served during anaphylaxis in the mast cell tumor mice. Kidneys, lungs, small intestine and liver are all thought to be capable of metabolizing free serotonin(11), and, in these organs, anaphylaxis was associated with a striking decrease in the elevated serotonin levels. A similar reduction was not noted in spleen or in mast cell tumors. Presumably in these tissues serotonin was either not released or, if released from cells, was not subsequently metabolized. Reduction in serotonin content of whole blood is accompanied by a fall in number of platelets during anaphylaxis in the rabbit(4). However, no evidence of release from tissue has been obtained(5). In tissues of mice bearing mastocytomas the decreases in serotonin concentration were striking.

The high serotonin content observed in tissues of mice with mast cell tumors suggest that such experimental animals might prove useful in the study of effects of serotonin in a situation where the amine is chronically present in large amounts. The mechanisms by which serotonin is bound to or released from tissues remain poorly understood. In view of the striking changes observed in tissue serotonin content, further studies utilizing organs of mice with mast cell tumors may help clarify these mechanisms.

Summary. Tissues of mice bearing mastocytomas are rich in serotonin. A significant decrease in serotonin content occurred in kidneys, lungs, small intestine and liver of these mice following anaphylactic shock.

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Absorption of Cholesterol-4-C^{14} Oleate* (25482)

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Studies(1,2) using blood cholesterol levels as an indicator of cholesterol absorption in chicks and rats have demonstrated that feeding of free cholesterol leads to a higher blood cholesterol level than feeding of the long chain fatty acid esters of cholesterol. Vahouny and Treadwell(3) examined lymph after feeding of different cholesterol esters and found that free cholsterol was absorbed more rapidly than the long chain fatty acid esters of cholesterol. They suggested that only free cholesterol was transferred from the lumen into the mucosa. Pihl(4) reported that fed cholesterol esters were extensively hydrolyzed in the intestine and were not absorbed to a greater extent than free cholesterol. Favarger and Metzger(5) fed D-cholesterol oleate and demonstrated hydrolysis of this labeled ester in the lumen of intestine. They also found that in the intestinal wall of such animals the labeled cholesterol was present principally in the free form. When free D-cholesterol was

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TABLE I. Cholesterol and Cholesterol-4-C^{14} of Lymph and Intestine after Feeding Cholesterol-4-C^{14} and Cholesterol-4-C^{14} Oleate.

Tissue*	Free	Free-C^{14}	Ester	Ester-C^{14}	Total	Total-C^{14}	Ester / Total	Ester-C^{14} / Total-C^{14}	Administered C^{14}-recovered
	mg						%		
Fed cholesterol-4-C^{14} oleate									
Lymph	1.2	.3	3.6	1.9	4.8	2.2	75.0	86.4	5.3
	±.3†	±.1	±.5	±.3	±.4	±.3	±2.1	±3.2	±.6
Small intestine	14.1	2.4	2.6	.8	16.7	3.2	15.6	25.0	7.7
	±.6	±.3	±.3	±.2	±.5	±.3	±1.8	±2.2	±.6
Fed cholesterol-4-C^{14}									
Lymph	1.9	.6	6.7	4.1	8.6	4.7	77.9	87.2	11.4
	±.3	±.2	±.4	±.3	±.5	±.3	±3.0	±3.1	±.7
Small intestine	14.4	4.3	2.6	1.4	17.0	5.7	15.3	24.6	13.8
	±.5	±.3	±.2	±.2	±.6	±.4	±1.2	±2.1	±.8

* Values are avg for 5 rats killed 6 hr after test meal. Cholesterol-4-C^{14} and cholesterol-4-C^{14} oleate administered/rat was 41.3 mg as free sterol (5000 cpm/mg).

† Stand. error of mean.

fed they were unable to demonstrate any esterified labeled cholesterol in intestinal lumen or wall. Evidence has been presented(6) that free cholesterol after transfer from the lumen is mixed with a pool of free cholesterol in the intestinal wall, then a major part is esterified and transferred to lymph. According to this concept, cholesterol esterification occurs at site of transfer of cholesterol from mucosa to lymph. The importance of this esterification step in cholesterol absorption has been questioned(4,5) since fed cholesterol esters are not absorbed to a greater extent than free cholesterol. However, if only free cholesterol can enter the intestinal wall, cholesterol esters must be hydrolyzed in the lumen prior to transfer of cholesterol into intestinal mucosa. If so, the esters might be absorbed at a slower rate than free cholesterol rather than the reverse.

