THE PRODUCTION OF RUNT DISEASE IN RATS THYMECTOMIZED AT BIRTH*

By ALAN C. AISENBERG, M.D., BARBARA WILKES, and BYRON H. WAKSMAN,‡ M.D.

(From The John Collins Warren Laboratories, Huntington Memorial Hospital, the Departments of Medicine, and Bacteriology and Immunology, Harvard Medical School, and the Neurology Research Laboratory, Massachusetts General Hospital, Boston)

Plates 96 to 103

(Received for publication, July 13, 1962)

In the course of experiments designed to study tolerance, Billingham and Brent observed the development of a disease 1 to 2 weeks following the injection of immunologically competent homologous lymphoid cells into newborn mice (1). This usually fatal illness was termed "runt" disease and has since been observed in the rat (2–4), the chicken (5), and the rabbit (6). The clinical and histologic pictures, and the overwhelming evidence that runt disease represents an immunologic attack by the grafted cells on the host have been considered in detail in several recent comprehensive reviews (7–9). In the adult animal it has been possible to produce a graft *versus* host reaction only after immunologic manipulations which permit survival of the transplanted lymphoid cells. This has been achieved by injection of parent strain cells into an F_1 hybrid recipient (10–14), by irradiation of the recipient (15–17), and by parabiotic union which permits a prolonged exchange of cells (18).

Recent work from several laboratories (19–21) has established that adult animals, thymectomized in the 1st week of life, have a depression both of antibody formation and of several delayed immune responses. In particular, the homotransplantation reaction of mice (20) and of rats (22) has been found to be severely impaired. This immunologic defect in the thymectomized animal is correlated with a lack of development of lymphoid follicles in the lymph nodes and the white pulp of the spleen (23). It appeared to us that such animals afforded an opportunity to study several aspects of runt disease.

If current concepts of the pathogenesis of runt disease are correct (7–9), *i.e.* the basic disturbance in the process is an attack on the host by the graft, then thymectomy of the newborn should not impair the development of runt disease

* This investigation was supported by grants C-4644 of the National Cancer Institute and E-1257 of the National Institute of Allergy and Infectious Diseases, Public Health Service. This is publication No. 1088 of the Cancer Commission of Harvard University.

‡ National Neurological Research Foundation Scientist.

produced by the neonatal injection of homologous lymphoid cells. Our first experiments were designed to test this point and to serve as a baseline for our second study. It seemed plausible that thymectomy would also permit the homologous lymphoid graft to survive in the adult animal and allow the subsequent development of a graft *versus* host reaction. Therefore, the second group of experiments was undertaken to establish whether it was indeed possible to induce this reaction with homologous lymphoid cells in adult animals thymectomized at birth. Finally, it was hoped that a comparison of the histologic picture of runt disease in normal and thymectomized animals would allow some conclusions as to which of the complex histologic alterations of runt disease are primary and which are secondary.

Methods

Animals.—Albino Sprague-Dawley derived rats (CD strain), obtained from Charles River Breeding Laboratory, Brookline, Massachusetts, were used as recipients in all experiments. Donor animals were Long-Evans (hooded) rats, 3 to 4 months of age, obtained from Rockland Farms, New City, New York. Neither strain is inbred.

Preparation of Cells.—Spleen cells were prepared for injection by minor modification of the method of Billingham and Brent (1). With sterile technique, the entire spleens were pressed through a cytosieve (obtained from Macalaster Scientific Corporation, Cambridge, Massachusetts) with the aid of a teflon homogenizer pestle. Hank's solution was added dropwise to the splenic tissue during the process of sieving, a final volume of 5 to 7 ml per spleen being used. The cell suspension was centrifuged once at 500 *g* in an International model PR-2 refrigerated centrifuge, and the supernatant fluid containing cytoplasmic debris discarded. The cells were then suspended in Hank's solution to give a final concentration of 650 to 750 $\times$ 10^6 cells per ml. Fifty units each of penicillin and of streptomycin (both obtained from Microbiological Associates, Bethesda) were added per ml of final suspension, and macroscopic clumps of cells were dispersed by passing the cells through a No. 22 gauge needle. All suspensions were counted with a hemocytometer, only structurally intact nucleated cells being counted, and were used immediately Each cell suspension was prepared from pooled cells of either 4 donor animals (2 male and 2 female) or 6 animals (3 male and 3 female).

Thymectomy.—All animals were thymectomized on the 3rd day of life. Under ether anesthesia, the skin was incised, the soft tissues of the neck retracted, and a sternal window approximately a millimeter wide made and extended down to about the level of the third rib. (If the window is extended too far, difficulty is experienced with herniation of the lung into the wound). The bilobed thymus was then freed with the aid of a sharp cotton-tipped stick, beginning with its inferior margin. Only animals were used in which a satisfactory removal of the two intact thymus lobes was obtained. The wound was closed with silk and painted with collodion. The operative mortality was about 25 per cent, but an additional group of animals, approximately 25 per cent, died because of maternal neglect or cannibalism in the week following operation. Those dying within 7 days of operation have been discarded from the present study. At autopsy each rat was examined for thymic remnants in the gross and any mediastinal tissue present was always submitted for histologic examination.

Experiments in Newborns.—Two experiments involving a total of 14 litters of Charles River strain (Sprague-Dawley) rats were carried out. On the 3rd day of life, 4 or 5 animals of each litter were thymectomized. Immediately following operation, the thymectomized animals and the 4 unoperated litter mates were injected with 65 to 75 million hooded (Long-Evans) spleen cells intraperitoneally. The injection was made through the muscles of a lower extremity with

a No. 25 needle, and the site of injection coated with collodion. The animals were weaned on the 24th day of life. Clinical observations were made daily and the animals were weighed every other day. The animals were autopsied when moribund or immediately after death, and the tissues fixed in buffered formalin. Surviving animals were autopsied after 60 days.

Experiments on Adults.—Sprague-Dawley rats 9 to 13 weeks of age, thymectomized on the 3rd day of life, and normal controls of the same age received an injection of 800 to 1000 million spleen cells into a lateral tail vein. In our experience, thymectomized rats are quite sensitive to ether anesthesia and we have also found it important to use a slow rate of injection with these large cell volumes. Clinical observations and histologic study were done as in the experiments on newborns, with the exception that surviving animals were sacrificed 18 to 20 days post-injection in the last two adult experiments.

Four operated animals, which displayed gross and microscopic evidence of residual thymus in excess of 250 mg at autopsy and no lymphocyte depletion of spleen and lymph nodes, have been considered as non-thymectomized in the results which follow. (They did not develop clinical or histologic evidence of runt disease). Four additional thymectomized animals, while showing no gross or microscopic evidence of residual thymus at autopsy, failed to show lymphocyte depletion, and were dropped from further consideration although one developed an early lymph node lesion. Animals without lymphocyte depletion, despite adequate thymectomy, have been described (23), and such animals are not immunologically impaired.

RESULTS

Experiments in Newborn Rats—Clinical Observations.—Clinical data on the 14 litters of Sprague-Dawley rats injected on the 3rd day of life with hooded adult spleen cells are summarized in Table I. The incidence of fatal runt disease was similar in thymectomized and non-thymectomized animals in Experiment A, while in Experiment B a somewhat higher incidence was observed in the thymectomized group. In addition to the animals listed in Table I, 2 injected, non-thymectomized rats (one in litter A-3 and one in A-6) developed a non-fatal, chronic form of the runting syndrome. Animals of some litters, thymectomized or not, tended to have a high incidence of runt disease (A-8, B-1, B-2); in other litters the disease incidence was low among both operated and non-operated rats (A-1, A-7, B-3).

Runt disease, as observed clinically, was similar in thymectomized and non-thymectomized animals. In both groups the majority of animals became sick between the 17th and 21st days. The character and intensity of specific clinical features in the two groups were closely comparable. Weight loss was almost always present. Crusting lesions of the eyes, nose, and snout, ruffling of the fur, and the high-stepping unsteady gait described by others (8, 9) were commonly observed; and more extensive dermatitis and diarrhea were frequent. In both operated and non-operated animals, a fulminating form of runt disease occasionally appeared with onset less than 14 days after cell injection, and death within a few days before much skin change or weight loss had taken place (24). In occasional animals the onset of otherwise typical runt disease occurred more than 30 days after the injection of homologous cells.

