on days 18, 19, 22, 23, 24, 35, 36 and 41 after grafting. The percentage of host type mitoses was similar to that found in grafts from one-day-old donors. In an additional experiment, recipient (AKR×T6)F1 mice were thymectomized when aged 7 weeks, and one week later grafted with one-day-old AKR thymuses. Four grafts were examined 14 days after grafting and a mean percentage of 29% host mitoses found. In 3 grafts examined 21 days after grafting, 99.5% mitoses were host type.

Discussion. After grafting, the donor-type mitotic cells in normal thymus grafts in normal hosts were gradually completely replaced by mitotic cells of host origin. Since the lymphoid cell population of thymus grafts probably originates from local proliferative activity *in situ,* the present results indicate that most of the lymphoid cells in thymus grafts come, in time, to be derived from host cells.

A few lymphocytes in older thymus grafts may be long-lived survivors from the original donor cell population in the thymus at time of grafting, but if so these surviving cells must form a small percentage of the total population and apparently are incapable of mitotic activity in the grafts. It should be emphasized that in the first 2 weeks after grafting there was active proliferation of donor lymphocytes, but that this mitotic activity subsequently ceased.

These findings suggest that there may not be a permanent population of special lymphoid stem cells in the thymus, surviving for the life of the animal and continuing to give rise by mitosis to thymic lymphocytes. Instead it seems possible that the population of primitive cells in the thymus is continually replaced by immigrant cells of unknown type, entering from the circulation. Harris, Barnes and Ford (Ford, personal communication) using parabiotic CBA/CBAT6T6 mice have recently demonstrated a similar, but slower, entry of cells from the circulation into the pool of mitotic cells in the normal thymus.

Neither thymectomy of the adult host animal nor the use of adult rather than day-old thymus grafts materially affected the rate of replacement of the donor population of dividing lymphoid cells in thymus grafts by host cells.

Summary. After initial death of most donor lymphocytes in AKR thymus grafts in normal (AKR×T6)F1 mice, there was a temporary period during which active proliferation of surviving donor lymphocytes occurred. This population of dividing lymphocytes in the thymus grafts was subsequently completely replaced by host-type cells. The results suggest that thymic lymphoid stem cells are continuously replaced by cells entering the thymus from the circulation.

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Studies on Thiamine Absorption. (29023)

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In man, urinary output of thiamine as per cent of dose was shown to decrease as the dose was increased [see (1) for review]. Doses of thiamine up to 400 μg were readily absorbed by the rat(2), as based on urinary output, but larger doses were not(3). This suggested that proportionately less of the vitamin was being absorbed at the higher

dose levels. However, such dose-excretion studies revealed a net effect of absorption and excretion. Not accounted for was the possibility that ingested thiamine was absorbed in duodenum with a considerable quantity excreted *via* an enterohepatic route or secreted by intestinal mucosa into the lower intestinal sections and thus appeared in feces as "unabsorbed." Gassmann *et al*(4) recently demonstrated that the latter two routes are functioning and that duodenal mucosa has a great affinity for thiamine.

Studies were undertaken with chicks to determine the absorptive capacity of various intestinal sections for thiamine, and to correlate these observations with thiamine concentrations in the tract contents of chicks fed several dietary levels of thiamine, using thiamine-C^{14} supplementation.

Methods. Chicks 3 to 5 weeks of age were reared in wire-floored battery brooders using standard husbandry practices and were fed stock diets from 1 day of age. Ligated sections of the intestinal tract were prepared in chicks fasted overnight. The operative procedures, method of injecting and recovering samples in the loops have been described(5, 6). Absorption, *a,* is defined as the amount of thiamine, *d,* introduced into a ligated segment minus the amount recovered at time of sacrifice.

