40% diet without drinking water do not grow as well as those fed a 50% diet with drinking water. While this may be attributed to the somewhat lower caloric intake of the former group it is more likely the result of inadequate water intake since rats ingesting an equal volume of the same 40% diet with *ad libitum* water grew as well as the 50% group and even utilized the diet more efficiently (g gain/g dry diet consumed). Since the solid nutrient intake was equal for the groups fed the 40% diet (approximately 16 g) it may be deduced that under these conditions the minimal water requirement compatible with good diet utilization and growth is represented by the 40% + *ad libitum* water regime. This value corresponds to about 39 ml/rat/day. The total volume of a water soluble diet combining 39 ml of water and 16 g of solids into a single source would be 48 ml. This is equivalent to a diet containing 33% (w/v) solids and would represent the maximum dietary concentration capable of simultaneously satisfying the water and solid nutrient requirement of the weanling rat.

Summary. A chemically defined liquid diet was found to be well suited for studying water:nutrient interrelationships. When supplied as the sole source of water and solid nutrients such a diet can satisfy the nutritive requirements of the growing rat. In this study rats fed diets containing 30% (w/v) or 40% (w/v) solids as the sole source of water and nutrients appeared healthy and grew well. Under the same conditions a 50% (w/v) diet proved hypertonic, promoted slower growth and produced highly concentrated, hypertonic urine. More diluted (10% and 20%) diets were also inadequate, but, in this case, the cause was probably poor diet utilization. When drinking water was provided with a diet containing 50% (w/v) solids, good growth was obtained and urinary specific gravity and osmolarity were reduced.

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Reversal of Post-Thymectomy Wasting Syndrome with Multiple Thymus Grafts in Diffusion Chambers.* (31700)

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Previous experiments in this laboratory demonstrated that wasting syndrome in thymectomized mice can be reversed by intact multiple syngeneic or allogeneic thymus grafts (1,2). Osoba and Miller(3) and Levey *et al* (4) have shown that a non-cellular thymic factor can stimulate lymphoid maturation and development of normal immune capacity in thymectomized mice prior to the onset of wasting. The following studies were performed to determine whether thymic humoral mechanisms, apart from direct cellular mechanisms, are effective in reversing wasting syndrome and restoring immunologic capacity in thymectomized mice.

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Materials and methods. Thymectomy or sham-thymectomy was performed on inbred C3H/HeJ mice during the first 12 hours of life, employing hypothermic anesthesia. The onset of wasting was determined by the appearance of diarrhea, ruffled fur, hunched posture, a sustained weight loss of at least 7 days duration or a failure to gain weight for 10 days or more, and a progressive lym-

phopenia. Diffusion chambers were constructed(4) using Millipore®† filter membranes (pore size = 0.30 μ). Thymus for grafting was obtained from live donors less than 24 hours old.

Seventy-one mice with fully developed wasting syndrome were selected at random and divided into the following treatment groups:

Group I: Thirty-three mice were each grafted with 5 newborn syngeneic thymuses in a single diffusion chamber.

Group II: Twenty mice were each grafted with 5 newborn allogeneic thymuses in a single diffusion chamber, from mice in the same H-2 group (CBA/J into C3H/HeJ).

Group III: Eighteen mice were each grafted with 5 newborn allogeneic thymuses in a single diffusion chamber from mice in a different H-2 group (DBA/2J into C3H/HeJ). All chamber grafts were placed intraperitoneally.

Body weights, absolute lymphocyte counts and a record of signs of wasting were obtained on all mice before thymus grafting, at time of grafting, and thereafter at periodic intervals. Once mice had recovered from wasting syndrome, they were challenged twice with allogeneic skin grafts from donors of a different H-2 group. Mice grafted with allogeneic thymus also received skin grafts from the thymus donor strain. The discriminating spleen assay of graft-*versus*-host reactivity (5), was employed to determine the degree of immunologic capacity in all recovered mice and the presence of possible cell chimerism in mice grafted with allogeneic thymuses. Two to four months following recovery from wasting syndrome, splenectomy was performed on all mice, and host and donor components on these spleen were assayed in appropriate litters of 6- to 10-day-old hybrid mice(6). A section of each spleen was saved for histologic examination.

