Thymus, Peripheral Lymphoid Tissues and Immunological Responsiveness of the Pituitary Dwarf Mouse

Previous histological and autoradiographic studies from this laboratory have shown that in the pituitary dwarf mouse there exists a thymic deficiency with an early thymic involution and a decreased production of lymphocytes[1-3], thus indicating the need of more detailed morphological studies of the antibody-producing organs and of their relationships with the immunological capacity. In this preliminary paper, we report histological and autoradiographic investigations on thymus, spleen, lymph nodes and bone marrow, and immunological studies on the antibody-producing cells of the spleen.

Experimental. 40 mice of the Snell-Bagg strain with hereditary recessive pituitary dwarfism, and 29 normal litter mates were used for histology and radioautography. The animals were killed at different ages between 21 and 61 days of age. At the time of sacrifice total body, thymus and spleen weights were recorded. Carnoy-fixed material from thymus, spleen, peripheral and mesenteric lymph nodes, Payer's patches and sternal bone marrow was stained with hematoxylin-eosin and methyl-green pyronine. A group of dwarfs and normal litter mates received an i.p. injection of 0.8 μc of thymidine-H^3/g of body weight 24 h before sacrifice, and the % number of labelled nuclei was determined in thymus sections as previously reported[1-2].

A second experiment concerned an investigation of the immunological responsiveness of dwarfs in comparison to that of the normal mice, as judged by the capacity to produce antibody-plaque-forming cells according to the technique of JERNE et al.[4].

Seven 40-day-old dwarf animals and 5 normal litter mates were challenged with an i.p. injection of 0.1 ml of a suspension of sheep red cells in saline containing $4 \cdot 10^7$ cells/ml. 4 days after challenge the mice were killed and their spleens removed to assay the number of antibody-plaque-forming cells per million spleen cells. An i.p. injection of 0.8 μc of thymidine-H^3 was also given 24 h before sacrifice for additional autoradiographic studies of thymus and peripheral lymphoid tissues.

Results and discussion. The body weight and both the absolute and relative weights of thymus and spleen of the dwarf animals were found to be consistently lower than those of the normal litter mates (Table I), and at inspection the peripheral and mesenteric lymph nodes and the Payer's patches appeared small and atrophic. Histological examination of the thymus revealed in dwarfs an early involution with marked loss of lymphocytes and evident fibrosis in 11 out of 40 animals; in animals which still had an appreciable thymus we found a much thinner cortex and a predominance of the medulla with a larger number of Hassal's bodies. The mean % of labelled thymic nuclei in the dwarf mice was markedly lower (and mostly so in the outer cortex) than that of the normal animals in all age groups; in those showing extreme thymic involution, no evidence of labelling was found.

The germinal centres in the splenic nodules from dwarf mice were less prominent than those from normal litters; this was particularly true for the animals with marked involution of the thymus. The most striking differences in the splenic nodules of dwarfs were the hypotrophy of the lymphoid tissue surrounding the small germinal centres and the lower numbers of the large pyroninophylic cells and plasma cells distributed around the splenic nodules.

The germinal centres of the peripheral and mesenteric lymph nodes and of the Payer's patches of the dwarfs appeared to be extremely reduced, both in size and number. Furthermore, in the animals showing clear-cut signs of thymic involution, loss of small lymphocytes, proliferation of reticular tissue and lack of pyroninophylic cells and plasma cells in the medullary cords were the main findings.

The bone marrow from dwarf animals showed variable changes ranging from a moderate decrease of granulopoietic and erythropoietic activity to a severe cellular depletion. Also the megakaryocytes appeared to be in low numbers.

Table I. Body, thymus and spleen weight of normal litters and dwarf mice sacrificed between 21 and 61 days of life

	No. of mice	Body weight (g)	Thymus weight mg	Thymus weight % body weight	Spleen weight mg	Spleen weight % body weight
Normal	29	14.946	65.9	0.44	104.1	0.691
Dwarf	40	4.801	10.4[a]	0.20[a]	9.9	0.206

[a] 11 animals without any appreciable thymic residue were excluded.

