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Evidence for a Humoral Thymic Factor in Rabbits

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Summary. Rabbits thymectomized between 12 and 36 hours after birth subsequently show reduced levels of circulating lymphocytes together with a lowered immunological response to human γ-globulin. These effects of thymectomy can, to a large extent, be prevented by means of intraperitoneal autotransplants of thymus in a Millipore diffusion chamber. It appears possible that the epithelial-reticular cells of the transplant persist in the diffusion chamber and elaborate a humoral factor, or factors, which take part in the maturation of antibody-producing cells.

INTRODUCTION

Evidence for the existence of a humoral thymus factor has been provided by Levey, Trainin and Law (1963), Osoba and Miller (1964) and Law, Trainin, Levey and Barth (1964) who used Millipore diffusion chambers containing thymic tissue implanted in the peritoneal cavities of newborn thymectomized mice. It has also been shown by Aisenberg and Wilkes (1965) that a similar factor exists in rats.

The experiments which are described were designed to investigate the existence of a cell-free thymic factor in the rabbit which would be capable of altering the pattern of the immunological response of the animal. If this could be demonstrated, it would establish that such a factor of thymic origin is not peculiar to the mouse and rat but probably exists in most mammals.

MATERIALS AND METHODS

Forty-eight newborn rabbits were divided into three groups (I, II and III) so that controls for each experiment included litter mates. The fourteen members of Group I were thymectomized within 12–36 hours of birth under ether anaesthesia. The gland was exposed by splitting the sternum and was then carefully dissected from the front of the pericardium and great vessels under direct vision. In every case the tissue was confirmed to be thymus by histological studies. Aspiration techniques were found to be unreliable for total removal of thymic tissue but complete excision was performed with relative simplicity when the dissection technique as described above was used.

The fifteen experimental animals in Group II were similarly thymectomized but the thymus of each rabbit was immediately placed in a Millipore diffusion chamber which was then inserted into the animal's peritoneal cavity through a midline abdominal incision. The Millipore chamber consisted of a Perspex ring 10 mm in outside diameter,

6·4 mm in internal diameter and 1·6 mm in depth, sealed at each end with a nylon-reinforced Millipore filter disk of average pore size 0·45 μ and 100 μ thickness. The filter discs and the cement used for bonding them to the Perspex were obtained from the Millipore Filter Corporation, Bedford, Massachussetts.

The nineteen animals in Group III were not thymectomized and were used as normal controls.

Lymphocyte counts were performed on each of the thirty-five surviving rabbits when they were 2 months old.

At 3 months, five rabbits from Group I, six rabbits from Group II and seven rabbits from Group III were given three courses of immunization. The antigen used was human γ-globulin (Fraction II) obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio.

Each immunizing course consisted of:

1st day 10 mg γ-globulin in 1 ml normal saline by intraperitoneal injection.
2nd day 2·5 mg γ-globulin in 1 ml normal saline by intravenous injection.
3rd day, 4th day, 5th day } 10 mg γ-globulin in 1 ml normal saline by intravenous injection.

The first two courses were separated by an interval of 8 days while the second and third by one of 18 days. The animals were bled 10 days after their last injection and their sera tested for anti-human globulin using a modification of the tanned red-cell technique described by Boyden and Sorkin (1955).

All the rabbits were killed when they reached the age of 5 months. The chest was opened and the mediastinum searched to establish the presence of a thymus in the control animals and to confirm the absence of any thymic remnants in the thymectomized rabbits. One of the latter animals, in which a thymic remnant was found, was excluded from the experiment.

The Millipore diffusion chambers were removed from those animals bearing autotransplants, the contents fixed in formalin, sectioned, and then stained with haematoxylin and eosin.

RESULTS

Fig. 1 shows the lymphocyte counts in each of the three groups of animals at 2 months of age, and the mean of the count in each group is indicated. It will be seen that the mean count of the rabbits subjected to thymectomy alone is significantly lower than either of the other two groups. While the controls show a mean count of 3524 cells/mm^3 and those autotransplanted 3334 cells/mm^3, those animals deprived of thymic tissue attain a mean count of only 1706 cells/mm^3. An abnormally high lymphocyte count of 12,560 cells/mm^3 is excluded from Group II. This is found to make no difference to the statistical significance of the results.

Table 1 is the record of serum titres of anti-human globulin in thymectomized rabbits, thymectomized rabbits with an autotransplant and their controls. Their mean serum titres respectively are 139, 485 and 1470. It can be seen that the control group of animals and those thymectomized and bearing a thymic autotransplant produce titres substantially higher than those of the rabbits deprived of thymic tissue.

