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Runt Disease Produced in Thymectomized F1 Hybrid Mice Injected with Parental Strain Lymphoid Cells.* (27806)

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It has recently been shown that in mammals the thymus gland plays in early life an important role in development of immunological capacity. Guinea pigs(1), rabbits(2) and mice(3) thymectomized in early life show a decreased ability to produce circulating antibodies and thymectomy performed in mice during the neonatal period interferes with the capacity to reject either skin(4,5) or tumor (6) homografts. In addition, our recent reports seem to demonstrate that spleen cells derived from adult mice thymectomized shortly after birth fail to elicit graft *vs* host reactions, as determined by the Simonsen's assay of histocompatability(7). These results and the observation that mice of several inbred strains thymectomized shortly after birth fail to grow normally and regularly develop disease and wasting during the second or third month of life prompted us to study the development and course of the homologous disease induced by injection of normal lymphoid cells into mice previously submitted to complete thymectomy. For this purpose the production of immunological "runt disease" was attempted in young adult F1 hybrids by injection of lymphoid cells taken from normal adult donors isologous with one of the parent strains, and the effect of previous thymectomy performed in the F1 hybrid either immediately after birth or at 40 days of age upon the development and severity of the graft *vs* host reaction was studied. These experiments show that early thymectomy of the recipient F1 hybrid mice facilitates and increases the severity of the immunological disease produced by administration of normal splenic cells. Indeed, it is demonstrated that thymectomy performed as late as 40 days after birth appears to favor the development of homologous immunological disease.

Method. Mice of the A and C3H strains and F1 hybrids resulting from the cross between A and C3H and between A and C57Bl strains were used. Three experiments were performed and the following groups of F1 hybrid mice were prepared for each experiment: (a) sham-thymectomized, to be injected with parental spleen cells, (b) thymectomized, to be injected with same parental cells as group "a", and (c) thymectomized, to be kept as non-injected controls.

In a first experiment (A × C3H)F1 hybrid mice were operated upon during their first day of life and 35 days later groups "a" and "b" were injected intraperitoneally with approximately 200 million viable A strain spleen cells taken from 10- to 12-month-old donors. Mice of all groups were of approximately the same weight at time of injection. In a second experiment (A × C3H)F1 hybrid mice were operated upon at 40 days of age and 10 days later groups "a" and "b" were injected intravenously with approximately 200 million spleen cells donated by 6-month-old C3H mice. In a final experiment (A × C57Bl)F1 hybrid mice were submitted to surgery at 40 days of age and 10 days later groups "a" and "b" received intraperitoneally 150 million spleen cells obtained from 10- to 12-month-old A strain

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TABLE I. Effect of Thymectomy upon Susceptibility of F1 Hybrid Mice to Homologous Disease Due to Administration of Parental Strain Spleen Cells.

Host strain	Operation	Age at operation	Age at inj	Donor strain	No. cells inj (million)	No. animals died/No. in group	Survival period (days after inj, mean ± S E.)
(A × C3H)F1	Sham thymectomy	1-24 hr	35 days	A	200	2/10	28, 46
	Thymectomy	"	"	"	"	10/10	14 ± 1.3
	"	"	—	—	—	11/11	33 ± 2.9
(A × C3H)F1	Sham thymectomy	40 days	50 days	C3H	200	0/10	
	Thymectomy	"	"	"	"	0/9	
	"	"	—	—	—	0/12	
(A × C57Bl)	Sham thymectomy	40 days	50 days	A	150	8/14	32 ± 1.7
	Thymectomy	"	"	"	"	9/9	23 ± 2.4
	"	"	—	—	—	0/11	

donors. In all cases the donors were killed with ether and their spleens were made into suspensions of viable cells in Ringer-Locke's saline solution and immediately injected into the F1 recipients. Beginning when animals were 30 days old, body weight was determined twice a week until the end of experiment. In every group deaths were recorded in terms of days of survival after the time of injection of parent strain spleen cells. Spleen, liver and lymph nodes from dead animals and from living mice which survived 3 months after operation were prepared for histological studies.