Experiments in Newborn Rats—Histologic Observctions.—The lesions ob-

TABLE I

Production of Runt Disease in Thymectomized and Non-Thymectomized Neonatal Animals

Litter No.	Thymectomized and injected*						Injected* only					
	Incidence	Animal No.	Day* of onset	Day* of death	Weight loss	Skin‡ lesions	Incidence	Animal No.	Day* of onset	Day* of death	Weight loss	Skin‡ lesions
					gm						*gm*	
A-1	0/2						0/5					
A-2	1/4	i	16	21	9	+++	1/4	i		19	0	
A-3	2/2	i	20	23	10	+++	1/4	i	22	30	4	++
		ii	22	34	8	+++						
A-4	0/0						2/5	i		14	0	0
								ii	36	50	13	0
A-5	0/1						1/5	i	46	51	15	0
A-6	1/1	i	22	26	16	+++	1/4	i	20	26	13	+++
A-7	0/4						1/4	i		22	8	
A-8	1/1	i		16	0		4/4	i		15	0	
								ii		15	0	
								iii		22	2	+++
								iv		22	2	+++
A 1-8	5/15		19	24	9	+++	11/35		20	26	6	++
B-1	5/5	i	17	18	3	+++	3/4	i	18	20	0	+++
		ii	18	22	8	+++		ii	18	20	5	+
		iii	18	24	3	+		iii	18	22	11	++
		iv	20	23	12	+++						
		v	31	27	12	+						
B-2	5/5	i	17	19	8	++	4/4	i	17	21	8	++
		ii	18	22	4	+++	4/4	ii	17	22	13	++
		iii	18	28	5	+		iii	17	26	16	+++
		iv	18	32	5	++		iv	38	44	35	
		v	20	34	5	++						
B-3	1/4	i	29	36	10		0/4					

* Each animal received 65 to 75 million spleen cells from hooded rats on the 3rd day of life intraperitoneally. Days of onset and death are measured from time of injection.

‡ Skin lesions graded from 0 to +++ for maximal lesions.

TABLE I—*Continued*

Litter No.	Thymectomized and injected*						Injected* only					
	Incidence	Animal No.	Day* of onset	Day* of death	Weight loss	Skin‡ lesions	Incidence	Animal No.	Day* of onset	Day* of death	Weight loss	Skin‡ lesions
					gm						*gm*	
B-4	3/3	i	17	19	6	+	1/3	i	17	24	16	++
		ii	17	21	4	+++						
		iii	19	22	11	++						
B-5	0/0						1/4	i	17	27	10	+
B-6	4/4	i	12	14	1		1/3	i	26	29	22	+++
		ii	12	14	5							
		iii	19	22	10	+++						
		iv	21	28	12	+						
B 1-6	18/21		19	22	7	++	10/22		20	25	14	++

served, in both thymectomized and non-operated animals injected with homologous spleen cells, were exactly comparable to those described by other workers (5, 8). Spleen and lymph node lesions were present in almost all the animals. These consisted of a variable degree of replacement of parenchyma by masses of large pale cells, typical histiocytes, and more mature macrophages, apparently beginning as a proliferation of sinus reticulum cells in the splenic red pulp and the capsular, medullary, and intermediate lymph node sinuses. In some lymph nodes there was also an increase of large basophilic cells, predominant in the cortex, and in others hemorrhage was prominent. In the livers of about half the animals, there were mild to moderate periportal infiltrates of dark mononuclear cells and destruction of liver parenchyma in the infiltrated zone, also nests of basophilic blast-like cells in the liver lobules, and occasional foci of necrosis. The skin showed infiltrative lesions of the type described by Billingham *et al.* (8) in most of the cases examined. Finally, the thymus, in the unoperated animals, was depleted and showed a marked relative increase of reticulum cells. There were no lesions of the heart, gut, joints, eyes, pancreas, and salivary glands in the cases examined.

Experiments in Adult Rats—Clinical Observations.—Fifteen of 41 thymectomized rats and 4 of 31 normal controls developed a severe clinical disease following intravenous injection of 800 to 1000 million homologous spleen cells (Table II). The onset varied between the 9th and the 22nd day after injection. The average observed duration of disease until death was 4 days. A single animal whose illness began on the 22nd day developed weight loss and generalized dermatitis but had partially recovered by the 40th day when it was sacri-

TABLE II

Production of Clinical Runt Disease in Adult Animals

Experiment No.	Thymectomized and injected*		Injected* only	
	Incidence	Clinical data	Incidence	Clinical data
1-A	3/8	(i) 15d-23‡; S/K = 3.6; dermatitis, arthritis (ii) 19d-22†; S/K = 1.6; wry neck (iii) -22‡; S/K = 4.5; cyanotic	0/0	
1-B	3/5	(i) 17d-22‡; wgt. 438 to 270 gms; dermatitis, arthritis, gastrointestinal bleeding; S/K = 0.6 (ii) 17d-19 sac.; wgt. 396 to 360 gm; (iii) -9‡; no postmortem	0/2	
1-C	3/5	(i) 10d-19‡; wgt. 268 to 153 gm; S/K = 0.7; dermatitis, bleeding into muscles (ii) 14d-18‡; wgt. 192 to 136 gm; S/K = 0.5; dermatitis, gastrointestinal bleeding (iii) 22d-40 sac.; wgt. 178 to 110 to 140 gm; S/K = 1.0; generalized exfoliative dermatitis	1/5	(i) 15d-20‡; wgt. 204 to 135 gm; S/K = 0.6; dermatitis
2	5/17	(i) 9d-9‡; wgt. 150 to 150 gm; S/K = 3.0; pneumonia (ii) 13d-15‡; wgt. 286 to 200 gm; S/K = 0.6; gastrointestinal bleeding (iii) 18d-20‡; wgt. 240 to 190 gm; S/K = 0.4; gastrointestinal bleeding (iv) 12d-20 sac.; wgt. 208 to 166 gm; S/K = 1.2 (v) 15d-19 sac.; wgt. 180 to 147 gm; S/K = 0.6	2/11	(i) 9d-10‡; wgt. 198 to 160 gm; S/K = 2.0; dermatitis, gastrointestinal bleeding (ii) 15d-19‡; wgt. 206 to 152 gm; S/K = 0 8; dermatitis
3	1/6	(i) 11d-18 sac.; wgt. 389 to 338 gm; S/K = 2.0	1/13	(i) 10d-17‡; wgt. 224 to 175 gm; S/K = 1.5; dermatitis, gastrointestinal bleeding
1, 2, 3	15/41	(39 per cent); 15d-18‡	4/31	(13 per cent); 12d-17‡

d indicates day of onset of disease measured from day of injection.

‡ Day of death measured from day of injection.

Sac., day of sacrifice of severely ill animal.

S/K, spleen weight to left kidney weight (normal value 0.5 to 0.8).

* Normal adult animals and adult animals thymectomized on the 3rd day of life received 800 to 1000 million spleen cells from hooded rats intravenously.

ficed. Two rats became sick on the 9th day and died within 24 hours. While the time of onset and the duration of disease were similar in the thymectomized and non-thymectomized groups, the disease began more rapidly than in the newborns.

Substantial weight loss was the commonest clinical finding. In 9 of the 19 adult animals with disease, a dermatitis was present similar in type and distribution to that occurring in neonatal animals. In 8 of these adult animals there were crusting hemorrhagic lesions with loss of fur in other parts of the body. Seven animals showed extensive amounts of blood in the stomach and intestine at autopsy, and 2 of these also showed evidence of bleeding elsewhere; one displayed bleeding into the muscles of the feet and the other had petechial lesions in the intestine and thymus. (A count of blood platelets in this last animal gave a value of 20,000 mm^3). In 7 of the 19 sick adults a variable number of lymph nodes were hemorrhagic in the gross at the time of autopsy, an observation we have also made in our neonatal animals. Two rats developed polyarthritis, 1 a wry neck, and 2 the wobbly high-stepping gait described in the newborns. Spleen size varied from low normal to very large (see spleen to kidney weight ratios in Table II). Similarly the lymph node size varied from small to much enlarged. The weight of a single cervical lymph node (average of 4) in the diseased adult animals was: 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 35 mg, 65 mg, 72 mg, 75 mg, 90 mg. In the 4 sick non-thymectomized adults, the thymus weight was markedly decreased (140 mg, 180 mg, 240 mg, 330 mg) as compared with thymus weights of 350 to 550 mg for non-diseased animals of the same age.

There were no obvious clinical differences between adult runt disease in the 15 thymectomized animals and in the smaller number of diseased controls. One of the thymectomized animals had pneumonia at the time of autopsy. (In 4 late deaths among 45 non-injected thymectomized animals extensive pneumonia and chronic pulmonary suppuration were found).