Table II. Level of antibody-producing spleen cells after a primary i.p. injection of sheep red cells in normal litters and pituitary dwarf mice at 40 days of age

	Body weight (g)	Thymus weight (mg)	Spleen weight (mg)	Total cells/spleen	Plaque-forming cells/10^6 spleen cells
Normal					
1	10.808	55	101	$78.0 \cdot 10^6$	238.5
2	9.523	44	89	$53.6 \cdot 10^6$	298.5
3	10.103	44	100	$44.6 \cdot 10^6$	340.8
4	11.088	54	91	$63.2 \cdot 10^6$	234.1
5	17.650	81	155	$126.8 \cdot 10^6$	272.8
Dwarf					
1	7.108	12	24	$25.6 \cdot 10^6$	178.1
2	4.848	+[a]	11	$8.6 \cdot 10^6$	18.6
3	5.153	8	15	$8.2 \cdot 10^6$	78.4
4	5.368	5	10	$4.6 \cdot 10^6$	52.1
5	4.158	8	16	$8.0 \cdot 10^6$	20.0
6	3.758	[a]	8	$3.2 \cdot 10^6$	25.0
7	3.305	[a]	11	$1.15 \cdot 10^6$	52.1

[a] Animals without grossly appreciable thymus residue.

[1] C. BARONI and L. TIEPOLO, Bristol Lymph. Symp., April 1966 (E. Arnold Publishers Ltd.), p. 56.
[2] C. BARONI, Acta anat., in press.
[3] C. BARONI, Int. Symp. on Atherosclerosis and reticuloendothelial tissue. Como (Italy), Sept. 1966, in press.
[4] N. K. JERNE, A. A. NORDIN and C. HENRY, in *Cell-Bound Antibodies*, Wistar Inst. Symp. Monograph No. 3, 10 (Ed. V. DEFENDI; Wistar Inst. Press, Philadelphia 1966).

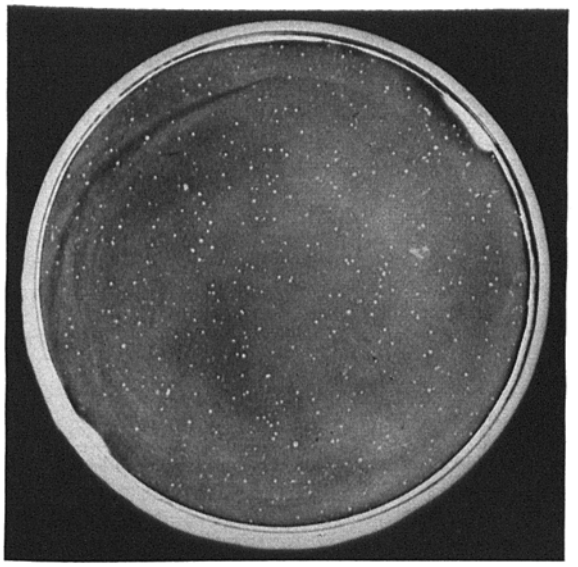

Fig. 1. Agar plate from a normal mouse. Note the high number of plaques produced by the antibody-forming cells of the spleen 4 days after receiving a primary i.p. injection of $4 \cdot 10^7$ sheep erythrocytes.

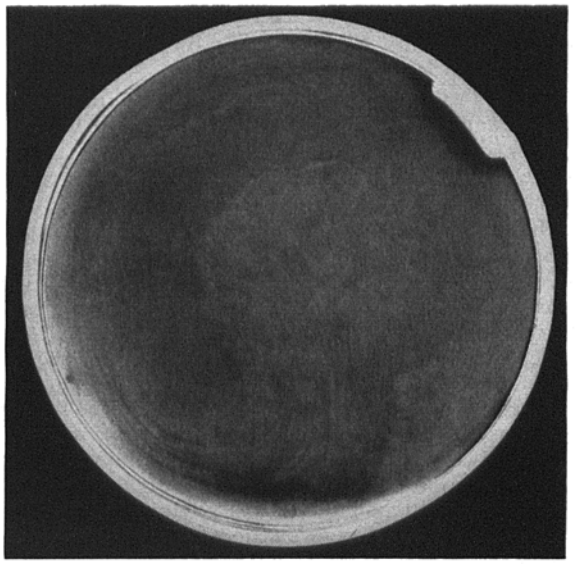

Fig. 2. Agar plate from a dwarf mouse. For comparison with Figure 1 note the extremely low number of plaques produced by the antibody-forming cells of the spleen 4 days after receiving a primary i.p. injection of $4 \cdot 10^7$ sheep erythrocytes.