Results. The results are summarized in Table I. In the first experiment, administration of 200 million A spleen cells to the (A × C3H)F1 mice operated upon at birth produced fatal runting disease in 20% of the sham-thymectomized animals. By contrast all the thymectomized mice died with signs of wasting disease, the mean survival period of the animals receiving the parental lymphoid cells being significantly shorter than that of the non-injected animals (14 ± 1.3 and 33 ± 2.9 days, respectively). In the second experiment where the operation was performed in 40-day-old (A × C3H)F1 mice and production of runting disease attempted with 200 million C3H spleen cells, all the animals grew steadily and were healthy during the entire observation period. In the final experiment, administration of 150 million A spleen cells to the (A × C57Bl)F1 hybrids operated at 40 days of age produced disease and death in 57% of the sham-thymectomized and in 100% of the thymectomized animals, after 32 ± 1.7 and 23 ± 2.4 days respectively. In this experiment none of the non-injected thymectomized mice showed growth alterations or disease. In all experiments mice dying in any of the 3 groups presented the characteristic signs of the runting disease, *i.e.*, loss of body weight, hunched appearance, poor condition of fur and sometimes diarrhea. Histologically, mice injected with parental cells, which developed the disease, showed characteristic atrophic changes of the lymphoid tissues. Histological study of the non-injected thymectomized mice also revealed alterations in the lymphoid tissues.†

Discussion. The results of the first experiment demonstrate that thymectomy performed immediately after birth in F1 hybrid mice increases their susceptibility to runting disease produced by injection of lymphoid cells from adult mice isologous with one of the parental strains. Early thymectomy without further treatment also caused disease and death in all mice. These animals failed to grow normally, developed roughing of the fur, diarrhea and a general appearance of wasting. This clinical syndrome has similarities to those commonly found in mice with immunological runt disease or homologous disease. Finally, all previously thymectomized mice died prematurely, probably as a result of intercurrent infections. It is therefore not surprising that these mice thymectomized immediately after birth were particularly susceptible to runting disease when injected with lymphoid cells from one of the parental strains. The deficiency resulting in mice thymectomized immediately after birth,

† To be published.

which was previously characterized by acceptance of tissue homografts, inability to form circulating antibodies, and failure of their lymphoid cells to elicit graft *vs* host reactions, is now further extended to include greater susceptibility and more precipitous course of the runting disease (graft *vs* host reaction) produced by administration of intact parental spleen cells than that observed in sham-operated animals.

Thymectomy in 40-day-old F1 hybrids did not alter their capacity to resist infections or to grow steadily, but in at least one strain combination, (A × C57Bl)F1 mice injected with A strain spleen cells, thymectomy increased susceptibility to runting disease, and significantly shortened the survival period usually seen after intraperitoneal injection of A parent strain cells. From previous experiments we know that thymectomy at this age does not interfere with the capacity of mice to reject skin or tumor homografts from a strain of mice differing from the grafted animals at the H-2 histocompatibility locus, but does prolong the survival of skin homografts interchanged between strains with a very weak histocompatibility difference(5,6). Thymectomy at 40 days of age might favor the development of runt disease simply because the total number of lympho-reticular cells in the thymectomized mouse has been reduced, leaving a relatively smaller target for the destructive adult parent spleen cells. Another alternative is that prior thymectomy, like sub-lethal total body irradiation(8) to the F1 recipient, may favor the development of the graft *vs* host reaction by decreasing the cellularity of the more peripheral lympho-reticular tissues such as the lymph nodes and spleen, thereby making room for the transferred lymphoid cells. It would be anticipated that under such conditions the homologous adult spleen cells might more readily become established and be able to launch an immunological attack on the recipient. Another hypothesis which is attractive to us is that this effect is observed because the thymus even for a period far beyond birth and perhaps extending throughout life is supplying lymphoid cells to the peripheral lymphoid tissue which replenish the substance of these peripheral organs and permit the intact animal to resist homologous disease to some degree. A similar conclusion has been drawn by Fichtelius(9) from studies of the distribution of injected thymic cells to peripheral lymphoid tissue such as the spleen. Whatever its mechanism, this quantitative and perhaps qualitative alteration of the lympho-reticular system produced by thymectomy would, indeed, facilitate its immunological destruction by the injected immunologically competent adult parental strain lymphoid cells.