Experiments in Adult Rats—Histologic Observations.—All the rats which showed obvious manifestations of disease *ante mortem* were found by microscopic examination to have conspicuous lesions (Table III). A number of additional animals, which had no symptomatic abnormality, presented microscopic evidence of disease. Of 35 animals examined histologically, with effective thymectomy as judged by depletion of small lymphocytes in the spleen and lymph nodes, 24 had clear-cut lesions of runt disease. However, of 30 animals which were unoperated (26 animals) or ineffectively thymectomized (4 with residual thymus and no depletion), only 5 had such lesions. As in the newborns, the spleen and lymph nodes were the primary sites of lesion formation in most of the affected adults. However, liver lesions were present in about half and pulmonary lesions also in half. Disease of heart, skin, joints, bowel, and kidney each affected 2 or 3 animals of the series, almost always in conjunction with

lesions of the more commonly involved organs. The changes observed are briefly described herewith:

Spleen.—In thymectomized animals, there was a partial to total absence of small lymphocytes from the splenic follicles and white pulp (23). Lymphocyte depletion is also observed as a feature of runt disease (8), but was not used as a histologic criterion of disease in our thymectomized animals. The runt disease lesion was a progressive replacement, first of the red pulp and later of the white

TABLE III

Involvement of Individual Organs in Newborn and Adult Rats with Runt Disease

Organ	No. of rats with lesions					
	Thymectomized*			Non-thymectomized‡		
	0	+	++ or +++	0	+	++ or +++
Newborn						
Spleen	2	2	13	3	0	7
Lymph nodes	1	2	15	0	3	6
Liver	7	9	1	5	4	1
Adults						
Spleen	19	7	9	27	0	3
Lymph nodes	16	12	7	27	2	1
Liver	23	5	4	26	2	1
Lung	14	2	6	17	3	0
Heart	18	0	2	15	0	1
Kidney	19	4	0	20	0	0
Bowel	5	1	1	4	1	0
Joints	0	1	2	3	0	0
Skin	2	3	1	2	0	0

* 20 newborns and 35 adult animals, thymectomized at birth.

‡ 10 non-operated newborns and 30 adults.

Lesions graded 0 to +++ for maximal lesions.

pulp as well, by masses of large, pale "reticulum cells," which in some animals looked more like histiocytes and in others more like typical macrophages (Figs. 1 and 2). At the time of death the lesions varied from isolated foci of these cells to nearly total replacement of normal splenic tissues by them. Frequently there were masses of large basophilic cells, particularly in the white pulp, and foci of active hemopoiesis were commonly observed. In many spleens we found small, ovoid, or irregular deposits of pale, eosinophilic material (non-staining with congo red (*cf* 25)) occasionally with central calcification.

Lymph Nodes.—Effective thymectomy was represented by absence of small

lymphocytes from the cortex with preservation of germinal centers and medullary plasmacytes. The principal lesion of runt disease was massive overgrowth of large pale cells, which sometimes took the form of reticulum cells multiplying in the walls of sinuses, sometimes of histiocytes invading the adjacent cortex, and sometimes of macrophages filling the lumen of the sinuses (Figs. 3, 4, and 5). A characteristic picture was the presence of small and large patches of these pale cells replacing portions of the lymph node cortex. Another was the alternation of masses of macrophages with the intact medullary cords filled with plasma cells. In advanced lesions, the architecture of the node was lost, and it became a formless mass of plasma cells, reticulum cells, and unidentified large basophilic cells. Hemolymph nodes underwent similar changes.

Thymus.—In the three thymuses examined in diseased non-thymectomized adults severe lymphocyte depletion, as described in neonatal runt disease (8), was observed.

Liver.—Five related changes were observed (Fig. 6). Most common were large periportal aggregates of histiocytes invading the adjacent liver parenchyma with or without some degree of parenchymal destruction. Clumps of plasma cells were frequently found in the portal triads or adjacent to hepatic veins. There were sometimes focal dilatations of sinusoids containing masses of mononuclear cells, often with a few polymorphonuclears. In these there were fragmentation of cells and formation of typical macrophages. From these sinusoidal lesions there was occasionally invasion of the parenchyma by histiocytes, with destruction of liver cells. Such lesions were occasionally centrolobular. Finally, small or moderate sized foci of necrosis were common.

Lung.—Areas of lymphoid tissue adjacent to the bronchi and large vessels were sometimes replaced by masses of large basophilic cells. More commonly there were focal or diffuse zones of infiltration by large pale cells like those seen in other organs (spleen, nodes) (Fig. 7). These again ranged in appearance from histiocytes and large reticulum cells to typical macrophages. Many bronchi in involved areas were filled with polymorphs and there was often destruction of bronchial epithelium. Some vessels contained plugs of mononuclear cells, and there were focal areas of necrosis, with or without associated hemorrhage. In some non-thymectomized adults, patches of large pale cells, analogous to those frequently found in lymph nodes, were often present in otherwise normal lung.

Joints.—Several animals presented mild to moderate lesions of wrists, ankles, and toes, consisting of synovitis, tenosynovitis, and periarthritis with, however, no pannus, periostitis, or evidence of joint destruction. The cellular infiltrate was mononuclear, often with a few polymorphonuclear leukocytes; and there was moderate proliferation of synovial cells (Fig. 8). Some tendon sheaths were observed to contain hemopoietic tissue.

Heart.—The heart in 3 rats showed a massive myocardial infiltration by histio-

cytes with obvious destruction of myofibers and nuclear fragmentation in the zones invaded by these cells and secondary polymorphonuclear infiltration in areas of severe destruction (Fig. 9). The endocardium was also involved in some rats.

Kidney.—The kidney in a smaller number of rats showed isolated zones of interstitial mononuclear cell infiltration. The tubules in involved areas were filled with neutrophils.

Bowel.—In 3 rats there were, in addition to lymphocyte depletion in the Peyer's patches, massive histiocytic infiltration of the lamina propria and formation of micro abscesses filled with polymorphonuclear cells (Fig. 10). One animal showed a large deep ulcer as well.

Skin.—We observed skin lesions much like those described by Billingham *et al.* (8); *i.e.*, massive infiltration of the dermis with lymphoid cells, reactive thickening of the epidermis, and intra-epidermal spongiosis and vacuolation. In some rats, the epidermal overgrowth gave rise to a psoriasiform lesion, with lengthening and fusion of the rete pegs, edema and cell infiltration in the tips of the dermal papillae, and micro abscesses in the epidermis (Figs. 11 and 12). In others there were deep cutaneous ulcerations, the basic lesion being obliterated by secondary changes.

DISCUSSION

The runting syndrome observed in neonatal rats in the present study was similar in its incidence and clinical features to that described by Woodruff and Sparrow (2) in the same strain combination and also closely comparable to that seen in experiments with inbred strains of rats (8) or mice (1, 5, 26). It is clear from the data reported here that thymectomy of the newborn rat does not ameliorate the incidence, severity, or clinical manifestations of runt disease produced in the newborn. Thymectomy of newborn rats and mice produces a severe impairment of several well standardized types of immunologic response, among them antibody formation (19, 27), delayed cutaneous hypersensitivity to tuberculin or to purified protein (22, 27), experimental autoallergic encephalomyelitis (22), and skin homograft rejection (20, 22). In particular, homograft rejection time is more than doubled in both rats and mice by thymectomy (20, 22). It appears to follow that immunologic competence of the recipient animal is not necessary for the runting syndrome. Such an inference is consonant with extensive evidence which indicates that the immunologic direction of runt disease is from donor to graft (7–9). Since, however, there exists a possibility that some types of immune response may not be affected by early thymectomy and since the immunologic impairment observed is not complete (20, 22, 27), this conclusion must remain qualified.

Findings in recent studies have suggested that a major part of the cells participating in the spleen and lymph node lesions of runt disease are of host

origin (28, 29). The present work eliminates the thymus as a possible source of these cells. Since in rats thymectomized at birth there is a massive depletion of small lymphocytes in the spleen and nodes, while reticulum cells, germinal center cells, and cells of the plasmacyte series remain intact (23), it is among the latter that we must look for the precursors of the basophilic and pale cells (8) of the runt disease lesions. Similarly, our experiments show that runt disease occurs independently of any hormonal action of the thymus (30).

Thirty-seven per cent of neonatally thymectomized adult rats and 13 per cent of the adult controls developed severe illness at a suitable time period following the injection of homologous spleen cells. Histologic lesions were noted in 69 per cent of the thymectomized group and 17 per cent of the controls. The time of onset and the clinical and histologic pictures all strongly suggest that we are dealing with an immunologic disease fully comparable to neonatal runt disease. It is probable that thymectomy facilitated the survival of the graft of immunologically competent homologous cells for a sufficiently long time to permit their reaction against the host. This disease mechanism in the thymectomized animal then is much the same as that of homologous disease produced by injection of parent strain lymphoid cells into the F_1 hybrid and the wasting syndrome which follows homologous transfer of cells to lethally irradiated animals (16, 31). The occurrence of similar disease in some immunologically intact adult controls may be attributed to the large dose of injected cells (800 to 1000 million), a much larger transplant than is usually employed in experiments of the present type. It is possible that such a graft can overwhelm the recipient's immune response, thereby permitting survival of the graft, a mechanism similar to that of parabiotic intoxication (18). There have been two preliminary reports of a disease in adult mice suggestive of graft *versus* host reaction following the administration of massive doses of normal (32) and pretreated (33) homologous lymphoid cells. It has also been shown that injection of homologous lymph node cells into the skin of immunologically intact adult recipients produces an appreciable lesion, even when the transferred cells are not preimmunized against homograft antigens of the recipient (34). This lesion, which is comparable in form to that under consideration, may reach considerable proportions over a period of 5 to 7 days before beginning to regress.