Necropsies were performed on mice that died during this study, and at the end of one year, all surviving mice were killed, the diffusion chambers examined, and a search made for thymic tissue in its normal anatomic location. Other lymphoid tissues and major organs were also examined.

Results. Control studies. Of 111 untreated, neonatally thymectomized C3H mice which developed full-blown wasting syndrome, 78 (70%) died within 4 months(1,2) and 103 (93%) were dead by the end of 8 months. The few remaining mice continued to survive with persistent signs of wasting. Wasting syndrome was not detected among the sham-thymectomized controls.

Experimental studies. Recovery. Four months after thymus grafting, 12 of the 33 mice (36%) in Group I, 7 of the 20 mice (35%) in Group II and 6 of the 18 mice (33%) in Group III had recovered from wasting syndrome. The remaining mice in each group died with typical wasting syndrome. All animals which recovered demonstrated normal physical appearance and remained healthy at the end of one year. More than half of all deaths occurred within 2 weeks of thymus grafting, and the remainder occurred by the end of one month. As in previous experiments(1,2), reversal of external signs of wasting usually preceded the return of absolute lymphocyte counts to normal.

Body weights. As illustrated by Fig. 1, all mice gained weight normally until approximately age 4 weeks, when the neonatally thymectomized mice stopped gaining and/or began losing weight. The untreated thymectomized mice and wasted mice which died after receiving multiple syngeneic or allogenic thymus grafts in diffusion chambers gained no more weight and continued to show signs of wasting until their death. The mice in all 3 groups which recovered from the syndrome began to gain weight within one week after thymus grafting and continued to gain slowly over the following weeks. Despite regaining normal physical appearance and the restoration of immune capacity, these mice never equalled the weight of sham-thymectomized controls.

Lymphocyte counts. Serial absolute lymphocyte counts on control and experimental mice are presented in Fig. 2. Sham-thymectomized mice achieved a normal adult level by several weeks of age, while neonatally thymectomized mice demonstrated a progres-

† Millipore Filter Corp., Bedford, Mass.

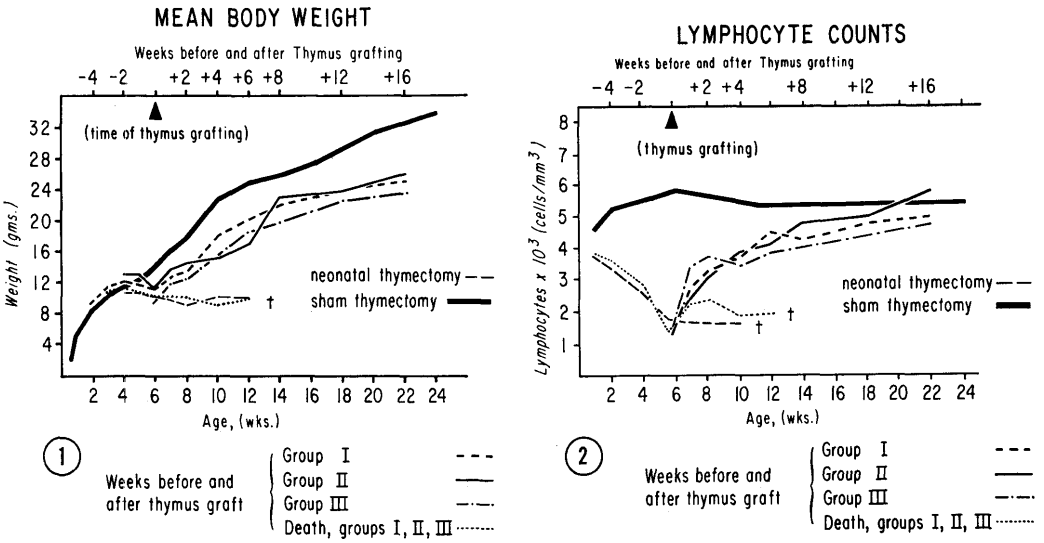

FIG. 1. Mean values for body weights of untreated thymectomized mice, sham-thymectomized mice and thymectomized mice which recovered after being grafted with thymus in diffusion chambers.

