bed prepared in the skin of 8-week-old Wistar recipients. The graft was maintained in position with Vaseline gauze and a light plaster cast. The cast was removed at 5 days, and animals kept under daily observation as long as the homograft showed any signs of survival.

Histology

Lymphocyte counts were performed on the tail blood, and their diameters were measured using the following criterion: small lymphocytes (6–8 μ), medium lymphocytes (9–10 μ) and large lymphocytes (11–12 μ). At the end of each experiment the rats were killed, and thymus, spleen and lymph nodes processed for histology. The sections were stained with haematoxylin and eosin and methyl-green–pyronine.

RESULTS

GROWTH AND LYMPHOCYTE COUNTS

Neonatally pinealectomized rats of both sexes gained steadily in weight from the 4th to the 8th week, although they tended to be lighter than sham-operated controls.

Analyses of blood smears, taken at 4, 5, 6, 7 and 8 weeks of age, revealed that both absolute lymphocyte and small lymphocyte counts were normal in rats pinealectomized at birth or at 6 weeks.

ANTIBODY PRODUCTION

Titres of mercaptoethanol-resistant and mercaptoethanol-sensitive antibodies in rats pinealectomized at birth or in adult life were comparable with those of sham-operated controls, showing that the extirpation of the pineal body did not significantly affect the antibody producing capacity. Even the booster failed to discriminate between the ability of pinealectomized and of control rats to manufacture antibodies (Fig. 1).

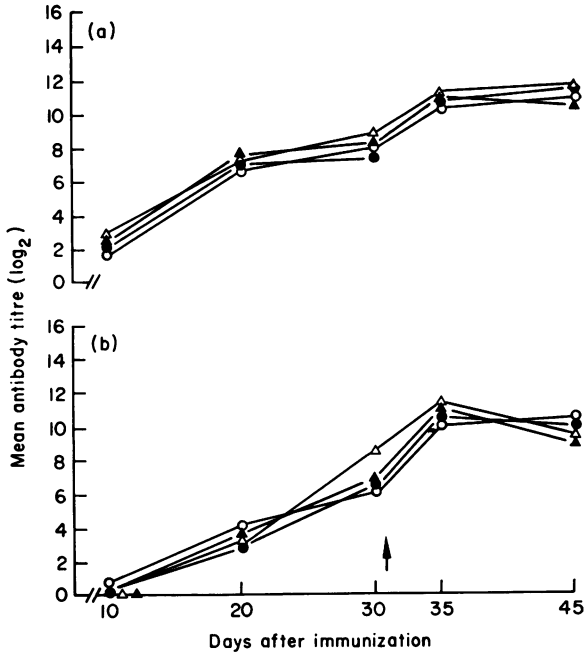

FIG. 1. Antibody production against BSA (passive haemagglutination method) in rats pinealectomized at birth or in adult life. (a) Pre-mercaptoethanol; (b) post-mercaptoethanol. Sham-pinealectomized at birth (●, ten rats); pinealectomized at birth (○, fourteen rats); sham-pinealectomized at 6 weeks (▲, fifteen rats); and pinealectomized at 6 weeks (△, eleven rats). The booster is indicated by arrow.

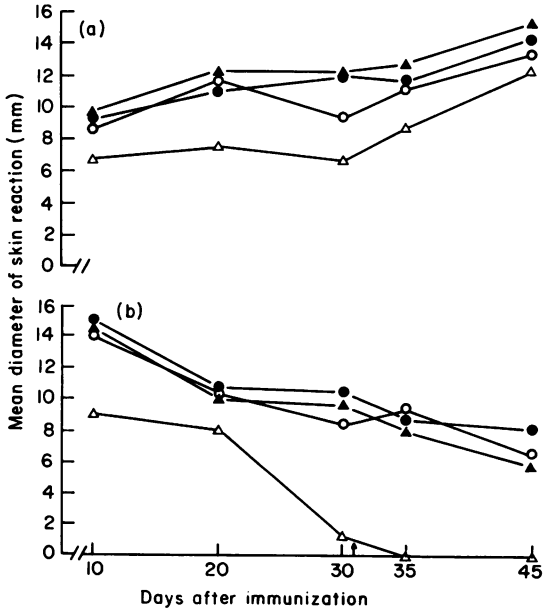

FIG. 2. Arthus (a) and delayed (b) reactions to BSA in rats sham-pinealectomized at birth (●, ten rats); pinealectomized at birth (○, fourteen rats); sham-pinealectomized at 6 weeks (▲, fifteen rats); and pinealectomized at 6 weeks (△, eleven rats). The booster is indicated by arrow.