The adult disease we have observed resembles the runt disease of our neonatal animals in many respects. The time of onset, duration of disease, and weight loss were comparable in the two groups of animals In each, fulminating disease of early onset and indolent disease of late onset were seen. The spleen and lymph node changes and the dermatitis were also closely similar in the newborn and adult animals.

There is also a close resemblance of the disease of our adult animals to other adult forms of the graft *versus* host reaction. Arthritis, which was present in 3 of our adult animals but not in the newborns, has recently been reported in

homologous disease in rats (35). Gastrointestinal bleeding was also a prominent feature in our adult animals, but not in newborns. Severe gastrointestinal lesions and focal eosinophilic spleen lesions such as we observed (25) are well known findings of the wasting syndrome (36). There is some suggestion that the gastrointestinal bleeding was due in part to a bleeding diathesis, since several of our adult animals showed evidence of bleeding elsewhere. Unfortunately, this aspect of the problem did not come to our attention until the present experiments were almost completed. (A single platelet count on one of the bleeding animals was markedly reduced.) Other investigators have reported that thrombocytopenia is a common finding in homologous disease (37). The severe myocarditis noted in 3 of the adults in the present series has not, to our knowledge, been described before, though small focal myocardial lesions have been observed (36) in the wasting syndrome. It is of considerable interest to note the more extensive organ involvement observed by us and others in adult forms of the graft *versus* host reaction (heart, joints, and intestine) when compared with the limited involvement of neonatal runt disease.

SUMMARY

Thymectomy of Sprague-Dawley rats on the 3rd day of life failed to influence the time of onset, incidence, clinical, or histologic picture of runt disease produced by the intraperitoneal injection of adult Long-Evans spleen cells. The fact that severe immunologic impairment of the host by thymectomy does not modify runt disease was felt to be consistent with the current view that the direction of the immunologic event in this syndrome is graft *versus* host.

Following the injection of 800 to 1000 million Long-Evans spleen cells into adult Sprague-Dawley rats, a severe illness comprised of dermatitis, gastrointestinal bleeding, arthritis, weight loss, and death ensued in 37 per cent of adults thymectomized neonatally and 13 per cent of normal controls. Histologic lesions were observed in 69 per cent of adequately thymectomized animals and 17 per cent of normal controls, and involved lymph nodes, spleen, liver, lungs, kidneys, joints, heart, and skin. The time of onset and the histologic and clinical pictures are consistent with the adult disease being a typical graft *versus* host reaction.

BIBLIOGRAPHY

1. Billingham, R. E., and Brent, L., A simple method for inducing tolerance of skin homografts in mice, *Transplant Bull.*, 1957, **4,** 67.
2. Woodruff, M. F. A., and Sparrow, M., Further observations on the induction of tolerance of skin homografts in rats, *Transplant. Bull.*, 1957, **4,** 157.
3. Billingham, R. E., and Silvers, W. K., The induction of tolerance of skin homografts in rats with pooled cells from multiple donors, *J. Immunol.*, 1959, **83,** 667.
4. Nisbet, N. W., Heslop, B. F., and Zeiss, I. M., Manifestations of runt disease in rats, *Proc. Univ. Otago Med. School*, 1960, **38,** 11.

5. Simonsen, M., The impact on the developing embryo and newborn animal of adult homologous cells, *Acta Path. et Microbiol. Scand.*, 1957, **40,** 480.
6. Porter, K. A., Immune hemolysis: a feature of secondary disease and runt disease in the rabbit, *Ann. New York Acad. Sc.*, 1960, **87,** 391.
7. Simonsen, M., Graft versus host reactions. Their natural history, and applicability as tools of research, *Progr. Allergy*, 1962, **6,** 349.
8. Billingham, R. E., Defendi, V., Silvers, W. K., and Steinmuller, D., Quantitative studies on the induction of tolerance of skin homografts and on runt disease in neonatal rats, *J. Nat. Cancer Inst.*, 1962, **28,** 365.
9. Nisbet, N. W., and Heslop, B. F., Runt disease, *Brit. Med. J.*, 1962, **I,** 129, 206.
10. Trentin, J. J., The immunological basis for induced tolerance to skin homografts in irradiated mice receiving bone marrow transfusions, *Transplant. Bull.*, 1957, **4,** 74.
11. Uphoff, D. E., Genetic factors influencing irradiation protection by bone marrow. I. The F_1 hybrid effect. *J. Nat. Cancer Inst.*, 1957, **19,** 123.
12. Schwartz, E. E., Upton, A. C., and Congdon, C. C., A fatal reaction caused by implantation of adult parental spleen tissues in irradiated F_1 mice, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 797.
13. Cole, L. J., and Ellis, M. E., Delayed deaths in sublethally x-rayed F_1 hybrid mice injected with parental strain spleen cells, *Science*, 1958, **128,** 32.
14. Kaplan, H. S., Nagareda, C. S., Rosston, B. H., and Brown, M. B., Comparative activity of isologous versus homologous mouse bone marrow on lymphoid tumor production following irradiation, *Proc. Soc. Exp. Biol. and Med.*, 1958, **98,** 384.
15. Barnes, D. W. H., Ford, C. E., Ilbery, P. L. T., Koller, P. C., and Loutit, J. F., Tissue transplantation in the radiation chimera, *J. Cell. and Comp. Physiol.*, 1957, **50** (suppl. 1), 123.
16. Trentin, J. J., Tolerance and homologous disease in irradiated mice protected with homologous bone marrow, *Ann. New York Acad. Sc.*, 1958, **73,** 799.
17. Koller, P. C., Davies, A. J. S., and Doak, S. M. A., Radiation chimeras, *Adv. Cancer Research*, 1961, **6,** 181.
18. Eichwald, E. J., Lustgraaf, E. C., and Strainer, M., Genetic factors in parabiosis, *J. Nat. Cancer Inst.*, 1959, **23,** 1193.
19. Archer, O., and Pierce, J. C., Role of thymus in development of the immune response, *Fed. Proc.*, 1961, **20,** 26.
20. Miller, J. F. A. P., Immunological functions of the thymus, *Lancet*, 1961, **2,** 748.
21. Arnason, B. G., Janković, B. D., and Waksman, B. H., Effect of thymectomy on "delayed" hypersensitive reactions, *Nature*, 1962, **194,** 99.
22. Arnason, B. G., Janković, B. D., Waksman, B. H., and Wennersten, C., Role of the thymus in immune reactions in rats. II. Suppressive effect of thymectomy at birth on reactions of delayed (cellular) hypersensitivity and the circulating small lymphocyte, *J. Exp. Med.*, 1962, **116,** 177.
23. Waksman, B. H., Arnason, B. G., and Janković, B. D., Role of the thymus in immune reactions in rats. III. Changes in the lymphoid organs of thymectomized rats, *J. Exp. Med.*, 1962, **116,** 187.
24. Anderson, N. F., Delorme, E. J., and Woodruff, M. F. A., Induction of runt disease in rats by injection of thoracic duct lymphocytes at birth, *Transplant. Bull.*, 1960, **7,** 93.