FIG. 2. Mean absolute lymphocyte counts in peripheral blood of untreated thymectomized mice, sham-thymectomized mice and thymectomized mice which recovered after being grafted with thymus in diffusion chambers.

sive lymphopenia, reaching a low plateau between 1,000 and 2,000 cells/mm^3 by six weeks of age. The mean absolute lymphocyte counts remained at this low level in untreated, neonatally thymectomized mice. Although mice that died in Groups I, II, and III showed some increase in lymphocyte counts after thymus grafting, this rise was only transient and most of these mice were again markedly lymphopenic before death.

All mice which recovered from wasting showed comparable rises in lymphocyte counts whether they received syngeneic or allogeneic thymus grafts in diffusion chambers. This inincrease in lymphocyte counts occurred gradually: The mice were still lymphopenic at 4 weeks and mean lymphocyte counts did not approach normal levels until 8 to 14 weeks after thymus grafting. Although mean absolute lymphocyte counts eventually did approach normal, the lymphocyte/granulocyte ratio remained reversed for some mice, at 40/60 rather than the normal 60/40 usually observed in healthy C3H mice.

Skin graft survival. It can be seen from Table I that sham-thymectomized mice demonstrated normal first and second set rejection of skin allografts. Previous studies have shown that untreated neonatally thymectomized mice often retain skin allografts until death(1,2,7). All mice which recovered from wasting after receiving syngeneic or allogeneic

TABLE I. Mean Skin Graft Survival Time in Sham-Thymectomized Mice and Wasted Mice Grafted with Thymus in Diffusion Chambers.

Skin donor strain	Host strain		Mean skin graft survival time ± S.D., days*			
			Sham-thymectomy	Group I	Group II	Group III
BALB/c	C3H	1st set	12.0 ± 1.1 (20)	12.4 ± .8 (12)	14.5 ± 1.4 (7)	14.0 ± 2.2 (6)
		2nd "	6.8 ± 1.0 (20)	8.4 ± 1.1 (12)	8.1 ± 1.0 (7)	10.3 ± 2.0 (6)
CBA	C3H	1st "	14.8 ± 2.4 (20)		14.4 ± 2.1 (7)	
		2nd "	8.3 ± .9 (20)		9.6 ± 1.6 (7)	
DBA/2	C3H	1st "	11.2 ± .8 (20)			13.5 ± 1.4 (6)
		2nd "	6.4 ± .6 (20)			7.8 ± .8 (6)

* No. of mice grafted given in parentheses.

TABLE II. Discriminating Spleen Assay of Graft-*Versus*-Host Reactivity of Wasted Mice Grafted with Thymus in Diffusion Chambers.

No. recovered	Donor assay strain: Thymus donor strain	Donor assay strain: Host strain	Donor strain spleen indices, mean and range: Control	Donor strain spleen indices, mean and range: Experimental
12 (Group I)	C3H	C3H	2.35 (2.10-3.22)	2.05 (1.76-3.00)
7 (Group II)	CBA	C3H	2.26 (1.88-2.98)	2.60 (2.21-3.40)
6 (Group III)	DBA/2	C3H	2.46 (1.90-3.46)	1.94 (1.72-2.41)

TABLE III. Discriminating Spleen Assay of Graft-*Versus*-Host Reactivity of Wasted Mice Grafted with Thymus in Diffusion Chambers.

No. recovered	Host assay strain: Thymus donor strain	Host assay strain: Host strain	Host strain spleen indices, mean and range: Control	Host strain spleen indices, mean and range: Experimental
7 (Group II)	CBA	C3H	2.42 (1.70-3.27)	1.01 (.72-1.10)
6 (Group III)	DBA/2	C3H	2.61 (2.11-3.43)	.83 (.70- .98)

thymus grafts in diffusion chambers demonstrated normal primary and secondary immune responses to skin allografts. There did not appear to be any significant difference among groups or between experimental groups and sham-thymectomized controls. Mice which recovered from wasting after receiving allogeneic thymus in diffusion chambers also rejected skin from the thymus donor strain in normal fashion. Mice which died following thymus grafting did not survive long enough to receive skin allografts.