Methods and materials. Rats with a thoracic duct fistula were prepared and given saline to drink(7). Twenty-four hours after operation each animal received, by gastric intubation without anesthesia, 3 ml of aqueous emulsion containing 50 mg albumin, 150 mg glucose, 292 mg oleic acid, 279 mg sodium taurocholate and either 41.3 mg cholesterol-4-C^{14} (0.5 μc) or 69.4 mg cholesterol-4-C^{14} (0.5 μc) equivalent to 41.3 mg cholesterol-4-C^{14}. Cholesterol-4-C^{14} oleate was synthesized according to the method of Page and Rudy (8) and had a m.p. of 44°C. Lymph was collected for 6 hours after administration of test meal and the animals were then sacrificed. The intestine was removed and washed with saline. The saline washings from intestine were pooled with the collected feces of the respective animal. Lipid extracts of intestine, lymph, and combined feces and intestinal contents were prepared according to procedures described earlier(6,7). Free and total cholesterol of each extract were determined colorimetrically by the method of Sperry and Webb(9). C^{14}-activity of free and esterified cholesterol fractions of all lipid extracts was assayed as previously described (6,7).

Results (Table I). Six hours after test meal containing cholesterol-4-C^{14} oleate, 5.3% or 2.2 mg of the fed cholesterol was present in lymph and 7.7% or 3.2 mg in the intestinal wall. Of the total C^{14}-cholesterol in lymph, 86.4% was esterified while only 25.0% of the total C^{14}-sterol was present as ester in the intestinal wall. The group fed free cholesterol-4-C^{14} had approximately twice the amount of C^{14}-cholesterol in both lymph (11.4% or 4.7 mg) and small intestine (13.8% or 5.7 mg) that was present in the group fed cholesterol-4-C^{14} oleate. The percentage of esterified cholestrol-4-C^{14} in lymph (87.2%) and small intestine (24.6%) of the cholesterol-4-C^{14} group, were the same as in the group fed the C^{14}-ester. There were no differences in chemical cholesterol levels of the intestine of the 2 groups. However, lymph of the cholesterol-4-C^{14} group had

TABLE II. Recovery of Fed Cholesterol-4-C^{14} and Cholesterol-4-C^{14} Oleate from Feces and Contents.

Free	Free-C^{14}	Ester	Ester-C^{14}	Total	Total-C^{14}	Administered C^{14}-recovered, %
mg						
Fed cholesterol-4-C^{14} oleate*						
10.7 ± 1.4	6.4 ± 1.6	23.5 ± 3.1	22.2 ± 2.6	34.2 ± 4.0	28.6 ± 3.4	64.3 ± 5.0
Fed cholesterol-4-C^{14}*						
29.4 ± 3.6	26.2 ± 4.0	2.0 ± .6	Tr	31.4 ± 3.1	26.2 ± 4.0	63.4 ± 6.0

* See Table I for amount of sterol fed.

larger amounts of both free and ester cholesterol than lymph of the C^{14}-ester group.

Examination of feces and contents following feeding of cholesterol-4-C^{14} oleate Table II) indicated that while extensive hydrolysis of the fed C^{14}-ester had occurred in the intestinal lumen, a large portion (78.0%) of the fed material was still present as unhydrolyzed ester. There was also considerably more *unlabeled* free cholesterol than *unlabeled* ester cholesterol. In the group fed free cholesterol-4-C^{14} virtually all cholesterol recovered from the lumen and feces, was present as free C^{14}-cholesterol. Also, there was very little unlabeled esterified cholesterol present in the contents. Total unlabeled sterol present in feces and contents of both groups was approximately the same. Total recovery of fed cholesterol-4-C^{14} oleate from feces and contents, lymph and intestine was 82.3%. Thus, 17.7% could not be recovered and may have undergone intestinal transformation as reported earlier(6). The balance data on the cholesterol-4-C^{14} group indicate that 11.4% of the fed dose was not recovered.