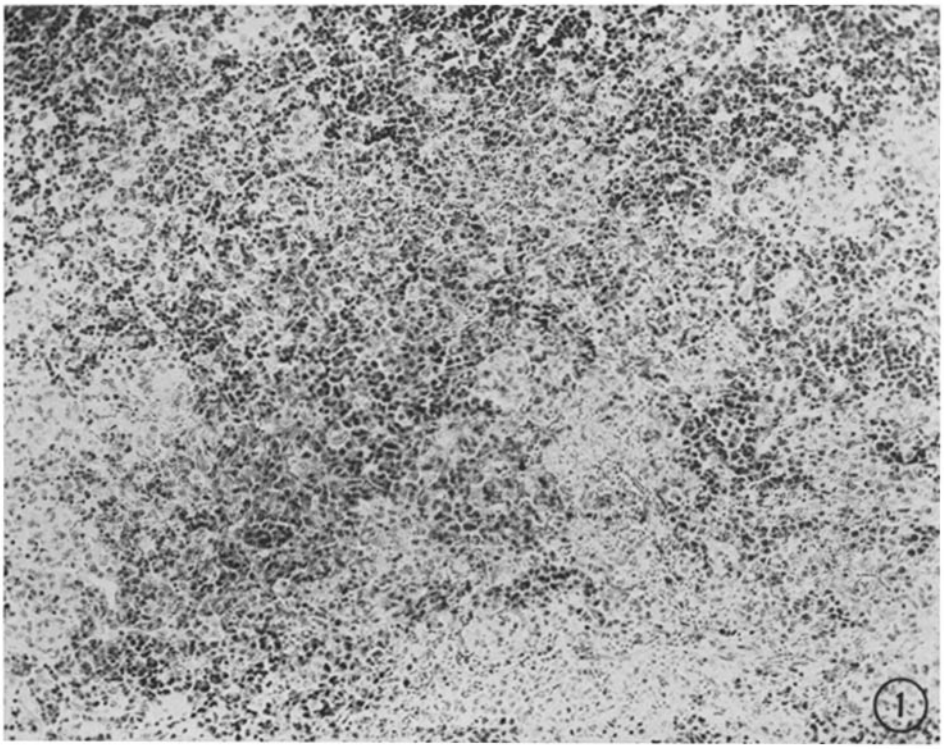
25. Congdon, C. C., and Urso, I. S., Homologous bone marrow in the treatment of radiation injury in mice, *Am. J. Path.*, 1957, **33,** 749.
26. Billingham, R. E., and Brent, L., Quantitative studies on tissue transplantation immunity. IV. Induction of tolerance in newborn mice and studies on the phenomenon of runt disease, *Phil. Tr. Roy. Soc. London, Series B,* 1956, **242,** 439.
27. Janković, B. D., Waksman, B. H., and Arnason, B. G., Role of the thymus in immune reactions in rats. I. The immunologic response to bovine serum albumin (antibody formation, Arthus reactivity, and delayed hypersensitivity) in rats thymectomized or splenectomized at various times after birth, *J. Exp. Med.*, 1962, **116,** 159.
28. Davies, A. J. S., and Doak, S. M. A., Fate of homologous adult spleen cells injected into new-born mice, *Nature*, 1960, **187,** 610.
29. Howard, J. G., Mitchie, D., and Simonsen, M., Splenomegaly as a host response in graft-versus-host disease, *Brit. J. Exp. Path.*, 1961, **42,** 478.
30. Metcalf, D., The thymic lymphocytosis stimulating factor, *Ann. New York Acad. Sc.*, 1958, **73,** 113.
31. Van Bekkum, D. W., Vos, O., and Weyzen, W. W. H., The pathogenesis of the secondary disease after foreign bone marrow transplantation in x-irradiated mice, *J. Nat. Cancer Inst.*, 1959, **23,** 75.
32. Castermans, A., Reevaluation of a pretreatment given to adult animals to modify their responsiveness to skin homografts, *Transplant. Bull.*, 1958, **5,** 381.
33. Najarian, J. S., and Feldman, J. D., Induction of runt disease in adult mice by presensitized homologous lymphoid cells, *Proc. Soc. Exp. Biol. and Med.*, 1962, **110,** 16.
34. Brent, L., Brown, J., and Medawar, P. B., Skin transplantation immunity in relation to hypersensitivity, *Lancet*, 1958, **2,** 561.
35. Stastny, P., and Ziff, M., Homologous disease in the adult rat, a model for autoimmune disease, *Fed. Proc.*, 1962, **21,** 42.
36. Denko, J. D., Simmons, E. L., and Wissler, R. W., The histopathology of delayed death in irradiated mice treated with homologous cells, *Radiation Research*, 1959, **11,** 557.
37. Oliner, H., Schwartz, R., and Dameshek, W., Studies in experimental autoimmune disorders. I. Clinical and laboratory features of autoimmunization (runt disease) in the mouse, *Blood*, 1961, **17,** 20.

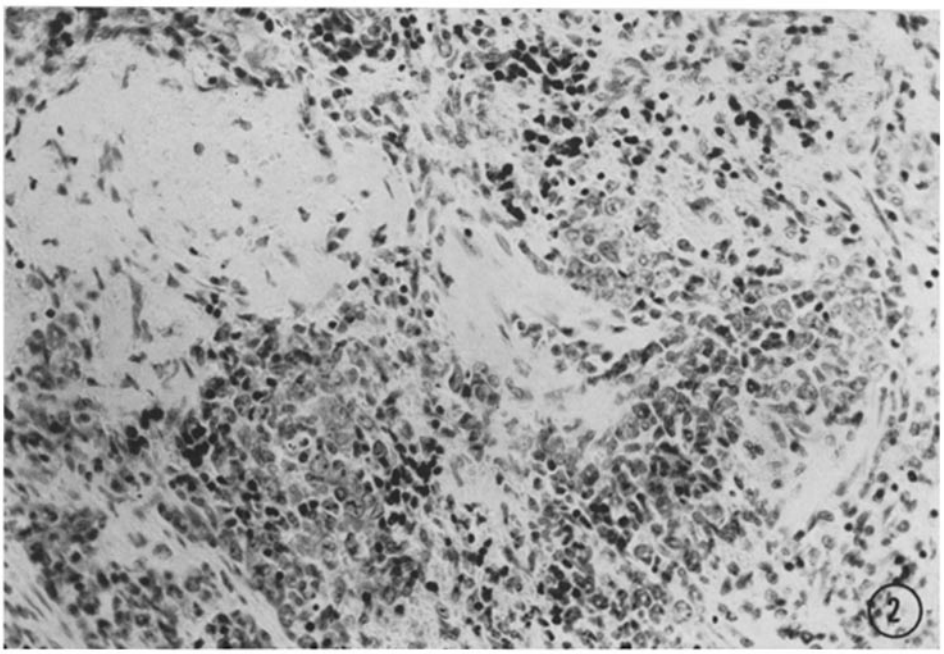
EXPLANATION OF PLATES

All photographs represent sections, stained with hematoxylin-eosin, of tissues from rats thymectomized at birth and injected with homologous spleen cells as adults, except Fig. 1 from a non-thymectomized control which developed runt disease. In each case the animal died of the disease process or was sacrificed when moribund.

PLATE 96

FIGS. 1 and 2. Spleen lesions. Fig. 1 from non-thymectomized rat, shows moderate (++) infiltration of red pulp with large pale reticulum cells. × 150. Fig. 2 shows a typical deposit of homogeneous eosinophilic material. × 300.