Discriminant spleen assays. The discriminating spleen assay of graft-*versus*-host reactivity on mice that recovered from wasting in Groups I, II and III indicates the presence of host-type immunocompetent cells able to react against foreign antigen (Table II). The mean host strain spleen index exceeded 2.0 in most instances. Cells reacting as thymus donor-type could not be demonstrated in any of the mice which were grafted with allogeneic thymus in diffusion chambers (Table III).

Histology. Thymus remnants were not identified grossly or microscopically in the mediastinum of any mouse which recovered from the syndrome. Because of post-mortem autolysis of tissues, it was not possible to examine the histology of approximately half the mice that died in each group. In the present study, as in previous ones(1,2,7), untreated neonatally thymectomized mice had a hypoplastic lymphoid system. The normal follicular structure of lymph nodes and spleen was usually absent, germinal centers were rare, Peyer's patches were atrophic and there was a paucity of small lymphocytes in all lymphoid organs.

No significant differences in lymphoid morphology could be detected between surviving mice which received syngeneic *or* allogeneic thymus grafts in diffusion chambers. Despite apparent recovery from wasting syndrome, surviving mice had significant lymphoid hypoplasia. Lymphoid tissues showed a moderate to marked depletion of small lymphocytes; lymphoid follicles and germinal centers were smaller and fewer in number than in normal mice. This is in contrast to previous studies which demonstrated normal lymphoid architecture in some mice which recovered from wasting syndrome after receiving *intact* thymus grafts(1,2). Lymphoid tissues of mice dying with wasting syndrome despite thymus grafts in diffusion chambers were indistinguishable from those of untreated neonatally thymectomized mice.

Diffusion chambers removed from mice that died contained only necrotic tissue or scattered epithelial-reticular cells and numerous small lymphocytes with pyknotic or fragmented nuclei. This finding is in contrast to the diffusion chambers removed at the end of one year from mice which recovered from the syndrome. These chambers were usually encapsulated in fibrous connective tissue and appeared to be intact. Histologically, tissue removed from them was composed of epi-

thelial-reticular cells scattered in amorphous debris or arranged in clumps. Lymphocytes were not identified in this tissue.

Discussion. These experiments have demonstrated that wasting syndrome produced by neonatal thymectomy can be reversed by grafting multiple newborn syngeneic or allogeneic thymuses in diffusion chambers. Recovery is documented by return of normal physical appearance, disappearance of signs of wasting, restoration of normal peripheral blood lymphocyte counts, normal first and second set rejection of allogeneic skin grafts, and normal immunologic capacity of lymphoid cells in graft-*versus*-host reactions. In every instance recovery from wasting sydrome was associated with restoration of immune capacity.

Since the diffusion chambers remained intact (pore size = 0.30 μ), presumably thymus donor cells did not have the opportunity to reach the host's circulation and must have exerted their effect by non-cellular means. It has been previously demonstrated that newborn or fetal thymus contained in a diffusion chamber can promote normal immunologic maturation in neonatally thymectomized mice prior to the onset of wasting(3,4). In the present experiments, presumably a thymic humoral factor was elaborated in the chamber and passed into the host's circulation; this factor(s) in some manner stimulated maturation of the hypoplastic lymphoid system in the wasted mouse, development of which was associated with recovery from wasting syndrome.

Thymuses grafted within diffusion chambers appeared to be less successful in reversing wasting syndrome than intact multiple thymuses, grafting of which was followed by recovery in 53% to 66% of affected mice. Although in previous studies syngeneic thymus was more effective in reversing wasting than allogeneic thymus(1,2), in the present investigation similar results were obtained with both syngeneic and allogeneic thymuses grafted within diffusion chambers. This seems to indicate that the humoral substance(s) elaborated by the thymus which promotes maturation of the lymphoid system is not strain specific and is effective in the absence of direct cellular mechanisms of action of the thymus. The work of Osoba(8) also supports this thesis. These experiments are somewhat analogous to those reported by Miller *et al*(9), who restored immunologic capacity to adult thymectomized, irradiated mice with syngeneic thymuses in diffusion chambers.

