magnification of ×1000, to detect the mitotic figures. The number of adrenal medullary cells was determined as follows: the sections were photographed and the negative films were projected on a paper sheet of uniform thickness; the pictures of the medulla were cut out and weighed. In 1 out of 6 sections the nuclei of the cells were counted with the microscope. The number of the cells per whole adrenal medulla was determined according to the following relation: $A:B = X:C$, where: A is the total number of cells counted in all the sections examined; B is the weight (in mg) of the paper pictures corresponding to the sections examined; C is the weight (in mg) of all the paper pictures; X is the number of the cells in the whole adrenal medulla. The data obtained were corrected using the equations reported by HAUG[4] to obviate the error due to the presence of the same cell in several contiguous sections.

As shown in the Figure, the number of mitoses in the adrenal medullary cell decreases in a linear fashion with the log of the age until 4 months, the reverse behaviour is shown by the number of chromaffin cells, as well as the body weight; both of these parameters, in fact, increase until 4 months and then reach a plateau. The ratio between the number of mitoses observed in the presence of colchicine and that observed in the absence of colchicine is almost costant (2.6–2.2).

Unilateral adrenalectomy in the adult rat does not result in a substantial increase of the number of either mitoses or cells per whole adrenal medulla (Table). This result is therefore in agreement with that reported by McKAY and McKAY[5], who showed no increase in the volume of the remaining medulla after unilateral adrenalectomy in the rat. The lack of hyperplasia in the remaining adrenal medulla could be explained by the presence of extramedullary chromaffin tissue.

The observation that mitotic activity occurs in the chromaffin cells of the adrenal medulla of the mouse is in agreement with analogous findings obtained in the rat[1,2,6,7], the golden hamster[8] and the rabbit[9]. These results therefore demonstrate that a proliferative ability persists in the chromaffin cells of the adrenal medulla during the postnatal life, although the adrenal medullary cells have been thought to be postmitotic cells[10], on the basis of their origin from sympatogonic cells. Since the mitotic activity is inversely related to the increase in the cell number, it appears that the only consequence of the observed mitotic activity is the postnatal growth of the organ. This seems to be particulary true in the first 4 months of the postnatal life, when a conspicuous variation in the number of both mitoses and cells occurs. The persisting mitotic activity in adult mice (4–12 months) is apparently associated with the slow increase in cell number and therefore could be responsible for additional growing processes (a continuous increase in body weight, although to a progressively reduced extent occurs in mice with aging); however, renewal processes, as acknowledged by some authors[8,9], cannot be excluded.

As shown by the presence of chromaffin reaction, mitoses occur in differentiated elements, thus excluding the possibility that immature cells remaining in the gland during the postnatal life are responsible for the observed mitotic activity[11].

Number of mitoses and of cells per whole adrenal medulla following unilateral adrenalectomy[a]

Days after unilateral adrenalectomy	No. of mitoses per whole adrenal medulla[b]	Cell number per whole adrenal medulla
5	2	24062
10	4	25200
15	3	23878

[a] 4 months old mice were used. [b] Mitoses were counted after colchicine treatment.

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Experimental Allergic Encephalomyelitis in T-Lymphocyte Deficient Rats

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Summary. Thymectomized, lethally irradiated rats reconstituted with syngeneic bone marrow were injected with rat brain in complete Freund adjuvant mixture. Both, they and sham-thymectomized, irradiated and bone marrow protected rats displayed a higher incidence of leg paralysis than normal non-irradiated animals. Thymectomy lowered the incidence of the disease.

The mechanism of experimental allergic encephalomyelitis (EAE) became rather obscured when it was found that the disease can be elicited without a mononuclear cell attack upon the neural tissue[2]. Recently it has also been shown that autoimmune thyroiditis developes in neonatally thymectomized rats given 3-methylcholanthrene[3]. This would suggest that the activity of circulating antibodies is at the bottom of at least these autoimmune

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Experimental allergic encephalomyelitis (EAE) in normal and T-lymphocyte deficient rats

Rats injected with encephalitogenic vaccine	No. of rats	No. of rats undergoing paralysis within days				Total paralyzed rats	Dead/ paralyzed rats
		8–14	15–21	22–28	29–35		
Normal	16	4	1		1	6/37%/	2/6
Thymectomized	10	1		1		2/20%/	1/2
Sham-thymectomized, irradiated, bone marrow reconstituted	11	5	2	1		8/72%/	6/8
Thymectomized, irradiated, bone marrow reconstituted	16	4	4	1	1	10/63%/	6/10

diseases. To obtain more information on mechanisms of EAE pathogenesis, we tried to induce the disease in T-lymphocyte deficient rats.

