

Changes in the properties of the plasma membrane were observed by workers who studied the proliferation of cells transformed by oncogenic viruses. Terskikh and Malenkov [4], working with a stationary culture of Chinese hamster fibroblasts, found that as early as 15 min after a change of medium, inducing cell proliferation, the permeability of the plasma membrane of the cell induced to divide underwent sharp changes. The results of the present investigation are evidence that the sharp increase in the intensity of synthesis of certain plasma membrane proteins is another of the earliest events taking place in the cell preparing for division.

LITERATURE CITED

1. I. D. Belyaeva, *Tsitologiya*, 15, 1297 (1973).
2. L. V. Orlova, N. V. Smirnova, and V. M. Rodionov, *Tsitologiya*, 12, 111 (1970).
3. V. V. Terskikh, in: *The Cell Cycle* [in Russian], Moscow (1973), p. 165.
4. V. V. Terskikh and A. G. Malenkov, *Tsitologiya*, 15, 7 (1973).
5. R. Baserga, *Cell Tissue Kinet.*, 1, 167 (1968).
6. P. Emmelot, C. J. Bos, E. I. Benedetti, et al., *Biochim. Biophys. Acta*, 90, 126 (1964).
7. C. H. Fiske and J. A. Subbarow, *J. Biol. Chem.*, 66, 375 (1925).
8. E. W. Gerner, M. C. Glick, and L. Warren, *J. Cell. Physiol.*, 75, 275 (1970).
9. D. M. Neville, *J. Biophys. Biochem. Cytol.*, 8, 413 (1960).
10. D. M. Neville, *Biochim. Biophys. Acta*, 133, 168 (1967).
11. J. L. Roti, S. Okada, and H. Eberle, *Exp. Cell Res.*, 76, 200 (1973).
12. M. R. Stetten, *J. Biol. Chem.*, 239, 3576 (1964).
13. L. Warren and M. C. Glick, *J. Cell. Biol.*, 37, 729 (1968).

MORPHOLOGICAL AND FUNCTIONAL RELATIONS BETWEEN THE THYMUS AND ADRENALS IN INBRED MICE

V. V. Zarudin and V. F. Semenov

UDC 577.391:612.017.1

A morphological investigation was made of the thymus, adrenals, and spleen of mice belonging to the CBA, C57BL/6, and AKR lines, and the immunologic reactivity of the mice to the sex antigen of a skin graft was studied together with determination of the plasma corticosterone level. A narrower cortical layer in the thymus, wide thymus-dependent zones in the spleen, and a higher corticosterone level were observed in CBA mice than in C57BL/6 and AKR mice, and no reaction was found in the former to sex antigen of the skin graft. Adrenalectomy on CBA mice led to a sharp increase in width of the cortex of the thymus, the appearance of numerous lymphatic follicles in the spleen, and the development of an immunologic reaction to sex antigen of the skin graft. In B mice of the CBA line atrophy of the adrenal cortex was observed, with a sharp decrease in the concentration of sudanophilic lipids.

KEY WORDS: *cortex of the thymus; adrenals; B mice; sex antigen.*

Adrenocortical hormones (glucocorticoids) are widely used in clinical and experimental practice to inhibit reactions of tissue incompatibility. Glucocorticoids act on the central organ of immunity — the thymus [7]. The cortex of the thymus contains cortisone-sensitive thymocytes, which on migration into the medullary layer become cortisone-resistant, like peripheral T lymphocytes [10]. It is accordingly interesting to study the effect of adrenocortical function on the thymus and T cell activity detectable by the transplantation immunity test, as well as to determine the morphological and functional state of the adrenals in B mice in the absence of the thymus. The dependence of the immunologic reaction on sex antigen

Departments of Pathological Anatomy and Microbiology, Smolensk Medical Institute. (Presented by Academician of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 85, No. 5, pp. 591-594, May, 1978. Original article submitted November 1, 1977.

of the skin graft on the plasma corticosterone level in inbred mice [4] was established previously. In the present investigation an attempt was accordingly made to study the possible role of morphological and functional interrelations between the thymus and adrenals in CBA, C57BL/6, and AKR mice, differing in their plasma corticosterone levels and in their immunologic reactions to skin graft sex antigen.