Stock diets were supplemented with thiamine-2-C^{14} chloride HCl (labeled in the thiazole moiety) which raised the vitamin level from 5.8 ppm (dry wt.) to 10.6 and 126.7 ppm thiamine. Diet preparation was as follows: 62.7 mg thiamine-2-C^{14}, 0.4 mc/mmole, was diluted to 150 mg with carrier thiamine, and dissolved in 3 ml methanol-water (50%); 0.1 ml of the solution was removed for preparation of standards and the remaining 2.9 ml mixed with 15 g sifted (#40 sieve) feed to yield a premix containing 9.67 mg thiamine/g. Aliquots of 0.5 g and 12.5 g of premix were added to stock diet to yield 1 kg of each preparation. Chicks were started on the diets when 3 weeks of age and 4 from each group, including controls, were sacrificed 4 days later while on full feed. The intestinal tract was removed intact from freshly-killed chicks, cut into desired sections and the contents collected, pooled according to treatment, and frozen at —20°C. These contents were later thawed, weighed into tared 12 ml graduated centrifuge tubes and extracted 4 times with 75% methanol. Extracts were pooled, brought to 25 ml in a graduated cylinder, and 1 ml aliquots pipetted into counting vials containing 16 ml toluene-ethanol phosphor [2000 ml toluene, 900 ml 100%

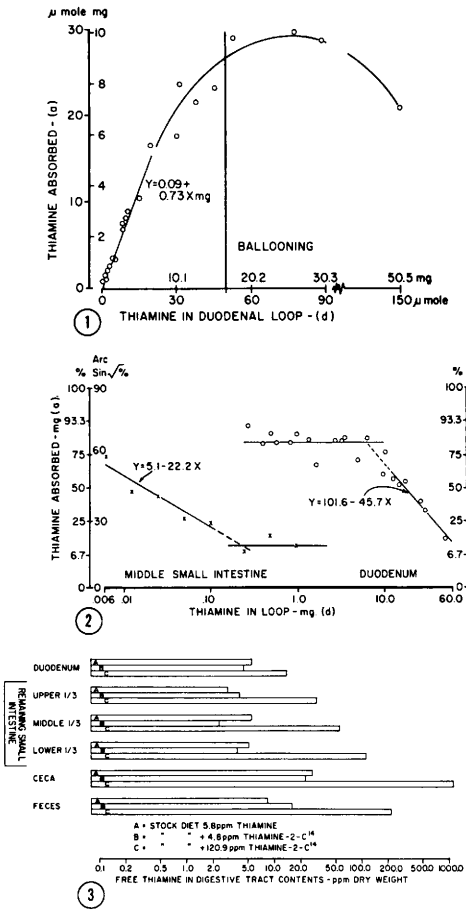

FIG. 1. Dose absorption curve for thiamine introduced into ligated duodenal loops of chicks. Absorption time = 1 hr. Values to right of vertical line at 50 μmole dose are influenced by accumulation of fluid in lumen, "ballooning." Each point represents 4 to 8 chicks.

FIG. 2. Dose-percentage absorption curves for duodenal and middle small intestinal loops in White Leghorn chicks 3 to 5 wk of age. Absorption time = 1 hr. Y = arc sin $\sqrt{\%}$; X = log dose in mg.

FIG. 3. Free thiamine concentration in digestive tract content of chicks fed stock diets supplemented with 4.8 and 120.9 ppm thiamine-2-C^{14}.

TABLE I. Recovery and Absorption of Thiamine from Digestive Tract of Chicks During Absorption Time of 1 Hour.

Site	[d] (μg)	No. chicks	Recovered μg at 1 hr	Absorbed in 1 hr* [a] (μg)	Absorbed in 1 hr* ([a]/[d]) × 100
Crop	250	8	229 ± 5†	6	3
Duodenum	250	8	37 ± 7‡	198	84
Remaining small intestine — Upper ⅓	250	8	199 ± 12‡	36	15
Remaining small intestine — Middle ⅓	250	8	219 ± 6‡	16	7
Remaining small intestine — Lower ⅓	250	8	244 ± 5	−9	−4
Ceca	250	8	201 ± 9‡	34	14

* Zero hour recovery for 9 chicks (235 ± 5) minus recovery at 1 hr = [a].

† Mean ± standard error.