Unlike the wasted mice which became cellular chimeras with the thymus donor strain after receiving multiple intact allogeneic thymus grafts(1,2), no donor-type cellular activity could be demonstrated by discriminant spleen assay of mice grafted with allogeneic thymus in diffusion chambers. Lymphoid tissues in some of the cellular chimeras described in previous experiments appeared normal(1,2). This is in contrast with the present studies which demonstrated persistent lymphoid hypoplasia despite overt recovery from wasting syndrome. In the first instance, the intact grafted thymus must have made a cellular contribution to the hypoplastic lymphoid system of the wasted mouse as well as providing a humoral stimulus. In the latter instance, cellular mechanisms were precluded by the diffusion chamber and the grafted thymus must have exerted its effect by stimulating potentially immunocompetent host cells. Although lymphoid morphology remained hypoplastic, immunologic capacity was restored.

These experiments have shown that the thymus can promote reversal of wasting syndrome without making a cellular contribution to the host.

Summary. Wasting syndrome in neonatally thymectomized mice is reversed by multiple newborn syngeneic or allogeneic thymus grafts in diffusion chambers. Recovery from the syndrome is associated with incomplete lymphoid tissue development but restoration of normal immune capacity in affected mice. Thymic humoral factor apparently responsible for these results does not appear to be strain-specific.

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Methylation of t-RNA: Effect of Vitamin B_{12} Deficiency in Rat. (31701)

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Dunn *et al*(1) first reported the presence of small amounts of methylated purines and pyrimidines in amino acid transfer ribonucleic acid in addition to the 4 main bases. The methyl groups of these bases were shown to be derived by methionine by transmethylation at the polynucleotide level of the previously formed t-RNA(2,3). Evidence for this was first obtained by Borek and his associates(4) by examination of the RNA that accumulated during methionine starvation of the auxotroph of *Escherichia coli* K_{12} W_6 which requires the essential amino acid. The newly formed t-RNA lacked the methylated bases which pointed to the possibility that methionine in the form of S-adenosylmethionine was the active methyl donor. This was later confirmed by both *in vitro* and *in vivo* systems using labeled methionine(5,6). Following these findings, attempts were made to study the enzymatic methylation of methyl-deficient t-RNA in cell-free systems using enzymes which are called "RNA methylases". Hurwitz and his group(7) were mainly responsible for isolating several of these enzymes which were shown to be specific for the individual bases. These enzymes were shown to be capable of further methylating an otherwise fully methylated t-RNA from a different organism(8,9). Trace amounts of methylated bases are also found to occur in ribosomal RNA(10) and enzymes which methylate ribosomal RNA have been discovered recently (11,12,13). The purpose of our present study was to examine the possibility of an involvement of vitamin B_{12} in a pathway of methylation of ribonucleic acids in rat liver. Preliminary results presented here lead us to the conclusion that a vitamin B_{12} requiring pathway of t-RNA methylation does exist.

Materials and methods. Young male rats (45-50 g) were divided into 3 groups and were fed a soya-lactose basal diet(14) devoid of vit. B_{12} and methionine. Group II received, in addition, cyanocobalamin (50 μg/kg diet) and Group III methionine (2 g/kg diet). The animals were maintained on these diets for a period of 8 weeks and were then sacrificed by decapitation after fasting overnight to deplete their liver glycogen. The livers were homogenized in 5 volumes of 0.25 M sucrose and the cell supernatant fractions were obtained by centrifugation at 105,000 $\times$ *g* for one hour in a Spinco Model L centrifuge after a preliminary centrifugation at 10,000 $\times$ *g*. A small portion of the supernatant was used as the source of the methylating enzyme while a major portion of it was utilized for the isolation of t-RNA by the phenol extraction procedure of Kirby(15). The isolated t-RNA was purified by dialysis against several volumes of tris buffer (1×10^{-3} M and pH 7.8). The purity of isolated t-RNA was assessed by its absorption spectrum in the U-V region. It showed a single peak at 260 mμ and the absorption minimum was at 232 mμ. The ratios of A_{280}/A_{260} and A_{232}/A_{260} were 0.53 and 0.52 respectively.

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