EXPERIMENTAL METHODS

Altogether 235 inbred mice (160 CBA, 45 C57BL/6, and 30 AKR mice) were used. The mice were obtained from the Stolbovaya nursery of inbred animals, Academy of Medical Sciences of the USSR. The body weight of the animals was 18-21 g and their age 3-4 months. There were three series of experiments and in each series organs and tissues of 20 control and experimental mice were investigated histologically. In series I mice of strains CBA, C57BL/6, and AKR were used and morphological changes were studied in the thymus, spleen, and adrenals by the ordinary staining method (hematoxylin-eosin, Van Gieson), and also a special method (Brachet's method, Sudan III). Besides these morphological investigations, the plasma corticosterone level was determined and the immunologic reaction to sex antigen in females after transplantation of skin from a male of the corresponding strain was studied. The corticosteroid level in the blood plasma was determined by the method of Usvatova and Pankov [5]. Skin grafting and assessment of the taking of the skin grafts were carried out by the method described previously [2]. The skin grafts were transplanted onto 15 mice of each strain.

In the experiments of series II bilateral adrenalectomy was performed on CBA and C57BL/6 mice. Sodium chloride solution was given to the adrenalectomized animals. Three days after adrenalectomy, skin grafts from males of the same strain were transplanted on to 15 female CBA mice. Histological investigations of the thymus and spleen were carried out 5, 10, and 16 days after adrenalectomy. A histological investigation was undertaken of the adrenals and spleen of B mice 1.5, 2.5, and 3.5 months after thymectomy. The method of obtaining B mice of the CBA strain was the same as that used previously [5]. Completeness of removal of the thymus from the B mice was verified histologically and the T cell immunodeficiency was verified by transplantation of skin allografts from C57BL/6 mice. Some CBA mice after thymectomy and lethal irradiation were given injections of syngeneic bone marrow in a dose of 10 million nucleated cells from donors treated with hydrocortisone in a dose of 2.5 mg daily for 3 days. To determine the presence of cells with the θ marker in the bone marrow, anti- θ -serum was used in the cytotoxic test [1]. The anti- θ -serum was obtained by repeated immunization of AKR mice with CBA mouse thymocytes.

EXPERIMENTAL RESULTS

In the experiments of series I, in CBA mice, unlike in C57BL/6 and AKR mice, a narrow cortical layer was regularly found in the thymus (Fig. 1a, b), whereas in the spleen the thymus-dependent periarteriolar zone, consisting of small lymphocytes, was wider. The lymphatic follicles in the CBA mice were difficult to distinguish; they contained no reactive centers, whereas in the C57BL/6 and AKR mice the lymphatic follicles were large and consisted mainly of large lymphocytes and blast cells. Histological examination of the adrenals of mice of these strains also showed differences. For instance, in the CBA mice on staining with Sudan, many lipid granules were constantly found in the adrenal cortex, especially in the columnar zone. The lipid granules were round in shape and almost completely filled the cytoplasm of the cells. In the C57BL/6 and AKR mice the number of lipid granules was much smaller. A study of the plasma corticosteroid level showed a higher content of the hormone ($80 \pm 2.5 \mu\text{g} \%$) in the CBA mice than in the C57BL/6 mice ($35.8 \pm 2.8 \mu\text{g} \%$) or AKR mice ($33.2 \pm 3.4 \mu\text{g} \%$; $P < 0.01$). No immunologic reaction to sex antigen was present in the CBA mice, whereas in the C57BL/6 and AKR mice it was strong. The mean period of survival of the skin grafts on the C57BL/6 and AKR mice was 21.9 ± 0.9 and 15 ± 2.5 days, respectively.

In the experiments of series II, after bilateral adrenalectomy marked widening of the cortex of the thymus was observed in the CBA mice (Fig. 1c). In C57BL/6 and AKR mice the cortical layer of the thymus either was unchanged after adrenalectomy or was not so sharply increased. In the adrenalectomized CBA mice, lymphatic follicles with wide reactive centers, consisting mainly of large lymphocytes, were more numerous in the spleen at all times of investigation than in intact animals. The periarteriolar thymus-dependent zone remained wide and contained cells of the small lymphocyte type. In the C57BL/6 mice after adrenalectomy, wide periarteriolar thymus-dependent zones appeared in the spleen after adrenalectomy. Char-