The results of these experiments are in agreement with the demonstration by Papermaster *et al.*(10) that in young chickens hormonal suppression of the development of the bursa of Fabricius permitted more vigorous graft *vs* host reactions than were observed in untreated chickens following transplantation of adult chicken spleen cells. These results confirm and extend to adult life in mice the recent observations of Parrott(11)‡ that mice thymectomized at birth were susceptible to production of "runt disease" by injection of homologous cells when the recipient animals were approximately 2 weeks of age, whereas non-thymectomized controls were not susceptible.

Summary. Thymectomy performed immediately after birth increases the susceptibility of (A × C3H)F1 recipients to runt disease (homologous disease) produced by injection of A strain parent cells. Thymectomy performed as late as 40 days after birth enhances the susceptibility of (A × C57Bl)F1 recipients to homologous disease with A strain donor cells but has no demonstrable effect on susceptibility of (A × C3H)F1 recipients to C3H strain donor cells. The basis for these observations in terms of a reduction of available lympho-reticular mass and lympho-reticular reserve in the thymectomized animal is discussed. It is suggested from these observations that thymectomy even far later than the neonatal period has a significant effect on the integrity of the lympho-reticular system in mice.

‡ The paper of Parrott appeared after these studies had been completed and submitted for publication.

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EEG Studies of Normal and Encephalomalacia Chicks.* (27807)

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Although review of the literature fails to show the use of EEG in the study of function or abnormalities of the chicken brain, we have used this technic for studying nutritional encephalomalacia, one of the vitamin E deficiency syndromes. This condition, as described by Pappenheimer, involves primarily the cerebellum and to some extent the medulla and cerebrum, sparing the optic lobes (1-3). We have compared EEG records of standard tracings, photostimulation and pentylenetetrazol-induced convulsions, using normal birds, and chicks with various stages of nutritional encephalomalacia.

Administration of diets deficient in Vit. E induces in chicks certain well known syndromes, *i.e.,* encephalomalacia, exudative diathesis and muscular degeneration. In former studies of vitamin deficiencies, each bird usually showed more than one of these syndromes. Recent experiments have made it possible to differentiate nutritional factors pertinent to each syndrome by modifying the diets and producing single syndromes in individual experimental chickens. For example, selenium protects against exudative diathesis(4). Linoleic or arachidonic acid is necessary for development of encephalomalacia(5). Selenium and antioxidant protect chicks from muscle degeneration when they are fed a low sulfur containing diet(6). Diets deficient in arginine protect chickens from myopathy when they are on myopathy-producing diets(7).

Chickens fed for 2 or 3 weeks on diets which produce encephalomalacia exhibit various clinical signs which include ataxia, uncontrollable bicycling movements, coma and death. At the time our tracings were made a variety of these signs were evident.

Material and methods. Nichol 108 cockerels were placed on encephalomalacia producing diets, with controls kept on basal diets. EEG tracings were made from birds of each group selected at random from ages 4 to 20 days. Suitable restraining containers eliminated the use of any sedating drugs during the procedure. Med-Art Subdermal electrodes, shaped like a hook were passed through a pinch of skin over either side of the head, electrode #1 on the left, #2 on the right. An indifferent electrode, #3 was inserted over the posterior head region. A Grass 8 channel EEG machine was utilized, recording 2 or 3 birds simultaneously. Paper speed and voltage are indicated in the figures. Grass model PS 1 photostimulator was mounted with light source 5 inches directly before the birds; light intensity was 16. Photostimulation with and without blindfolds, and at varying frequencies lasted for periods of 10 seconds.

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