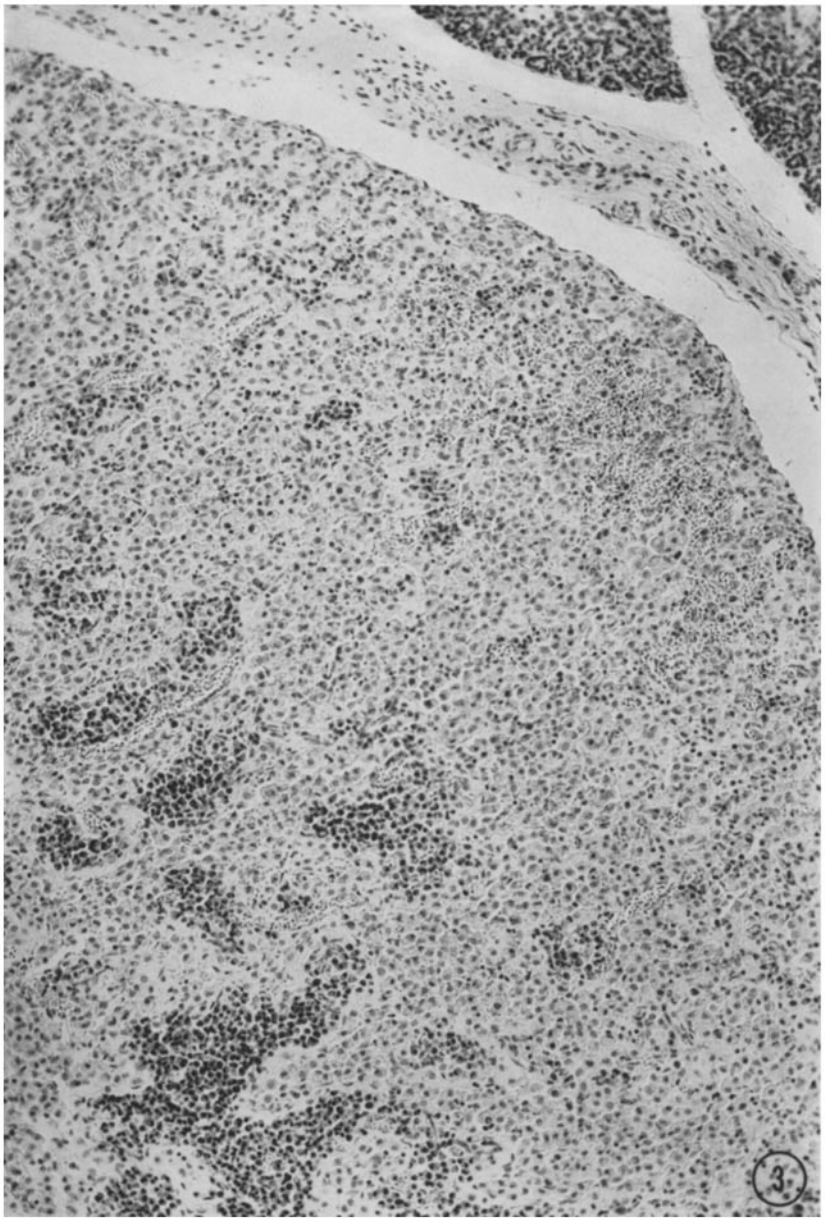




(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 97

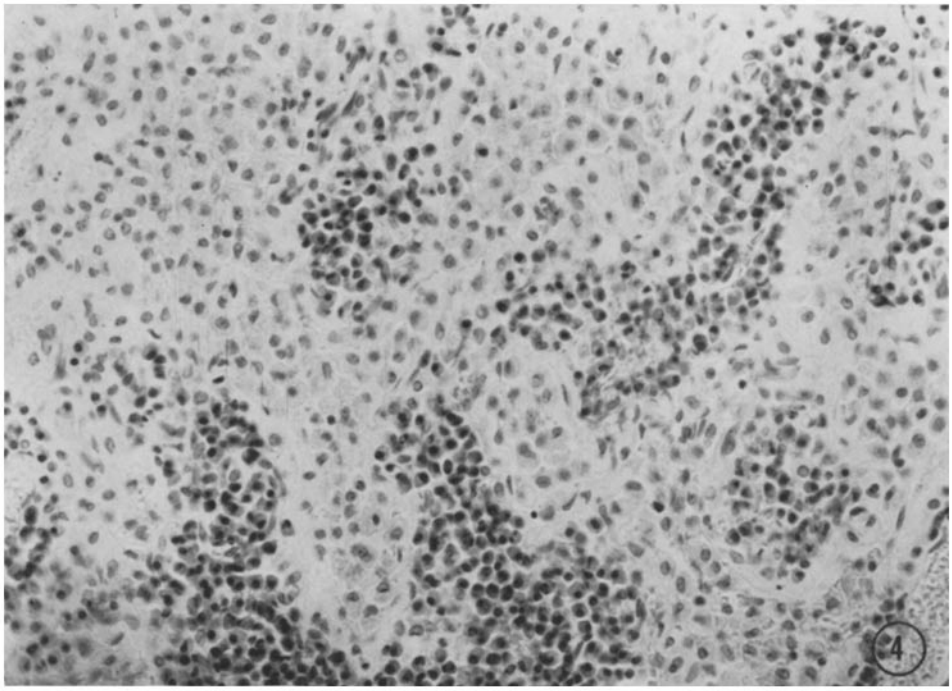
FIG. 3. Maximal lymph node lesion. Complete replacement of lymph node cortex and of major portion of medulla by large pale cells. Some of the medullary cords of plasma cells are preserved. × 150.

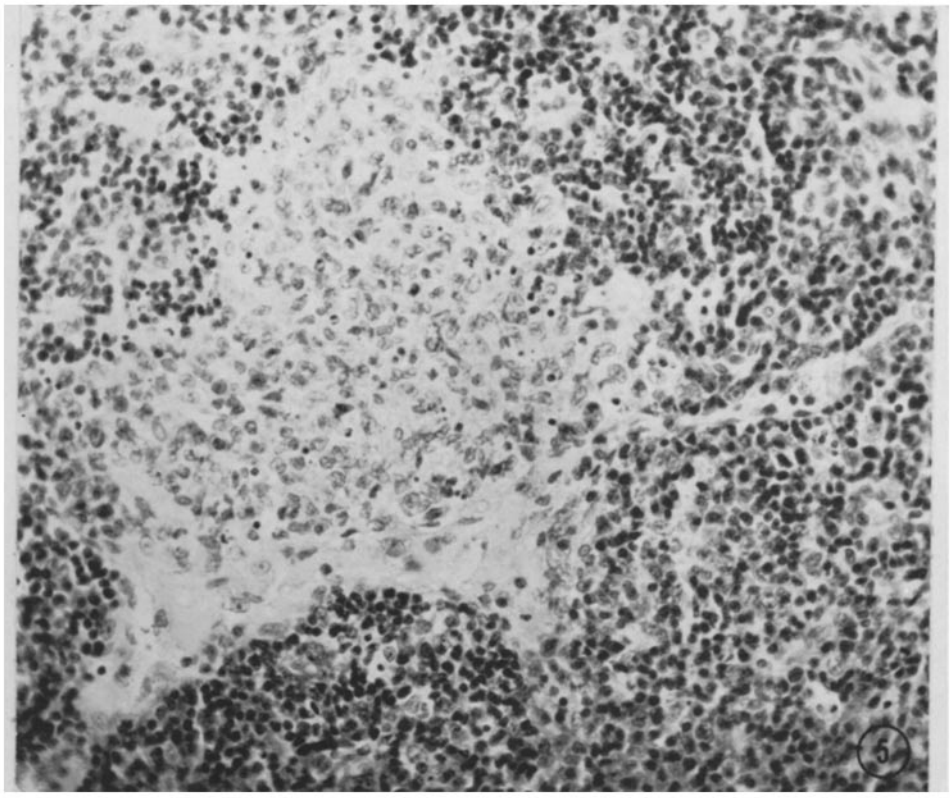


(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 98

FIGS. 4 and 5. Lymph node lesions. Fig. 4 shows high power view of same node illustrated in Fig. 3. Pale cells present typical morphology of fully developed macrophages. Fig. 5, from another animal, shows focus of proliferating pale cells resembling histiocytes, developing from intermediary sinus in cortex. × 300.





(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 99

FIG. 6. Liver, ++ lesion. Low power view shows infiltrative lesion near portal triad and focal intrasinusoidal deposits of hyaline material. Higher power shows intrasinusoidal aggregate of histiocytes with beginning infiltration of liver parenchyma. × 150 (above) and × 300 (below).