Discussion. Results of our study have confirmed, and extended earlier observations(2,3) and provided more definitive data regarding the first step in absorption of cholesterol. There is extensive hydrolysis of fed cholesterol esters in the lumen of intestine, but virtually no esterification of fed free cholesterol occurs in this area of the intestine. These findings agree with those of Pihl(4) and Favarger and Metzger(5). If fed cholesterol-4-C^{14} oleate were absorbed preferentially without being hydrolyzed in the lumen, it would be expected that most of the C^{14}-activity in the intestinal wall would reside in the esterified fraction. Also, there would be very little C^{14}-activity in the free fraction of lymph. The data indicate that following feeding of either cholesterol-4-C^{14} oleate or cholesterol-4-C^{14} most of the C^{14}-cholesterol was transferred from the lumen into the intestinal wall as free sterol. Regardless of whether the free or the ester were fed, amount of C^{14}-ester esterified in intestinal wall was the same. The data on lymph show that most of the fed C^{14}-free or C^{14}-ester was present in lymph as C^{14}-ester. This would suggest, in line with our previous findings(6), that the esterified fraction of lymph originates from the free cholesterol pool of the mucosa and that esterification occurs in transfer of this sterol from intestinal wall to lymph. The greater amounts of cholesterol-4-C^{14} in lymph and intestine of the animals fed free cholesterol-4-C^{14} than when cholesterol-4-C^{14} oleate was fed, must be due to rate-limiting hydrolytic reaction occurring in the lumen of intestine prior to entrance of cholesterol into the intestinal wall.

Summary. Cholesterol-4-C^{14} oleate and cholesterol-4-C^{14} were fed in a test meal to lymph fistula rats. The data indicate that free cholesterol was absorbed to a greater extent than esterified cholesterol. This study also provides evidence to support the concept that preliminary hydrolysis is obligatory for absorption of cholesterol esters and that only free cholesterol can enter the intestinal wall. The significance of these findings is discussed.

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Tolerance of F_1 Hybrid Skin Homografts in the Parent Strain Induced by Parabiosis.* (25483)

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Immunological tolerance allowing homotransplantation of tissues and organs has been achieved by exposing animals to donor strain antigens prior to delivery or during immediate neonatal period(1). However, our recent reports indicate that weanling Z mice, injected with viable spleen cells taken from (Z x Ce) F_1 hybrid individuals, became tolerant and accepted skin grafts from (Z x Ce)F_1 donors (2). Similarly, older female mice of A and C_{57}Bl (Subline 1) strains were made tolerant of male skin isografts either by intravenous injection of male spleen cells or by establishing a parabiotic union of adult females with isologous males(3). Further, using the technic of parabiosis, it has also been demonstrated that tolerance of homologous tissue transplants, induced during neonatal period, can be transferred to normal adult mice of the same strain. In the latter experiment, the previously non-treated parabiont lost its ability to reject homologous tissue and, like its partner, accepted the homologous skin graft (4). Therefore, it seems that under certain well-defined conditions, induction of immunological tolerance in mice may not be limited to antigen exposure during embryonal life or immediately after birth. Our incidental observations indicate that, contrary to expectation, parabiosis performed between certain F_1 hybrid mice and individuals of either parent strain were successful in most instances and resulted in induction of tolerance in the parent parabiont whereby this animal lost its capacity to recognize and reject homologous skin grafts taken from hybrid donors of the corresponding F_1 cross. It is the purpose to document and further extend these observations.

Method. Highly inbred mice of the Z§, Ce, A, C_{57}Bl (Subline 1) and their reciprocal F_1 hybrids were used. In one group of experiments parabiosis was established between (Z x Ce)F_1 hybrids and Z animals, and between (Z x Ce)F_1 and Ce. In this group mice were from 40 to 70 days of age at time of parabiosis. In another group parabiosis was performed between (A x Z)F_1 and Z, and between (A x Z)F_1 and A mice. These animals were from 30 to 46 days old when placed in parabiosis. In a third group (Z x C_{57}Bl)F_1 and Z, and (Z x C_{57}Bl)F_1 and C_{57}Bl mice were similarly united in parabiosis. These animals were from 30 to 50 days old when parabiosis was established. In all instances parabiotic animals were of the same sex. Each preceding group had its own controls, consisting of parabiotic pairs of either parental strain, *i.e.*, Z to Z and Ce to Ce for the first group, Z to Z and A to A for the second, and C_{57}Bl to C_{57}Bl and Z to Z for the third group. Parabiosis was of the celomic type(5-6), consisting essentially of surgical union of animals through midlateral incisions comprising skin, muscle and peritoneal cavities. Parabiotic pairs were housed individually in plastic mouse cages with free access to Purina Fox Chow and tap water. Parabiosis was maintained from 20 to 45 days, after

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§ Z is used to simplify designation of mice of the C_3H strain, originally obtained in 1956 from Dr. John J. Bittner's mouse colony.