not associated with the microsomes. The daily injection of smaller amounts of these compounds resulted in no significant effect on protein metabolism. The data appear to indicate that these agents may, under certain circumstances, have a deleterious effect on the protein balance of the organism.

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Thymic Control of Cellular Differentiation in the Immunological System* (32705)

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In mice, removal of the thymus at birth results in lymphopenia and deficient immunological responses (1). Similarly, when the immunological system of animals thymectomized in adult life is damaged by irradiation, recovery of immune responsiveness is impaired when compared with that of intact, irradiated controls (1). However, little is known about the precise functions of the thymus at the cellular level. The results of recent studies on the various classes of cells responsible for immune responses now make it possible to examine the stage in differentiation for which the presence of the thymus is essential. Differentiation in the immunological system is based on at least three classes of cells. The most differentiated class consists of antibody-producing cells which arise by proliferation and differentiation from antigen-sensitive precursors (2,3). Antigen-sensitive cells appear to have limited proliferative potential (4), suggesting that their numbers may be replenished from a yet more primitive precursor. Evidence for the existence of such precursors has been obtained from studies of bone marrow and fetal liver. These tissues contain no demonstrable antigen-sensitive cells (4); nonetheless, when bone marrow or fetal liver cells are given to heavily irradiated mice there is eventual recovery of immunological responsiveness (5,6). Thus, bone marrow and fetal liver contain a class of precursors which are not sensitive to antigen, but which have the capacity to differentiate, giving rise to antigen-sensitive cells.

Mice thymectomized at birth contain very few antigen-sensitive cells (7). However, when antigen-sensitive cells from normal mice are injected into neonatally thymectomized mice they yield a normal number of plaque-forming cells (8). This experiment indicates that the thymus plays little, or no role in the differentiation of antigen-sensitive cells to plaque-forming cells. My experiments were designed to determine whether or not the thymus plays a role in the differentiation of bone marrow precursors to antigen-sensitive cells.

A direct assay for the marrow precursors of antigen-sensitive cells is not available. Therefore, these precursors were studied indirectly by injecting normal bone marrow into heavily irradiated mice and observing the appearance of their antigen-sensitive descendents in these recipients at varying intervals of time after marrow transplantation. The role of the thymus was assessed by comparing thymectomized, and intact, irradiated mice in this system.

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Materials and Methods. Inbred CBA mice were thymectomized at 7 to 8 weeks of age; 2 weeks later these and intact controls, of the same age, received 950 rads from a ^{137}cesium irradiator (9). Marrow suspensions were obtained from the femora of normal isogeneic mice, and 5×10^6 nucleated cells were injected intravenously into each recipient within 2 hours of irradiation. At varying times after irradiation and marrow transplantation, the presence of antigen-sensitive cells was detected by injecting 10^8 sheep erythrocytes intravenously into each mouse and determining the number of plaque-forming cells (10) in the spleen 6 days later. Background numbers of plaque-forming cells were obtained by assaying the spleens of thymectomized and intact, irradiated recipients not injected with sheep erythrocytes. In some groups of mice, diffusion chambers constructed (11) from Millipore filter material were implanted intraperitoneally 12 days after irradiation. The filter material had a pore size of 0.1 μ and the chambers were found to be impermeable to AKR lymphoma cells. The chambers contained two lobes of thymus obtained from newborn isogeneic donors. In control animals, the chambers were left empty, or filled with isogeneic lymph nodes or spleen.

Results. The results obtained in intact, irradiated recipients and thymectomized, irradiated recipients are compared in Fig. 1. The number of plaque-forming cells per spleen 6 days after the injection of sheep erythrocytes is shown as a function of time after irradiation and marrow transplantation. In both groups there was no increase in plaque-forming cells over the background number for approximately 10 days. Thereafter, the plaque-forming cell response in the intact animals increased exponentially, reaching a plateau about 22 days after irradiation. In contrast, in the thymectomized, irradiated mice there was only a slight increase in the number of plaque-forming cells between days 10 and 20, and no further increase occurred during the remainder of a 70-day period of observation.

The effect of implanting thymus-filled diffusion chambers into thymectomized, irradiated mice is shown in Fig. 2. The number of plaque-forming cells in the spleens of mice implanted with thymus-filled chambers was significantly higher than that found in thymectomized controls bearing chambers that were either empty or filled with spleen or lymph nodes.