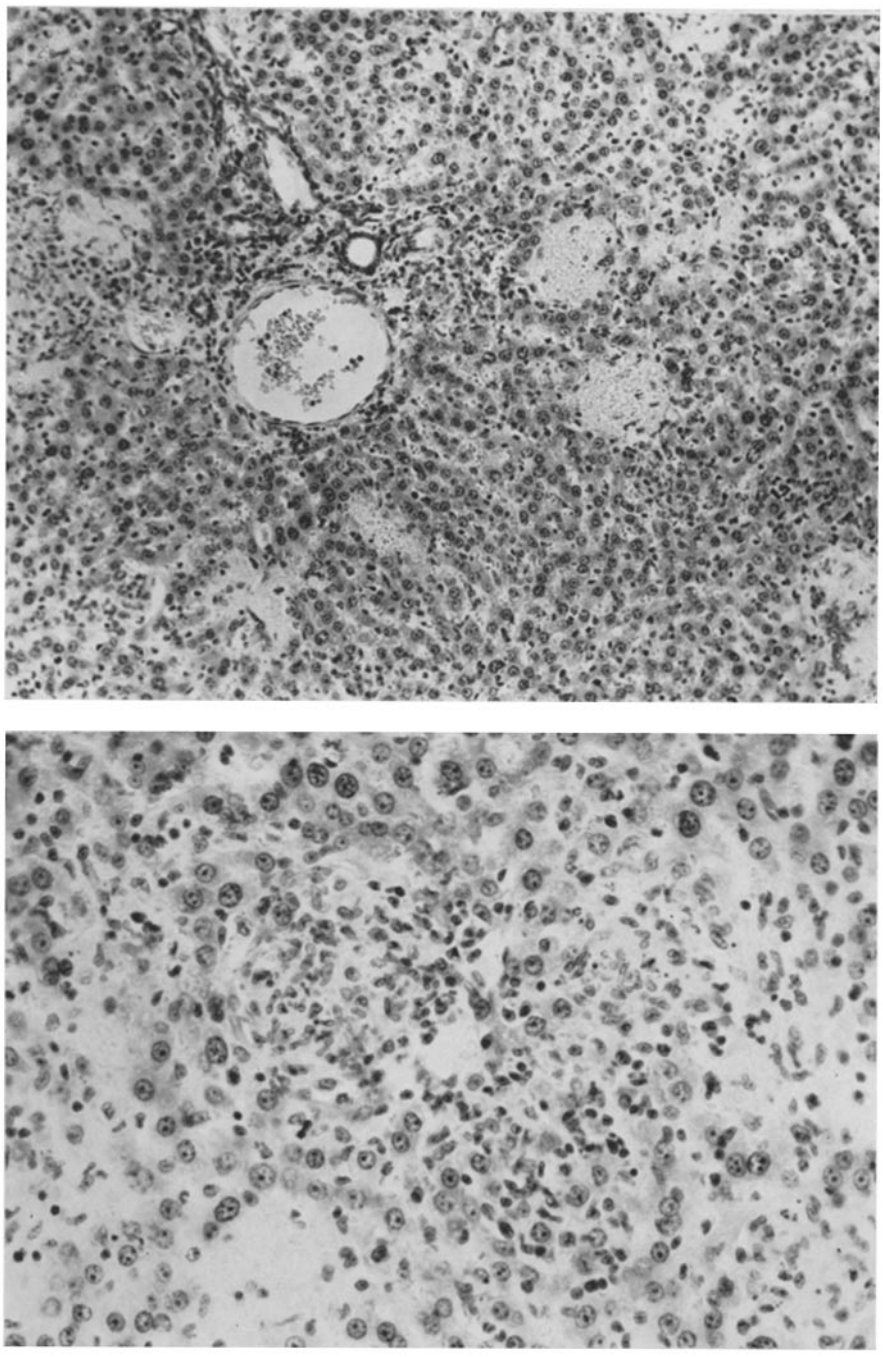


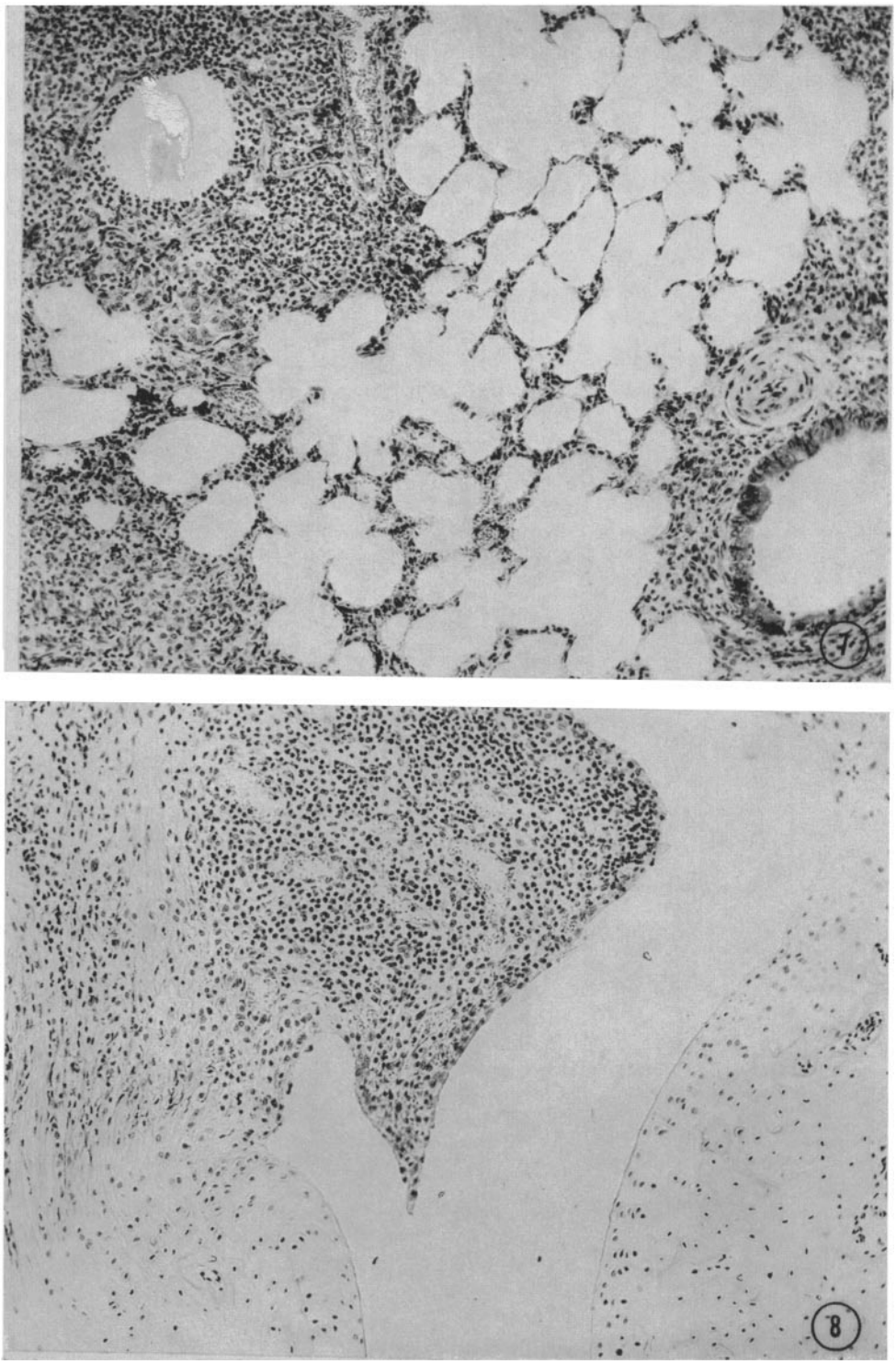
FIG. 6

(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 100

FIG. 7. Lung, ++ lesions. Infiltrative masses of large mononuclear cells, especially near bronchus, with focus of polymorphs (upper left). × 150.

FIG. 8. Wrist joint, +++ lesion. Synovitis, without pannus formation or alteration of cartilage. × 150.



(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 101

FIG. 9. Heart, +++ lesion. Massive infiltrative lesion, almost entirely histiocytic in character, with partial or total destruction of myofibers in zone of infiltration. × 150 (above) and × 300 (below).

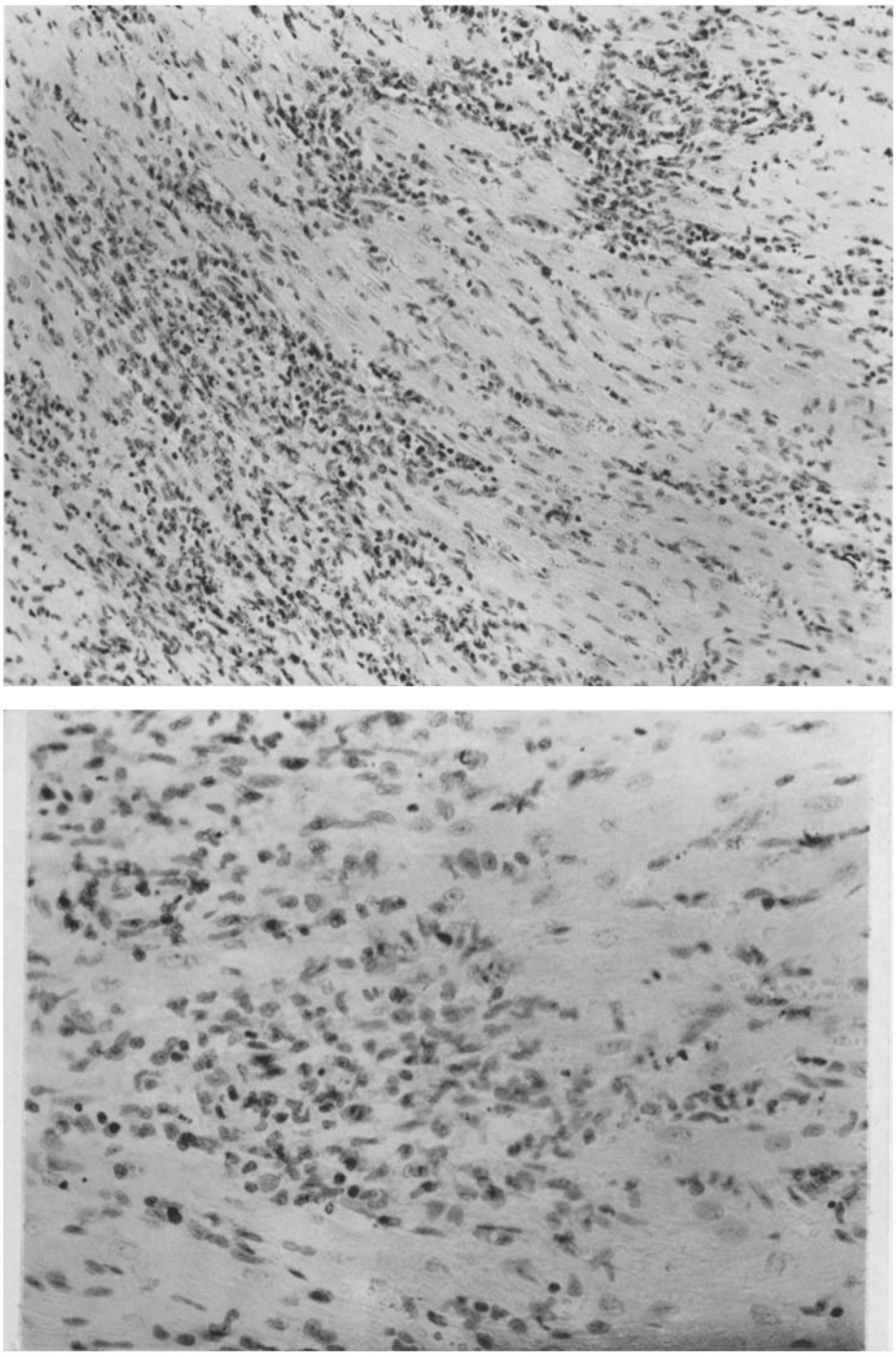


FIG. 9
(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 102

FIG. 10. Characteristic lesion of small bowel. Histiocytic infiltration of lamina propria and formation of microabscesses (arrows). High power view shows that these are filled with polymorphonuclear leukocytes. × 150 (above) and × 300 (below).