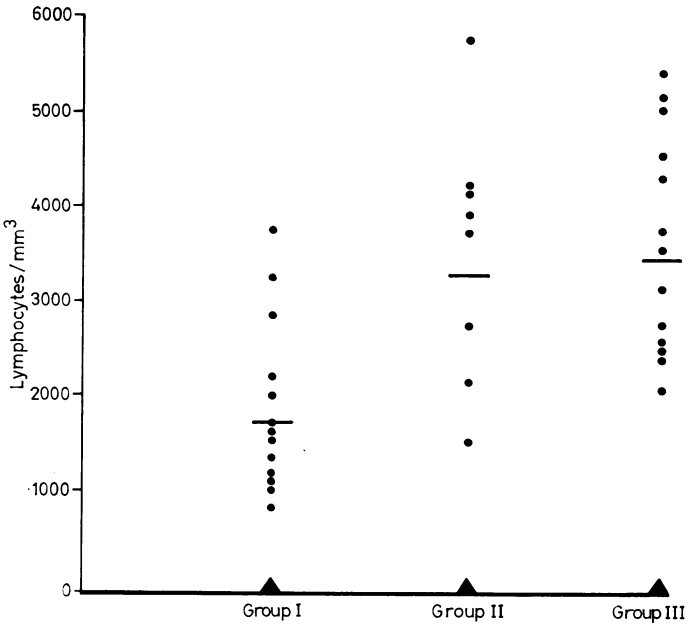


FIG. 1. Absolute lymphocyte counts (●) in 2-month old rabbits, thymectomized at birth (Group I), thymectomized at birth and autotransplanted with thymus in diffusion chamber (Group II), and their normal controls (Group III). The horizontal bar indicates the geometric mean.

The means for Groups I and II differ significantly at the 1 per cent level. Using logarithms of the counts, $t = 3{\cdot}78$, d.f. $= 21$, $P < 0{\cdot}01$.

The means for Groups II and III do not differ significantly. Again using logarithms of the counts, $t = 0{\cdot}42$, d.f. $= 21$, $P > 0{\cdot}6$.

TABLE 1

SERUM TITRES AND GEOMETRIC MEANS OF RABBITS THYMECTOMIZED, THYMECTOMIZED WITH AUTOTRANSPLANT AND CONTROL RABBITS IMMUNIZED AGAINST HUMAN γ-GLOBULIN

Group I (thymectomy only)	Group II (thymectomy with autotransplant of thymus in diffusion chamber)	Group III (normal controls)
320	5120	10240
160	640	1280
160	640	1280
80	320	640
80	160	640
—	80	—
Mean 139	Mean 485	Mean 1470

The means of Groups I and III differ significantly at the 1 per cent level. Using logarithms of the titres, $t = 3{\cdot}4$, d.f. $= 13$, $P < 0{\cdot}01$. The means of Groups I and II differ at the 10 per cent level, as do the means of Groups II and III. Again using logarithms of the titres, $t = 1{\cdot}71$, d.f. $= 13$, $P = 0{\cdot}10$.

Fig. 2 shows the histological features of the contents of Millipore diffusion chambers recovered from the peritoneal cavity of two of the rabbits after 5 months. The auto-transplanted thymic tissue at that time appeared to consist mainly of epithelial-reticular cells with only occasional small foci of residual thymocytes. Fig. 2(b) shows one of these foci.

All Millipore chambers removed after 5 months appeared to be intact, with the filter disk firmly adherent to its Perspex ring.

DISCUSSION

The results of these experiments strongly suggested that thymectomy performed between 12 and 36 hours after birth caused a significant reduction in the number of circulating lymphocytes and produced an alteration in the immunological response of the rabbit as demonstrated by a reduction in its capacity to produce antibody to human γ-globulin. That this effect is not peculiar to human γ-globulin has been shown by the work of Good, Dalmasso, Martinez, Archer, Pierce and Papermaster (1962), when they demonstrated a similar immunological unresponsiveness towards bovine serum albumin in rabbits thymectomized within the first 5 days of life.

In contrast to our findings with rabbits, Aisenberg and Wilkes (1965), using neonatally thymectomized rats, have shown that homografts of thymic tissue in Millipore diffusion chambers failed to restore peripheral blood lymphocyte levels to normal but did produce a partial restoration of the delayed hypersensitivity reaction to bovine serum albumin.