Discussion. These results indicate that the thymus plays an important role in cellular differentiation in the immunological system during the growth of transplanted marrow cells in heavily irradiated recipients. Its influence is either on the step of differentiation leading from antigen-insensitive precursors to antigen-sensitive cells, or on some other, as yet unknown, properties of the antigen-insensitive precursors present in bone marrow. In addition, the observation that implantation of thymus tissue in diffusion chambers is effective indicates that a humoral factor is

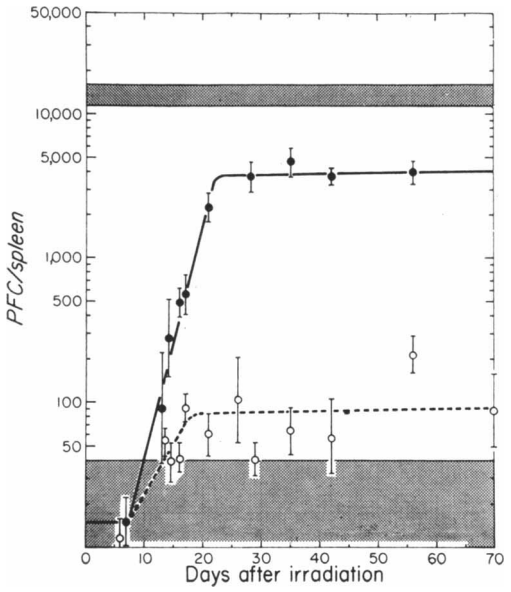

FIG. 1. Plaque-forming cell response to sheep erythrocytes in intact (———) and thymectomized (— — —) irradiated CBA mice. Each point represents the geometric mean and standard error for a group of at least seven mice injected with antigen on that day. The stippled band across the bottom of the graph represents the background value obtained in intact and thymectomized, irradiated mice not injected with sheep erythrocytes, while a similar band across the top represents the number of the plaque-forming cells found in intact unirradiated mice 4 days after injection with 10^8 sheep erythrocytes.

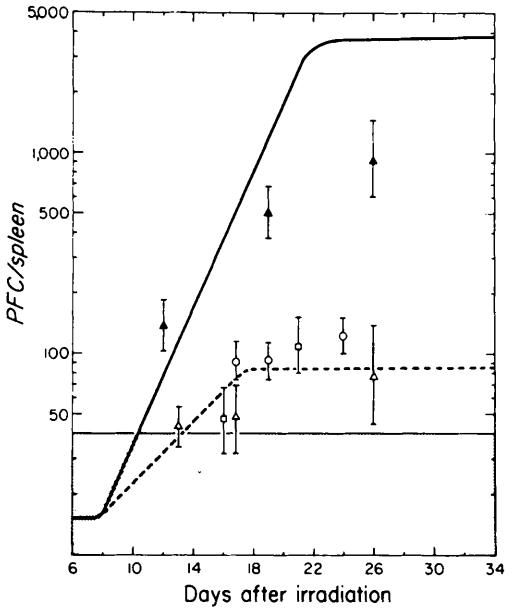

FIG. 2. Plaque-forming cell response to sheep erythrocytes in thymectomized, irradiated mice implanted with thymus tissue (▲), lymph node (△) or spleen (□) in diffusion chambers, or empty diffusion chambers (○) on day 12 after irradiation. Each point represents the geometric mean and standard error for a group of at least seven mice injected with antigen on that day. The results obtained for the above groups are compared with the recovery curves (shown in Fig. 1) of plaque-forming cells in intact (————), and thymectomized (- - -) irradiated mice. The stippled band across the bottom of the graph is the background number of plaque-forming cells detected in intact and thymectomized, irradiated mice not injected with sheep erythrocytes.

responsible. Finally, the experimental design described provides a system in which the effect of the thymus on cellular events in the immunological system may be studied.

Summary. Thymectomized and intact (nonthymectomized) adult mice were heavily irradiated and given isogeneic bone marrow. In contrast to the intact mice, thymectomized mice failed to recover the capacity to produce normal numbers of plaque-forming cells after antigenic challenge. However, when cell-impermeable diffusion chambers containing thymus were implanted into thymectomized mice, these animals produced significantly larger numbers of plaque-forming cells than did control mice bearing empty diffusion chambers or chambers containing lymph node or spleen. Since the thymus does not influence the differentiation of antigen-sensitive cells into plaque-forming cells, these results indicate that the influence of the thymus is directed at the differentiation of antigen-insensitive precursors in bone marrow to antigen-sensitive cells. The capacity of thymectomized mice bearing thymus-filled diffusion chambers to produce plaque-forming cells indicates that the thymic influence is mediated by a humoral factor.

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