Materials and methods. Rats of WVM strain (derived from Wistar stock) of both sexes were used. They were thymectomized at the age of about 4 weeks. Through an incision in the neck, the thymus was sucked out of the thorax. 2 months after thymectomy, the rats were irradiated with a dose of 750 R. Irradiation constants were: 220 kV, 15 mA, filters 0.5 mm Cu and 1.0 mm Al, distance to target 40 cm, dose rate 100 r/56 sec measured in air. Within 4 h after irradiation, the rats were given i.v. 60×10^6 syngeneic bone marrow cells suspended in 1 ml of buffered physiological saline. 2 months after irradiation and bone marrow reconstitution, the animals were given intradermally 10 doses of 0.1 ml of the rat brain homogenate in complete Freund adjuvant mixture (Difco). Control groups consisted of sham-thymectomized, irradiated and bone marrow reconstituted rats, only thymectomized rats and normal rats. All controls, as also the experimental group, were given the encephalitogenic vaccine.

Results. As shown in the Table, irradiated rats either thymectomized or sham-thymectomized before irradiation and bone marrow reconstitution, showed a higher incidence of paralysis after the encephalitogenic vaccine injections than normal and thymectomized non-irradiated controls. This seems to suggest that irradiation favours the incidence and development of EAE. Thymectomy alone exerted a slight lowering of the EAE incidence in comparison with normal controls. Severity of the disease, as estimated after the mortality in sick rats, seems to follow the incidence of EAE.

Inspection of the brain and medulla sections stained with haematoxylin and eosin revealed mononuclear cell infiltrations and extravasates only in the group of normal control rats which fell ill after the inoculation of the encephalitogenic vaccine. In the paralyzed animals of all other groups, no cellular infiltration was observed.

Discussion. The enhancing effect of irradiation upon the development of EAE was expected in view of the earlier findings of a favorable activity of irradiation upon the incidence of the disease[4]. However, this effect of irradiation was also visible in the rats made deficient in T-lymphocytes, thus supporting the reports mentioned in the introduction that there is no essential need for a mononuclear cellular immune reaction to induce EAE[2]. This appeared to be further supported when no cellular infiltration could be found in the brain and medulla of T-lymphocyte deficient, severely paralysed rats.

The conclusion that B-lymphocytes had to be primed with the encephalitogenic vaccine in thymectomized, irradiated bone marrow recipients in the absence of T-lymphocytes appears erroneous. One possible explanation could also be based upon the assumption that the immunocytes transferred with the bone marrow were already primed with selfneural tissue antigens in normal bone marrow donors which had an intact thymus. The injection of the vaccine would act accordingly, as the booster antigen injection.

It is questionable if the lower sensitivity to the encephalitogenic vaccine in thymectomized nonirradiated rats could be ascribed to the lack of priming due to the T-lymphocyte deficiency after the thymus removal in young adult animals.

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Inflammatory Factors Produced by Sensitized Guinea-Pig Peripheral Blood Lymphocytes

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Summary. The supernatants obtained from stimulated tuberculin-sensitive guinea-pig peripheral blood lymphocytes contain factors that induce a cutaneous inflammatory response in normal guinea-pigs similar to the tuberculin reaction and inhibit the migration of normal guinea-pigs peritoneal exudate cells. There appears to be a correlation between the presence of in vitro migration inhibitory activity and inflammatory activity in vivo.

The production of soluble mediators such as migration inhibition factor (MIF) or skin reactive factor (SRF) has been demonstrated in the guinea-pig using lymph node cells[2,3] or peritoneal exudate lymphocytes[4,5] stimulated with antigens such as PPD or BGG.

Since it is generally accepted that the production of these mediators is an in vitro correlate of delayed hypersensitivity[6] it would be expected that circulating mononuclear cells i.e. those found in the peripheral blood, would be involved in cutaneous delayed hypersensitivity responses. In addition the in vivo assay of these mediators (as SRF) should correlate with in vitro results as assessed