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Renal Lysozyme Levels in Mice Thymectomized at Birth.* (32594)

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Neonatal thymectomy in mice induces a syndrome of wasting which closely resembles classical runt disease. The latter is a form of graft-*versus*-host (GVH) reaction produced by injecting neonates with adult lymphoid cells of a different histogenetic origin. The resemblance includes weight loss, lethargy, diarrhea, hyperplasia of the reticuloendothelial system with terminal lymphoid depletion, and death. Phagocytic activity as measured by elimination of carbon particles from the blood is increased in GVH disease(1) and in athymic runts(2). Furthermore, the immunologic competence both of athymic runts and of animals undergoing a GVH reaction is reduced (3). Recent work by Troup and Walford(4) indicated that a striking increase in renal lysozyme occurs in mice undergoing classical runt disease. In the present report we show that renal lysozyme is also elevated in thymectomized mice and that this elevation correlates with the runting phenomenon.

Materials and methods. Inbred C3H mice were employed. Each newborn litter was randomly divided into individuals to be either thymectomized or sham-operated. In addition, 2 litters were set aside as non-operated controls. Thymectomy was performed within 24 hours of birth by the method of Sjodin *et al*(5), with Fluothane anesthesia. Sham-operated mice were similarly treated except that the thymus was not actually removed. All mice were weaned at 21 days of age, segregated according to sex into cages of from 1 to 4 mice, and maintained on standard mouse chow, supplemented for the first 10 days with wheat germ. Non-sterile water containing Tetracycline was given *ad lib*. A non-sterile, but clean environment was maintained.

The mice were weighed individually at weaning, at 6, 8, and 10 weeks of age, and at time of sacrifice. Thymectomized mice were sacrificed by cervical dislocation when characteristic symptoms of runting, including weight loss(6,7), developed. Diarrhea alone was not considered sufficient evidence of runting. Sham-operated and normal mice, and thymectomized mice without symptoms of wasting, were sacrificed along with the thymectomized wasted mice.

Immediately following sacrifice of a mouse both of its kidneys were removed, quick-frozen, and stored at —65°C. The spleen was weighed and a gross autopsy examination was performed. Representative hematoxylin and eosin-stained sections of liver and spleen were prepared from individuals of various ages within experimental and control groups. Renal lysozyme activity was determined as reported previously(4).

For statistical analysis, only those mice exhibiting overt symptoms of wasting were considered runts. Animals thymectomized at birth that did not waste before sacrifice were considered thymectomized non-runts, and were treated as a separate group. The Fisher ratio

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TABLE I. Comparison of Mean Renal Lysozyme Activity and Mean Spleen Weight in Experimental Thymectomized Runted and Non-Runted Mice and Control Sham-Operated and Non-Operated Mice.

	Control mice		Experimental mice	
	Non-operated	Sham-operated	Thymectomized non-runted	Thymectomized runted
Number of animals	7	15	26	14
Mean age at death (wk)	13.0	13.0	12.5	12.0
Mean spleen wt (% body wt)	0.42	0.46	0.84	1.17
Mean renal lysozyme activity*	1.28 ± 0.11	1.09 ± 0.06	2.07 ± 0.21†	5.52 ± 0.94‡

* Mean renal lysozyme activity is expressed in mg lysozyme equivalents per mg of protein ± S.E.M.

† Significantly different from the two control groups $P < 0.01$.

‡ Significantly different from the two control groups and from thymectomized non-runt group $P < 0.001$.

was calculated from the within and between column mean squares and employed to estimate significance levels.

Results. Of the 40 animals surviving the operative procedure of thymectomy, 14 (35%) developed frank signs of wasting. They were sacrificed between 8 and 14 weeks of age. 2 of these had diarrhea at death, whereas 4 of those which had been thymectomized but nevertheless classified as non-runts on the basis of body weights manifested diarrhea at sacrifice. 6 spontaneous deaths occurred between weaning and sacrifice. No attempt was made to determine the cause of these deaths. However, all occurred within the thymectomized group.