‡ Dunnett's allowance value A calculated from std dev of control group with 8 d.f. and on a 1 group comparison = 13 μg, thus values less than 222 (235-13) μg are significant at $P \leq .05$.

ethanol, 9 g 2,5-diphenyloxazole, 300 mg 1,4-bis-2(5-phenyloxazolyl)-benzene]. Average radioactivity in cpm was obtained on 3-10 minute readings with 2 controls per series in an automatic liquid scintillation counter. Counting efficiencies were determined by use of corrected counts and counts were converted to thiamine equivalents on the basis of specific activity of thiamine-C^{14} standard (587 cpm/μg). Thus, C^{14} counts represent thiamine-C^{14} and/or its metabolites. Moisture content was determined on pooled samples dried to constant weight in a vacuum oven. Thiamine was analyzed in methanol extracts by the thiochrome method described by Snell and Snell(7).

Statistical analyses were performed according to Snedecor(8) using Dunnett's(9) "t" (1-tailed "t" test) to determine significance of experimental data from control values, or Tukey's "Q"(8) for significance among experimental groups. The distribution of percentages tends to be binomial in form, so such values were transformed to angles = arc sin $\sqrt{\%}$(8), and these latter values were plotted and/or analyzed statistically.

Results. Ligated loop studies. Sections of the digestive tract varied in their capacity to absorb thiamine. In duodenal loops 84% of 250 μg of thiamine was absorbed in one hour, as compared to the 7 to 15% values obtained in the upper ⅓ and middle ⅓ sections of the small intestine and in the ceca. Apparently, no thiamine was absorbed from the lower ⅓ section of the small intestine or from the crop in the 1-hour absorption period (Table I).

Thiamine was introduced in amounts ranging from 250 μg to 50 mg, in 1 ml volume, to determine the absorptive capacity of the duodenum. A dose-absorption curve was obtained with a linear portion of the curve encompassing doses up to approximately 10 mg (Fig. 1). Higher doses produced a gradually curving phase, plateau and finally reversal of the *d vs a* curve. Associated with the plateau and reversal portion of the curve was a ballooning of intestine, in which an accumulation of fluid occurred within the ligated loops, presumably due to thiamine toxicity or osmotic imbalance.

The much smaller capacity for thiamine absorption in the middle ⅓ section, as evident from the data in Table I, was characterized using doses ranging from 6.25 μg to 10 mg. Approximately 75% of the 6.25 μg dose was absorbed in 1 hour from middle ⅓ sections (Fig. 2), and relatively lesser amounts of thiamine were absorbed as the dose was increased. At doses exceeding 100 μg, in the middle ⅓ section, thiamine absorption appeared to remain constant at about 14%; yet these same amounts of thiamine were readily absorbed from the duodenal loops (Fig. 2). The linear portions of the two curves were approximately 1000 log units apart. The points indicating that 50% of the thiamine was absorbed were 16.7 μg and 16,200 μg for the middle ⅓ and duodenal loops, respectively. Fluid accumulation did not occur over the range of doses used to characterize the absorptive capacity of the middle ⅓ section for thiamine; yet a minimum percentage of thiamine was being absorbed at the higher doses.

TABLE II. Total Free Thiamine and C^{14} from Thiamine-C^{14} in Digestive Tract of Chicks Fed 4.8 and 120.9 ppm of Thiamine-2-C^{14} Added to Stock Ration Containing 5.8 ppm of the Vitamin.

	Thiamine in ration – ppm dry wt					
	5.8 + 4.8 = 10.6			5.8 + 120.9 = 126.7		
	C^{14} ppm*	Free thiamine ppm*	C^{14}/free B_1	C^{14} ppm*	Free thiamine ppm*	C^{14}/free B_1
Duodenum	6.3	4.5	1.4	30	14	2.1
Remaining small intestine — Upper ⅓	4.0	3.9	1.0	41	31	1.3
Remaining small intestine — Middle ⅓	1.4	2.3	.6	59	59	1.0
Remaining small intestine — Lower ⅓	1.7	3.7	.5	116	119	1.0
Ceca	5.2	23.2	.2	938	1090	.9
Excreta (urinary + intestinal output)	8.8	15.9	.6	325	218	1.5

* Dry wt.