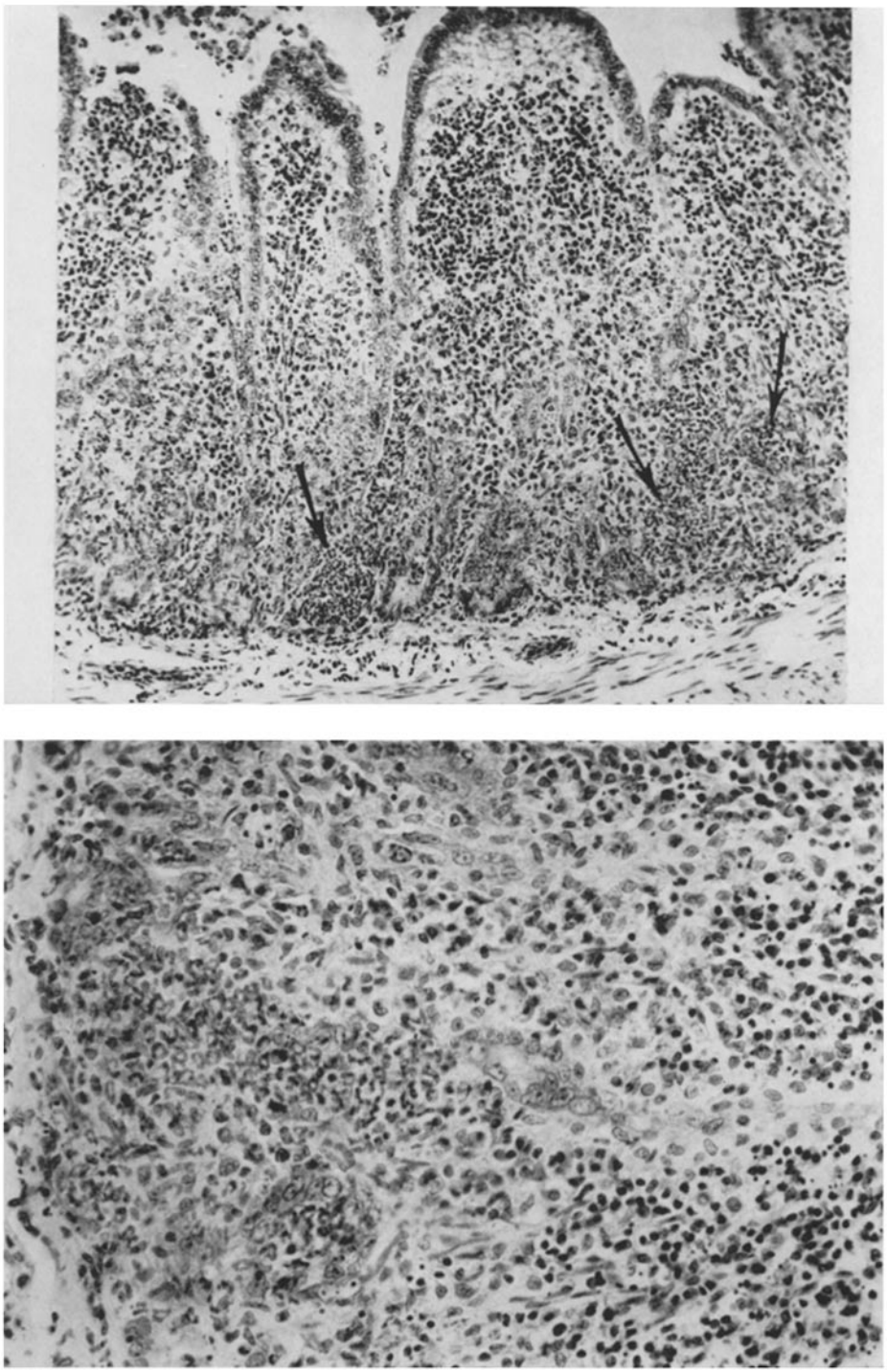
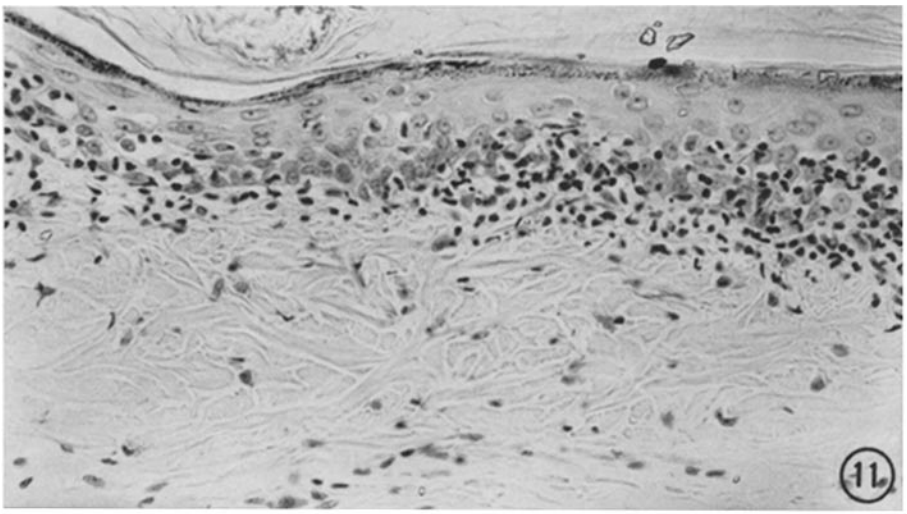


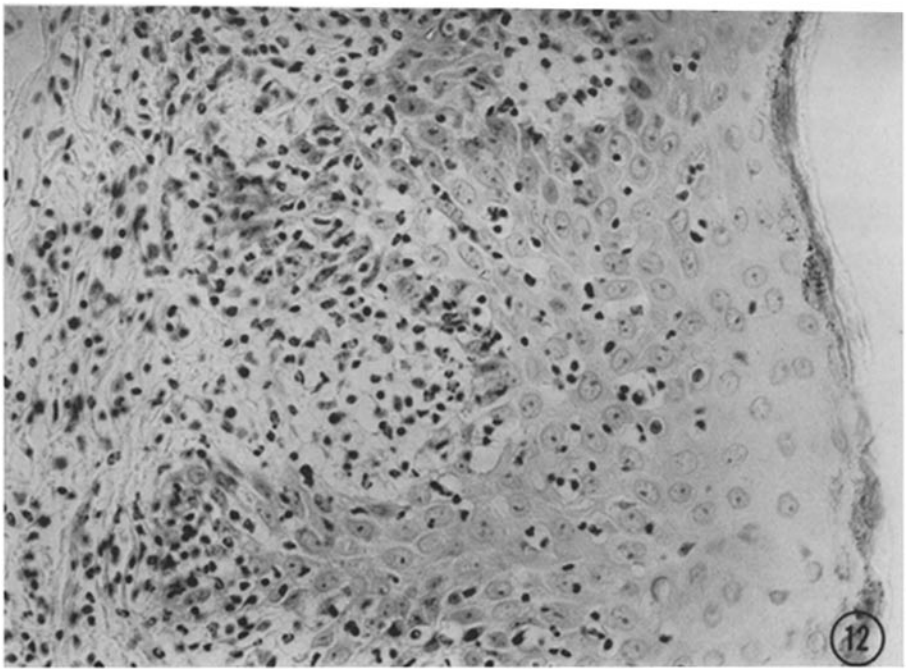
FIG. 10

(Aisenberg *et al.*: Runt disease in thymectomized rats)

PLATE 103

FIGS. 11 and 12. Skin sections from tail and ankle respectively illustrating characteristic lesions graded ++ and +++. Fig. 11 shows lymphocytic and histiocytic infiltration of upper dermis with invasion of epidermis and formation of spongiform vesicles. Fig. 12 shows extension of same process to produce psoriasiform lesion with acanthosis, elongation and fusion of rete pegs, edema of apices of dermal papillae. There is no parakeratosis, however. × 300.





(Aisenberg *et al.*: Runt disease in thymectomized rats)