ARTHUS REACTIVITY AND DELAYED HYPERSENSITIVITY

Neonatal pinealectomy did not influence the development and expression of either Arthus or delayed reactivity (Fig. 2). However, pinealectomy performed on 6-week-old rats caused a transient diminution in Arthus reactivity. The subcutaneous oedema was less pronounced in those animals.

Rats pinealectomized at 6 weeks and sensitized with BSA plus adjuvant showed a decrease of the delayed response, which lasted a shorter time than in the controls (Fig. 2).

SKIN HOMOGRAFT REJECTION

Rats pinealectomized at birth or in adult life rejected skin homografts rapidly in a manner indistinguishable from rejection in rats which were sham-operated at birth or at 6 weeks (Table 1).

TABLE 1

SKIN HOMOGRAFT REJECTION AND INCIDENCE OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN PINEALECTOMIZED RATS

Group	Homograft rejection		Allergic encephalomyelitis		
	No. of rats	Mean rejection time (days)	No. of rats	Degree of disease (mean score)	No. of rats with clinical signs of disease
Neonatally sham-pinealectomized	8	6·6	11	2·0	4
Neonatally pinealectomized	13	6·2	10	1·9	8
Sham-pinealectomized at 6 weeks	13	6·4	14	1·8	7
Pinealectomized at 6 weeks	11	7·9	10	0·5	0

INCIDENCE OF ALLERGIC ENCEPHALOMYELITIS

Histological examination revealed characteristic lesions in different parts of the central nervous system of all neonatally pinealectomized and sham-operated rats, and in animals sham-pinealectomized when 6 weeks old (Table 1). Allergic encephalomyelitis was less expressed in rats pinealectomized at 6 weeks. There were no lesions in five animals of this group, and the other five showed mild inflammatory foci in nervous tissue. None of the rats in this group developed neurological symptoms.

DISCUSSION

The mechanism which may account for the partial and temporary suppression of some immune reactions in rats pinealectomized in adult life is not clear. Since serotonin is probably the most effective oedemigenic agent in rats (Rowley and Benditt, 1956), and plays an ancillary role in the Arthus reaction (Brocklehurst, Humphrey and Perry, 1960), it may be assumed that the removal of the pineal in adult life is followed by a decrease in the total amount of serotonin, by a disturbance of normal serotonin distribution, and/or by some alterations of the metabolism of this amine. However, in explaining present results the force of this argument is somewhat weakened owing to the still current controversy over the function of serotonin in immune reactions in the rat (Austen and Humphrey, 1963).

Next to nothing is known about the function of serotonin in delayed hypersensitivity. O'Brien, Hughes and Newberne (1962) claimed that serotonin antagonists may reduce the clinical and histological signs of allergic encephalomyelitis. In this disease, cerebral vessels have increased permeability (Barlow, 1956). Since this permeability depends to some extent on serotonin (Liacopoulos, Halpern and Liacopoulos-Briot, 1957), then anti-serotonin drugs, by lowering vascular permeability through their action on serotonin, may prevent sensitized mononuclear cells from the circulation migrating towards the nervous tissue. A similar mechanism may be responsible for the reduced delayed skin reactions to BSA and lower incidence of demyelinating disease in rats pinealectomized in adult life. In addition, serotonin is involved in inflammation in the rat (Spector and Willoughby, 1965), and immune delayed reactions in fact represent inflammatory processes. Consequently, delayed reactions need not necessarily be the exclusive prerogative of sensitized cells. Serotonin may well play an ancillary role in the expression of a delayed response.

Another possibility that should be taken into consideration deals with observations that the extract from the pineal body induces lymphocytopoiesis (Urechia and Groza, 1927), and hyperplasia of lymphoid tissues (Milcou, 1957). Milin (1960) described the enlargement of lymph nodes in rats treated with aqueous extract of the epiphysis ('epiphysan'). He put forward the idea that the lymphocytopoietic effect of the pineal extract was due to the influence of extract on the hypophysis–adrenal system. The above data suggest that the pineal gland interferes in immune affairs *via* hormonal pathways. Since the embryonic derivation of the pineal is rather remote from immunologically reactive tissue, the slight immunosuppressive effect induced by pinealectomy in adult life might be attributed to the secretory function of the pineal body.

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