Feeding trials with thiamine-2-C^{14}. In chicks sacrificed while on full feed (5.8 or 10.6 ppm thiamine) the profile of thiamine concentration in the intestinal tract was found to have remained relatively constant (Fig. 3). However, in chicks fed the ration containing 126.7 ppm of thiamine there was a progressive and marked increase in thiamine levels of each intestinal section distal to the duodenum. Thiamine concentrations in the ceca were comparatively high in all groups, but exceptionally high, attaining 1000 ppm, in the group fed thiamine at 126.7 ppm in the diet. Thiamine in excreta appeared to reflect the admixture of cecal and intestinal contents.

The concentrations of C^{14} (thiamine-C^{14} and its metabolites) and thiamine (free thiamine by chemical analysis) in tract contents of the chicks fed diets containing thiamine-C^{14} are presented in Table II. In both groups, thiamine determined chemically did not account for all of the C^{14} in the duodenum, indicating that thiamine metabolites were present. The upper ⅓ and middle ⅓ sections of intestine in the chicks fed 10.6 ppm thiamine (4.8 ppm thiamine-C^{14}) had lower concentrations of C^{14} and thiamine than those found in the duodenum. The value of 2.3 ppm thiamine in the middle ⅓ section was 22% of the initial concentration in the feed. The 1.4 ppm value for C^{14} concentration in the middle ⅓ section was 22% of the 6.3 ppm value for C^{14} in the duodenum. This lower C^{14} value in absolute and relative amounts indicated that thiamine metabolites were absorbed in the middle section of the small intestine. In the lower ⅓ section, the concentrations of thiamine and its metabolites showed a slight increase, and in this section C^{14} accounted for 50% of free thiamine.

The pattern of C^{14} and thiamine concentrations was different in the digestive tract contents of chicks fed 126.7 ppm thiamine. The lowest concentrations of C^{14} and thiamine were found in the duodenal contents, and sections distal to this had contents with markedly higher values. All of the C^{14} activity was accounted for as thiamine (Table II). It appeared that the overwhelming concentration of non-metabolizable thiamine masked the detectability of small amounts of C^{14} metabolites that may have been present. That thiamine underwent degradation in these chicks was evident from the greater amount of C^{14} than thiamine, a 1.5/1 ratio, in the excreta (Table II).

Discussion. The data show that thiamine is absorbed from the duodenum and upper sections of the chick small intestine with the duodenum playing the major role. It had the largest capacity for thiamine absorption according to the ligated loop studies. The duodenum absorbed in 1 hour an amount of thiamine equal to 40 times the chick's daily intake from stock diets. Furthermore, it showed an absorptive capacity for the vitamin about 1000-fold greater than that in the middle ⅓ intestinal section. No evidence was obtained to indicate that thiamine was absorbed from the lower ⅓ sections. In contrast, the sections of the lower small intestine presented

a barrier to thiamine in the ligated loop studies.

Data from the feeding trials with thiamine-C^{14} indicated that the middle and lower sections of the small intestine are capable of secreting thiamine into their lumen. This was indicated in chicks fed moderate levels of thiamine by their higher concentrations of free thiamine in contents from the lower 1/3 intestine as compared to concentrations in the section preceding it. It was also indicated, and more evident, in those fed the very high levels of thiamine (127 ppm) by the progressive increase in concentrations of the vitamin in contents of sections distal to the duodenum. Likewise, this absorption profile added further confirmation to the important role played by the duodenum in thiamine absorption. The bile ducts empty at the junction of the duodenum and upper 1/3 section in chicks. Thus, excretion of thiamine via bile may account for some or all of the higher concentration of thiamine found in the upper 1/3 section, but the 2- and 3-fold increase in thiamine concentrations in the contents of lower sections indicated secretion of the vitamin occurred from intestinal mucosa.

With normal concentrations of the vitamin in feed, the lowest concentrations of thiamine in tract contents were found in middle sections of the small intestine in chicks (Table II) and in rats(10); yet at the highest feed level of 127 ppm, the lowest concentration of thiamine in tract contents was found in the duodenum. Apparently, thiamine absorption from feed by duodenal mucosa is more efficient at higher concentrations. However, thiamine at doses up to 10 mg was absorbed from water solutions in duodenal loops with equal facility as revealed in the curve representing "dose *vs* percentage absorbed." Tract contents from any one intestinal section in the chicks fed the diet containing 127 ppm thiamine contained at most about 1/10th of the 10 mg dose used in the ligated loop studies.