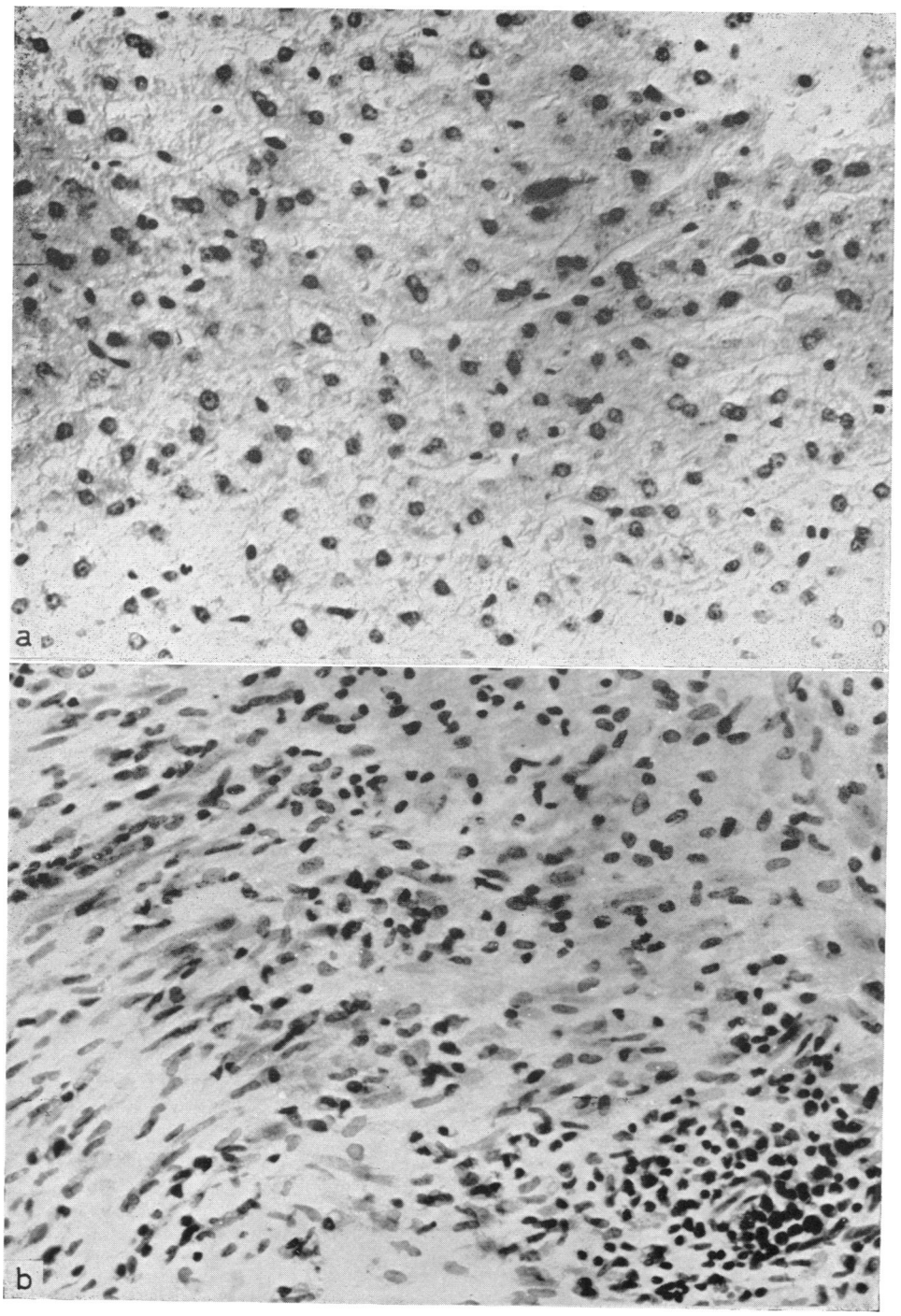
It has been assumed throughout this work that the Millipore diffusion chambers used prevented the passage of cells in either direction. Some doubt exists as to this impermeability as evidenced by Hays (1964) who showed that mouse lymphoma cells passed through Millipore membrane of average pore size 0·45 μ. However, Holub (1958), using diffusion chambers constructed with Millipore filter material of average pore size 0·8 μ, placed in the peritoneal cavity of rabbits and examined after 45 days, did not find any evidence of cell permeability. Studies on the permeability of the diffusion chambers used in the current experiment are being carried out in this laboratory and it is hoped the findings will be the subject of a further publication. Preliminary results indicate that the diffusion chambers used in our experiments were not cell permeable when tested in the peritoneal cavity of the rabbit.

This communication has therefore shown that, as in mice, the thymus enclosed in an apparently cell-tight chamber was capable of influencing two mechanisms. The first was the development of the potential to produce antibody to foreign serum proteins and secondly the maintenance of the circulating lymphocyte population of the animal at normal levels. Exactly how these effects are related needs further clarification.

Since the thymic remnant in the Millipore diffusion chamber showed a great reduction in the number of thymocytes and consisted largely of epithelial-reticular cells, it seemed possible that the epithelial-reticular cells elaborated a humoral factor, or factors, which influenced the immunological maturity of the animal.

FIG. 2. (a) Histological section of thymus enclosed for 5 months in Millipore diffusion chamber and recovered from neonatally thymectomized rabbit. The predominance of epithelial-reticular cells and scarcity of thymocytes will be noted.

(b) Thymus from another diffusion chamber at 5 months showing one of the occasional small foci of residual thymocytes.



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