CYTOPLASMIC FRAGMENTATION OF LYMPHOCYTES (CLASMATOSIS) IN ADRENALECTOMIZED MICE

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Marked inhibition of cytoplasmic fragmentation (clasmatosis) of lymphocytes in the spleen, lymph glands, and thymus is observed 7 days after the operation in adrenalectomized CBA mice. A return to the previous levels occurs after 12-14 days. The reciprocity of the time–effect curves for the weight of the lymphoid organs and for clasmatosis confirms the connection between this phenomenon and cytodifferentiation.

Clasmatosis or cytoplasmic fragmentation of lymphocytes consists of the separation of spherical anuclear fragments (leptons) from cells in various stages of maturation.

The systemic character of clasmatosis, the nonspecificity of the clasmatotic reaction to various factors, its kinetic features [2, 3, 9, 10], and also the effect of hormones on this phenomenon, as reflected by data in the literature [4, 6, 12], suggested that clasmatosis is adaptive in nature and that in vivo it is under corticosteroid control.

Since the most radical and effective method of changing the hormonal balance is by removing an endocrine gland (or glands) whose role in the particular phenomenon is to be studied, adrenalectomy was performed on mice and, by dynamic observations on the animals, an attempt was made to study the pattern of clasmatosis after total exclusion of adrenal function. No data on this problem are to be found in the literature.

EXPERIMENTAL METHOD

Male CBA mice weighing 18-20 g were kept before and during the experiment under standard conditions (10 animals in each cage, food and water ad lib.). Before the operation the animals were anesthetized with pentobarbital (1 mg/10 g body weight, intraperitoneally), and both adrenals were then removed through a standard skin incision in the lumbar region followed by division of the lumbar muscles allowing for the topography of the kidneys. To prevent bleeding, ligatures were applied to the vascular pedicles of the glands and the operation wounds were sutured in layers. A mock adrenalectomy was performed on the control animals (all stages of the operation except actual removal of the adrenal were carried out). During the postoperative period both the experimental and the control animals received 0.5% NaCl solution to drink.

Mice from the experimental and control groups were sacrificed five at a time at different times after the operation. The spleen and thymus were weighed on torsion scales. Films were prepared from the spleen, thymus, and cervical lymph glands and stained by the Romanovsky–Giemsa method. The ratio in per cent between leptons and nucleated cells was calculated after counting in the films under a magnification of 950 times [11]. The results were presented as time–effect curves for each lymphoid organ.

EXPERIMENTAL RESULTS

The time–effect curves for CBA mice given in Fig. 1 show that in all three organs clasmatosis became less active 2-3 days after adrenalectomy than in the intact animals and animals undergoing themock

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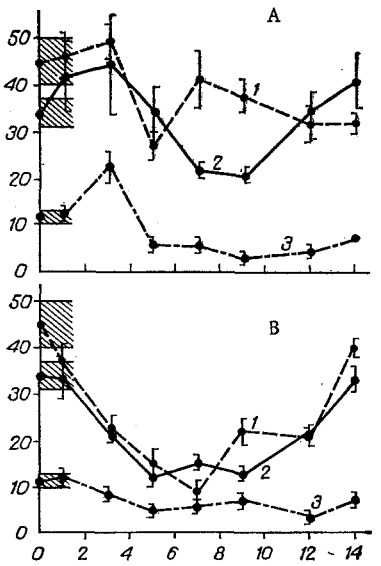

Fig. 1

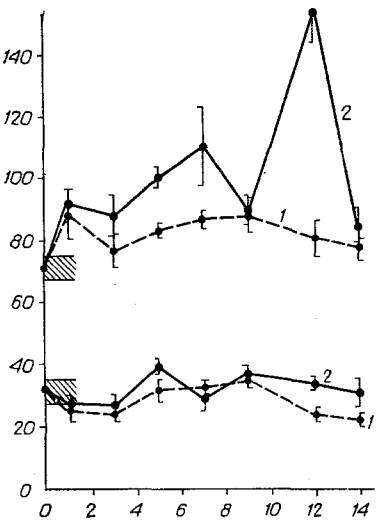

Fig. 2

Fig. 1. Effect of adrenalectomy on relative proportion of leptons in spleen (1), cervical lymph glands (2), and thymus (3) of CBA mice. Abscissa, time in days after operation; ordinate, number of leptons per 100 nucleated cells. A) mock operation; B) adrenalectomy. Each point represents mean for five animals ± standard error of the mean. Here and in Fig. 2, shaded areas represent levels of corresponding parameters in intact mice.

Fig. 2. Changes in weight of spleen (above) and thymus (below) in adrenalectomized CBA mice. Abscissa, time in days after operation; ordinate, weight (in mg): 1) mock operation, 2) adrenalectomy (± error of mean).

operation; by the 7th-11th day it reached its lowest level (about 20% of the initial value) after which (12th-14th days) it returned quickly to the normal values.

Changes in the weight of the spleen and thymus of the adrenalectomized CBA mice and mice undergoing the mock operation are given in Fig. 2. After adrenalectomy the weight of both organs increased, but whereas this increase for the thymus was small and could be detected only by comparison with the figures for the mice after the mock operation, the weight of the spleen rose rapidly in both absolute and relative figures to reach 200% of its initial level after 12 days. On the 14th day the weight of the spleen fell sharply to normal.

The increase in mass of the thymus and peripheral lymphoid organs in adrenalectomized animals is a long-established fact [5, 7, 8], and in the present case it was interesting only because it gave indirect evidence of the hormonal state of the animal, and because comparing weight with clasmatosis gave information on the ratio between the proliferative and differential components in the cytokinetics of the lymphoid cells at different periods after adrenalectomy. In particular, comparison of the time–effect curves for clasmatosis and the weight of the organs demonstrated marked reciprocity between them; this meant that clasmatosis was inversely proportional to the intensity of cell division. These mutual relations are determined by the nature of the action of corticosteroids on lymphocytes. Both the rapid restoration of the normal level of clasmatosis (12th-14th days) and the corresponding sharp decrease in weight of the spleen will be noted in Figs. 2 and 3.

The last of these findings was unexpected, for it could be explained only by restoration of the normal level of corticosteroids in the blood in the absence of the adrenals. Meanwhile, the presence of additional adrenal tissue which could account for this recovery is considered to be a characteristic feature only of rats [1] and not of mice. Accordingly, some of the adrenalectomized mice were investigated in the late periods after the operation (14th-21st days) to obtain anatomical and histological proof that the adrenals

can regenerate in these animals. The results showed that anatomical restoration of one of the removed adrenals took place in two of the seven adrenalectomized mice (in the normal position, mass of the regenerating organ about one-third of the usual); the results of histological examination of sections from these adrenals revealed a normal structure of the gland. In the remaining mice only nodules of "brown fat" were found in the perinephric cellular tissue. True regeneration of the adrenals, evidently due to their viable microscopic fragments, which in some cases remained in the perinephric cellular tissue, is thus not the rule and cannot be the explanation of the recovery of clasmatosis which was observed. It must be assumed that in this case hormonal compensation occurred on account of the additional adrenal tissue.

The reciprocity of clasmatosis and the weight of the lymphoid organs described above in the adrenalectomized animals, the systemic character of the response to this factor, and its kinetics are evidence in support of the hypothesis that clasmatosis is differential in nature and that the phenomenon is under the control of the adrenal cortex.

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