The chicks appear to have an absorption profile for thiamine similar to that in man and the rat, in that thiamine is absorbed primarily in the upper sections of the small intestine. In man, the evidence is indirect and is based on dose-excretion studies in which it was observed that urinary excretion of thiamine is maximal within 2 hours after dosing with thiamine salt(11), and that certain sustained-release coatings on vitamin tablets reduce urinary output of the vitamin (1,11). Also, excretion of thiamine in the urine is negligible when the vitamin is presented by retention enemas(1,12,13). Rats, in addition to showing the similarity indicated above(10), are similar to chicks in that their cecum plays a minor role, if any, in thiamine absorption. No growth response was detected on injection of thiamine into the cecum of thiamine-deficient rats if coprophagy was prevented, but a recycling of thiamine resulted in a growth response(14,15). Also, small amounts of thiamine appeared to be absorbed after cecal injection with thiamine-S^{35}(4), but when coprophagy was prevented $S^{35}O_4 =$ injection into the ceca did not label liver thiamine although thiamine-S^{35} was isolated from cecal contents(10).

Based on the probable similarity in the thiamine absorption profile for chick and man, the chick data suggest why proportionately less thiamine appears in urine when man is given "high" doses of thiamine. Apparently, thiamine is absorbed in the duodenum and recycled back into the intestinal lumen via the bile ducts which empty beyond the region of maximum absorption. If the dose is high enough, intestinal secretion may also occur and thus add to the thiamine which will appear in feces as "unabsorbed."

Summary. Thiamine absorption from intestinal tract of chicks was studied using ligated loops or analyzing tract contents for C^{14} and thiamine in chicks fed thiamine-2-C^{14}. In one hour about 84% of a 250 μg dose of thiamine was absorbed from chick duodenal loops, but only barely detectable amounts of 7 to 15% from ceca and upper and middle sections of remaining small intestine. No absorption occurred at this dose from crop or lower small intestine. A thiamine dose-absorption curve for duodenum was linear up to doses of 10 mg. Proportionately less thiamine was absorbed from middle small intestine as doses increased in ligated loops

from 6.25 to 100 μg; yet in duodenum almost 90% of the 100 μg dose was absorbed. Chicks fed 5.8 or 10.6 ppm thiamine in stock rations had a similar profile in intestinal tract concentrations with lowest value in middle ⅓ of small intestine. Chicks fed 127 ppm thiamine had progressively increasing thiamine concentrations in sections of small intestine distal to duodenum. The data indicate that thiamine is absorbed primarily in the upper small intestine and when fed at very high levels to chicks is secreted by intestinal mucosa of middle and lower sections of small intestine.

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Effect of Parenteral Heterologous Albumin on Transplanted Tumors in Mice.* (29024)

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The effect of RNase on tumor growth *in vivo* has been described by Ledoux for both ascites(1) and solid tumors(2). Mean survival time of the mice was increased from 15 to 185% depending on the tumor, amount of enzyme injected, and time of inoculation of the tumor cells. Similar results have been obtained by other investigators with other types of tumors.

Gazet and McKibbin† have shown that parenteral administration of heterologous RNA will significantly increase the percentage take of free 180 mouse sarcoma and Ehrlich's ascites tumor cells inoculated subcutaneously into mice. The question has arisen, therefore, as to whether heterologous albumin administered in a similar manner would have the same effect as heterologous RNA. The experiments reported herein were designed to answer this question.

Method and materials. All experiments were preformed on female Swiss mice aged 4 to 6 weeks and weighing approximately 20 g, maintained on Standard Mouse Breeders Purina chow with as much water as desired. In all experiments groups of 10 animals were used. The abdomen was shaved on the day prior to experiment, and all tumor cell suspensions were inoculated subcutaneously in the midline in this area. All drugs were injected subcutaneously in the back.

Two tumors, the mouse sarcoma 180 and Ehrlich's ascites tumor, were employed. In the case of the sarcoma, the cell suspensions were prepared from suitable donors under aseptic conditions as described by Cole *et al*

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