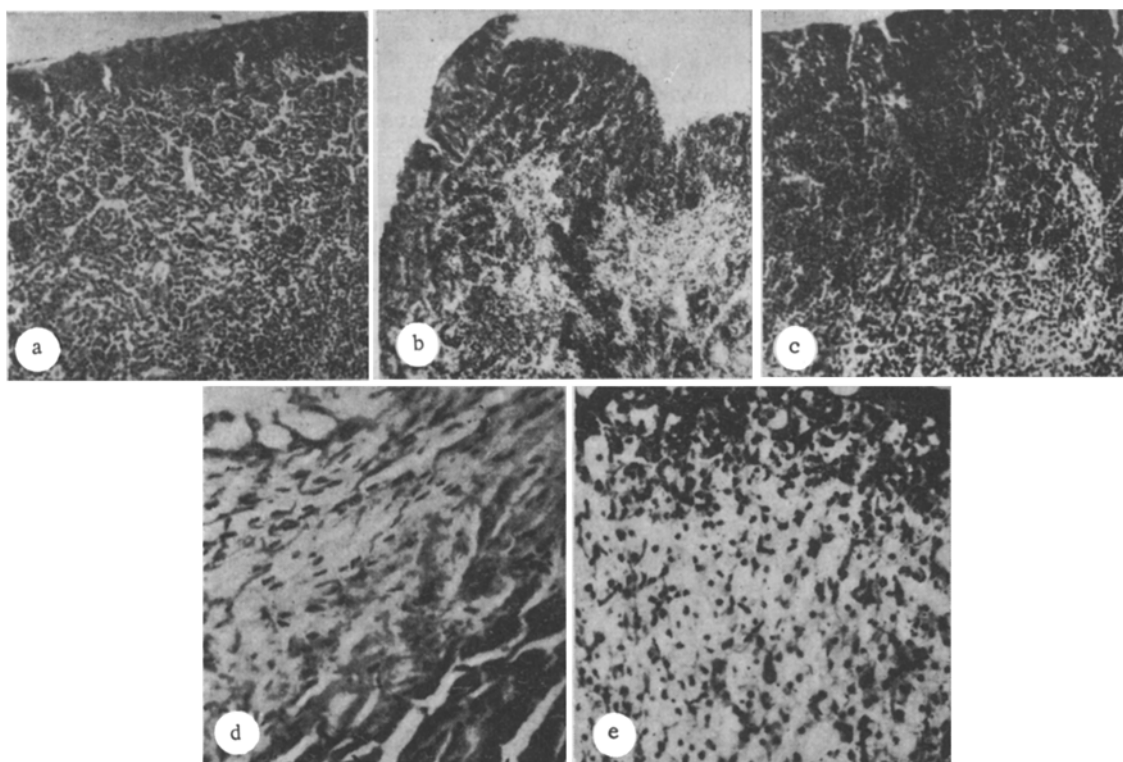


Fig. 1. Morphological changes in thymus of CBA and C57BL/6 mice and in adrenals of B mice (hematoxylin-eosin). a) Narrow cortical layer of thymus of CBA mouse (140 \times); b) wide cortical layer of thymus of C57BL/6 mouse (56 \times); c) marked widening of cortex of thymus in CBA mouse 10 days after bilateral adrenalectomy (140 \times); d) marked sclerosis of adrenal cortex in B mouse 2.5 months after thymectomy (280 \times); e) preservation of structure of adrenal cortex in B mouse protected against irradiation by syngeneic bone marrow of donors treated with hydrocortisone (140 \times).

acteristically, in adrenalectomized CBA mice, by contrast with the intact animals (series I), rejection of the skin grafts was observed toward the 25th day after transplantation.

In the experiments of series III, on histological examination of the spleen of the B mice, depopulation of the periarteriolar thymus-dependent zones and widespread amyloidosis of the spleen were observed on histological investigation. Special attention is drawn to the histological changes in the adrenals, the sharp atrophy of all zones of the cortical layer, and the development of focal or diffuse sclerosis (Fig. 1d). These changes were studied over a period of time. In the initial stages 1.5 months after obtaining the B mice, vacuolation of the cortical cells and atrophy of the cortical layer, with a sharp decrease in the lipid content, followed by the development of focal and later of diffuse sclerosis and hyalinosis, were observed. No significant changes could be found in the medullary layer of the adrenals under the light microscope. It is interesting to note that when syngeneic bone marrow from donors treated with hydrocortisone was used to protect the B mice against irradiation, the structure of the adrenal cortex at the above-mentioned times of investigation was not significantly changed (Fig. 1e), the boundaries between the layers were fairly clear, and a large quantity of pseudanophilic lipids could be seen in the cytoplasm of the cells of the cortical layer. Up to 20% of cells carrying the θ marker could be detected in the bone marrow of donors receiving hydrocortisone, in the cytotoxic reaction; this is evidence that cortisone-resistant T lymphocytes, which were evidently capable of delaying the development of the atrophic and sclerotic changes in the adrenal cortex of the mice, had entered the bone marrow of the donors [6].