Results are summarized in Table I. Significantly elevated mean renal lysozyme levels occurred both in the thymectomized runted group ($P<0.001$) and the thymectomized non-runted group ($P<0.01$) when compared to the two control groups. Also, thymectomized runted mice had higher renal lysozyme values than thymectomized non-runted mice ($P<0.001$). Values for sham-operated and non-operated mice were not significantly different from one another.

Renal lysozyme activity within the thymectomized runted group, though highest in mean value, showed a wider variation than in any other group (Fig. 1). The 2 runts with diarrhea had very high renal lysozyme values, whereas only 1 of 4 of the thymectomized non-runts with diarrhea showed a disproportionately high level. 1 sham-operated control animal appeared somewhat wasted at time of sacrifice. However, its renal lysozyme level fell below the mean for that group. No other animals in the 2 control groups manifested any signs of runting, nor did any have diarrhea. There was no correlation between age, sex, litter size, and renal lysozyme activities.

Individual relative weights of spleens (expressed as % of body weight) and variability of splenic weights between mice was greatest in the thymectomized runted group (Fig. 2). Compared to the 2 control groups, spleens from runts averaged a 3-fold increase in relative weights, from non-runts a 2-fold increase (Table I). All animals having diarrhea had elevated relative spleen weights (Fig. 2), although not all with diarrhea had increased enzyme activities (Fig. 1).

The spleens and livers of the thymectomized runted and non-runted mice were in fact not markedly different on histologic examination from those of the sham-operated controls. There were very small foci of cellular infiltration (microabscesses) in the livers of the majority of experimental mice, but also in an occasional control mouse. Decreased numbers of small lymphocytes and inactive germinal centers, increased amount of red pulp, and scattered neutrophil influx in lymphoid organs were only present to an equivocal degree in experimental as compared to control animals. A mild degree of histiocytic conversion was noted in some spleens from severely runted animals.

Discussion. The approximately 5-fold increase in renal lysozyme in athymic runted animals in the present experiments was less marked than in the classical runt disease induced by injecting neonates with histo-incom-

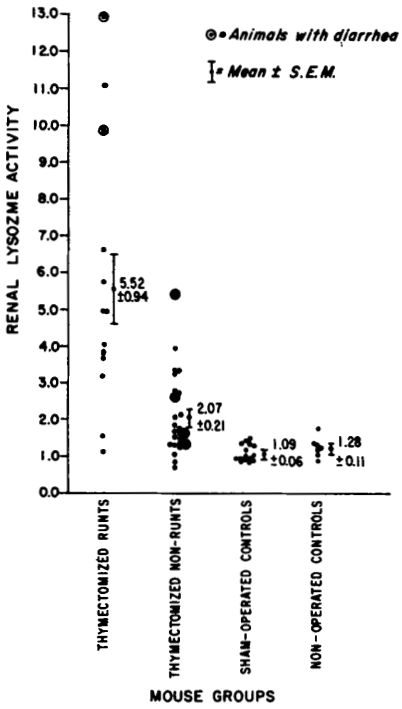

FIG. 1. Individual renal lysozyme activities in mice thymectomized at birth and in control mice, expressed as mg. lysozyme equivalents per mg. of protein.

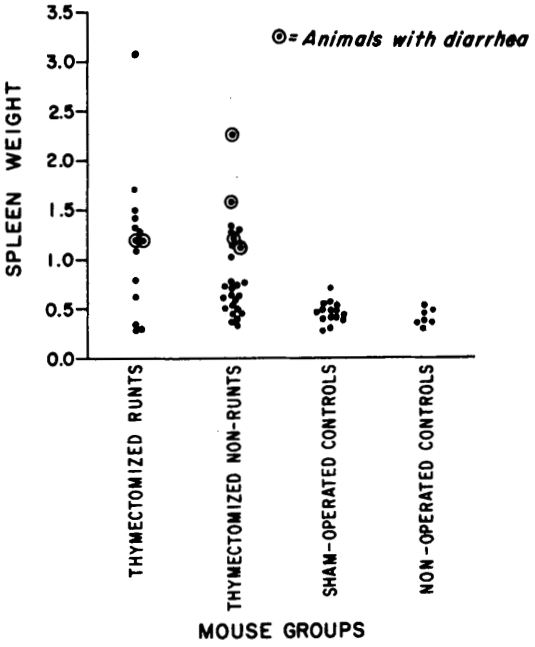

FIG. 2. Individual spleen weights in mice thymectomized at birth and in control mice, expressed as per cent of body weight.