As shown in Table II and in Figures 1 and 2, the capacity to produce plaque-forming cells in dwarfs with thymic and peripheral lymphoid tissue involution was markedly impaired. In 6 out of 7 animals the impairment was well evident; it should be noted that the only dwarf mouse showing a reasonably high level of plaque-forming cells was an animal whose thymus still showed histological and autoradiographic evidence of active function, and peripheral lymphoid tissues with less marked atrophy.

The possible action exerted by the thymus on other lymphoid organs has been extensively investigated, mostly by studying the effect of complete thymic removal in new-born mice. These studies showed a marked failure to gain weight, generally followed by wasting symptoms and premature death[5]. It has also been shown that there is frequently a terminal peripheral lymphocyte deficit, together with a reduction in the lymphocyte population of the peripheral lymphoid organs[6,7], and an impairment of several immunological capacities[5,7–13]. Taking these data together with the fact that the dwarf mice of our strain have a life span limited to 45–65 days, and with our experimental observations showing in these animals an early thymic involution, a deficiency in the production of thymic lymphocytes, a marked atrophy of the peripheral lymphoid tissues and a severe impairment of the capacity to produce antibody-plaque-forming cells in response to an injection of sheep red cells, it appears reasonable to suggest that this condition resembles the syndrome seen in mice thymectomized shortly after birth.

In conclusion, our results, showing an extreme atrophy of the peripheral lymphoid system associated with a marked decline in immunological capacity mostly in dwarfs with marked involution of the thymus, fit well with the generally accepted assumption of the important role played by these organs in the immune response. They also seem to confirm the assertion[10,12–14] that thymic function is not only involved in establishing the immunological potential during early life, but is also strictly related to the maintenance of an adequate proliferation of immuno-competent cells during adult life.

Riassunto. L'autore ha studiato la morfologia del sistema linforeticolare e lo stato di reattività immunitaria del topo Snell/Bagg con nanismo ereditario preipofisario. È stata riscontrata una notevole ipoplasia di tutto il sistema linforeticolare ed una severa diminuzione della capacità anticorpopoietica la cui causa viene fatta risalire con ogni probabilità al grave stato di deficit timico congenito. Si prospetta che il topo nano ipofisario sia portatore di una sindrome analoga a quella sperimentalmente indotta mediante timectomia neonatale.

C. Baroni[15]

Istituto di Anatomia Patologica dell'Università di Pavia (Italy), 9th December 1966.

[5] J. F. A. P. Miller, Lancet *2*, 748 (1961).
[6] J. F. A. P. Miller, Proc. R. Soc., B. *156*, 415 (1962).
[7] D. M. V. Parrott and J. East, in *The Thymus in immunobiology* (Ed. R. A. Good and A. E. Gabrielsen; Hoeber-Harper, New York 1964), p. 523.
[8] R. H. Levey, N. Trainin and L. W. Law, J. natn. Cancer Inst. *31*, 199 (1963).
[9] L. W. Law, T. B. Dunn, N. Trainin and R. H. Levey, in *The Thymus* (The Wistar Inst. Symp. Monograph *2*, 105, 1964).
[10] J. F. A. P. Miller, Science *144*, 1544 (1964).
[11] J. F. A. P. Miller, P. M. de Burgh and G. A. Grant, Nature *208*, 1332 (1965).
[12] J. F. A. P. Miller, Nature *208*, 1337 (1965).
[13] J. F. A. P. Miller, in Ciba Found. Symp. on the Thymus: experimental and clinical studies. 153 (Ed. G. Ev. Wolstenholme and R. Porter; London 1966).
[14] D. Metcalf, Nature *208*, 1336 (1965).
[15] The author wishes to thank Professor Cesare Cavallero and Professor Marco Fraccaro for their suggestions and criticism. The technical assistance of Miss L. Forni and Mr. G. P. Tonda of the Cattedra di Immunologia of the University of Milan is also greatly appreciated.