Consequently, C57BL/6 and AKR mice differ from CBA mice in possessing the following morphological features of their immune system: a wide cortex of the thymus, many lymphatic follicles and narrow periarteriolar thymus-dependent zones in the spleen. A high blood plasma

corticosterone level in CBA mice was combined with a high lipid content in the adrenal cortex, whereas in AKR and C57BL/6 mice the lipid content in the adrenal cortex was low. AKR mice are known to be distinguished by hypofunction of the adrenal cortex, with which the characteristic preleukemic changes in the thymus tissue are associated [9]. In the present experiments the CBA mice did not react to sex antigen, whereas in C57BL/6 and AKR mice a well-marked reaction to this antigen was observed, in agreement with data obtained by other workers [8].

On the basis of comparison of the morphological and functional data it can thus be postulated that adrenocortical function and the blood plasma corticosterone level in inbred mice may affect the thickness of the cortical layer of the thymus, the morphology of the spleen, and the intensity of the immunologic reaction induced by weak transplantation antigen. Adrenalectomy on CBA mice led to a sharp increase in width of the cortex of the thymus, to the appearance of many lymphatic follicles with wide reactive centers in the spleen, and to the development of an immunologic reaction to the skin graft sex antigen.

LITERATURE CITED

1. B. D. Brondz, Vopr. Onkol., No. 8, 64 (1964).
2. V. F. Semenov, Eksp. Khir., No. 1, 43 (1969).
3. V. F. Semenov and A. P. Bogdanov, Dokl. Akad. Nauk SSSR, 225, 704 (1975).
4. V. F. Semenov and O. V. Molotkov, Dokl. Akad. Nauk SSSR, 214, 1437 (1974).
5. I. M. Usvatova and Yu. A. Pankov, in: Modern Methods of Determination of Steroid Hormones in Biological Fluids [in Russian], Moscow (1968), p. 38.
6. J. J. Cohen, J. Immunol., 108, 841 (1972).
7. T. F. Dougherty, Physiol. Rev., 32, 379 (1952).
8. D. L. Gasser and W. K. Silvers, Adv. Immunol., 15, 215 (1972).
9. D. Metcalf, Cancer Res., 20, 1347 (1960).
10. J. D. Stobo and W. E. Paul, J. Immunol., 110, 362 (1973).

LOCALIZATION OF TAGGED CELLS IN THE ISLETS, ACINI, AND PERI-INSULAR ZONE OF THE MOUSE PANCREAS AFTER RESECTION

V. P. Krasnov

UDC 612.34-087.45

[³H]Thymidine was injected five times in the course of the 24-h period into CBA × C57BL/6 hybrid mice in which about 40% of the tissue of the pancreas has been resected. The number of tagged cells in the islets and acini did not change with an increase in the time elapsing after the last injection of thymidine (5-18 days). The localization of the tagged cells in the pancreatic islets in the later stages did not differ significantly from that observed 2 h after injection of the isotope into the animals. On examination of medium-sized and large islets as far removed as possible from the site of injury, a very similar number of tagged cells per unit area was found in the central, middle, and peripheral zones of the islets 2 h and 18 days after the last injection of the isotope.

KEY WORDS: *mouse pancreas; localization of tagged cells; peri-insular zone.*

The problem of the sources of formation of the pancreatic islets in intact animals and under experimental conditions still remains a matter for debate. Many workers consider that the formation of the cell population of the islets takes place on account of external sources: 1) by transformation of exocrine cells from the terminal portions [1, 2, 4-7, 9, 12, 13]; 2) from cells of the ducts [3, 10, 11, 14]; 3) from special precursor cells, also belonging to

Department of Anatomy, Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 85, No. 5, pp. 594-598, May, 1978. Original article submitted October 31, 1977.