patible lymphoid cells(4). This quantitative difference in levels of enzyme activity may be due simply to the much shorter time required for runting in the latter instance (10 to 16 days) than in the former (8 to 14 weeks). A 2-fold increase in enzyme levels was also noted in thymectomized mice which did not show obvious runting.

Mammalian leukocytes and macrophages contain high concentrations of lysozyme (8,9). The enzyme is also elevated in the extracts of reticuloendothelial tissues obtained from radiation-chimeras during periods of active lymphatic proliferation(10,11). Perri *et al*(12) showed that exogenously-administered as well as endogenously-produced lysozyme accumulates in the kidneys of rats and guinea pigs as its level in the blood rises. Renal lysozyme indeed appears to increase concomitant to any of a number of varieties of immune response(4). Its level is a good general indicator or parameter of immunological activity.

The elevated enzyme levels in post-thymectomy wasting disease attest to the existence of an immunological response on the part of the experimental animals. This response might be directed against environmental factors, for recent evidence(13,14) indicates that mice thymectomized at birth can in fact respond to later antigenic challenge with the production of antibodies. A 2nd possibility is that the immune response is anti-allergic in nature, in line with the concept of de Vries *et al*(15) that the fundamental lesion in post-thymectomy wasting disease is failure of immunocompetent cells to be able to recognize "self" antigens of the host. The demonstration by Brézin *et al*(16) of antinuclear antibodies in the sera of neonatally thymectomized mice strengthens this concept.

Summary. C3H mice thymectomized at birth showed significantly elevated renal lysozyme levels with the onset of wasting disease at 8 to 14 weeks of age. This finding supports the idea that wasting is associated with an active immune response, the nature of which is not yet established.

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Coxsackie B_4 Viral Nephritis in Mice and Its Autoimmune-Like Phenomena.* (32595)

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During a recent investigation on viral valvulitis in mice produced by Coxsackie B_4 virus(1), it was noticed that, besides in the valves and other tissues, antigen was found in the renal glomeruli and tubules of several chronically infected mice. Glomerulonephritis and tubular necrosis were noted on histologic examination in those animals where the presence of virus could be confirmed. It has long been thought that some renal glomerular diseases have an immunologic basis as demonstrated by the presence of gamma globulin and a component of complement believed to represent evidence of a hypersensitivity mechanism(2,3). However, little is known concerning the primary etiologic agent responsible for the provocation of hypersensitivity reaction in such cases.

A report of autoimmune reaction and the presence of virus-like particles in germ-free NZB mice by East *et al*(4) suggests the possible role of virus infection in "triggering" an autoimmune disease. Renal lesions similar to chronic membranous glomerulonephritis or lupus nephritis were frequent in these mice (5,6). The present report concerns the localization of globulin and Coxsackie B_4 antigen in the kidney of experimentally infected mice by use of the immunofluorescent technique.

Materials and methods. 36 HaM/ICR mice weighing 15-20 g were inoculated intraperitoneally with 0.1 ml of monkey-kidney culture fluid containing 10^5 $TCID_{50}$ of Coxsackie virus B_4(7). 24 littermates as controls were injected intraperitoneally with 0.1 ml of virus-free monkey-kidney culture fluid. The experimental and control animals were killed at weekly intervals for 8 weeks following the injection. Kidneys were then studied for histopathology and immunofluorescence of Coxsackie B_4 viral antigen and deposited globulin.

The direct immunofluorescent technique(8) was used to identify the viral antigen and

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