of DMEA, shorter than that required for hydrolysis of M-DMEA. There is no reason to suppose that reactions rates would be grossly different in the concentrations involved in the pharmacologic effects and while an enzymatic mechanism could speed hydrolysis of M-DMEA, one would then have to assume that hydrolysis was slowed for DMEA. There is a complete lack of correlation between duration of effects and *in vitro* life of what is assumed to be the active forms. This situation makes it seem doubtful that *in vivo* metabolism of these compounds is simply a matter of spontaneous ring hydrolysis, analogous to the reactions observed *in vitro*.

Specific points of difference between DMEA and M-DMEA may be mentioned. There was considerable difference in adrenergic blocking potency. While the potency of this action of haloalkylamines cannot be predicted solely upon rates and degrees of intramolecular change, there has not previously been reported such marked difference in activity between compounds with such similar structures and rates of imine formation. Rate of development of adrenergic blockade also varied, with DMEA producing maximum effects in seconds while M-DMEA required minutes. Finally, both compounds produced long-continued salivation but only with DMEA was this effect resistant to atropine antagonism.

Summary. Acute pharmacologic comparisons have been made of N, N-dimethyl, 2-chloro-2-phenylethylamine (DMEA) and N, N-dimethyl, 2-chloro-2-phenyl-1-methylethylamine (M-DMEA). The 2 differed in immonium ring stability *in vitro,* speed of onset and degree of adrenergic blocking activity, and production of atropine-resistant salivation. There was no relationship between duration of effects and *in vitro* life of intermediate ethyleneimmonium forms.

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Absorption and Distribution of Cholesterol-4-C^{14} in the Rat.* (24352)

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The peak in the specific activity-time curve for blood cholesterol after feeding a single dose labelled cholesterol to humans(1,2,3) and to rats(4) has been reported to occur during the second or third day. This delay in the postabsorptive peak compared to most other ingested material, has not been adequately explained. The finding fo Biggs(2) that in the rat the activity of intravenously injected chyle cholesterol-H^3 is selectively taken up by the liver points to this organ as one site of delay of newly absorbed cholesterol. The present investigation was undertaken in an attempt to analyse the dynamics of absorption and distribution of dietary cholesterol in the intact rat. The results obtained indicate that in the rat dietary cholesterol is rapidly absorbed from the intestinal lumen but is held up in the intestinal mucosa cells for a considerable time before being transferred into the thoracic duct lymph over a period of days. Further delay of appearance of newly absorbed cholesterol in the blood stream is caused

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by selective uptake of lymph cholesterol in the liver prior to its distribution throughout the body.

Methods. Male adult rats fasted for 24 hours were fed by intubation under light ether anesthesis 5 ml of a fluid test meal containing per ml 0.4 μc cholesterol-4-C^{14}, 50 mg olive oil, 52 mg glucose and 47 mg skim milk powder homogenized with an Ultra-Turrax(5). Total amount of cholesterol was determined to be 0.06 mg per ml.

Results. Absorption and distribution of cholesterol. In this series 7 animals were killed 3, 3, 6, 9, 12, 24 and 48 hours after feeding of the test meal. The small intestine was washed through with 40 ml of 0.9% saline. The contents of the small intestine, the stomach, colon and feces, the small intestinal wall and the liver were extracted and their lipids isolated. Table I summarizes the total radioactivity figures of these different fractions. Amount of activity absorbed has been calculated as the difference between activity fed and the sum of activity found in stomach, small intestinal washings, colon and feces. Prolonged washing of the intestinal wall with saline did not decrease the activity found in the intestinal wall. Of the activity found in a 0.25 M sucrose homogenate of small intestinal wall 5 hours after feeding the test meal 60, 30 and 10% was found in the microsome, mitochondria and supernatant fractions obtained on differential centrifugation according to standard procedures, indicating that the active cholesterol found in the intestinal wall actually had been absorbed into the cells. In Fig. 1 the figures for total activity found in the small intestinal wall and liver have been recalculated as per cent of activity absorbed in each case. Three hours after feeding the test meal 88% of the activity absorbed is found in the small intestinal wall and after 24 hours it still contained 26% of the absorbed activity.

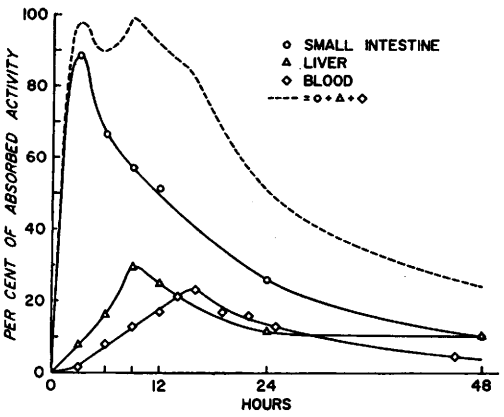

FIG. 1. Per cent of absorbed activity present in small intestinal wall, liver and blood at different times after feeding rats 0.3 mg 4-C^{14}-cholesterol incorporated into a well-balanced test meal.

Total activity present in the liver shows a maximum 9 hours after feeding at a level of about 25% of that absorbed.

A semilogarithmic plot of the curves for total activity content in small intestinal wall and liver against time shows an apparently linear character up to 24 hours after the feeding. From these curves the half time for the newly absorbed active cholesterol in the intestinal wall and the liver can be estimated to about 12 and 11 hours respectively.

In another series of experiments 9 rats were used for a study of the blood activity. Blood was drawn from the rat tail at different time intervals after feeding the test meal, the lipids extracted and the radioactivity assayed. The results are included in Fig. 1 calculated as per cent of activity absorbed contained in the

TABLE I. Distribution of Cholesterol-4-C^{14} in the Rat at Different Time Intervals after Feeding 0.3 mg Labeled Cholesterol Incorporated into Test Meal. Figures in % of activity fed.

Hr after feeding	Stomach	Washings of small intestine	Colon + feces	% absorbed	Wall of small intestine	Liver
3	22.2	17.3	13.9	46.6	41.2	3.6
3					42.0	
6	6.2	10.0	14.3	69.5	46.2	11.1
9	1.4	15.7	28.6	54.4	31.0	15.9
12	4.6	4.8	41.0	49.6	25.4	12.2
24	.9	4.5	43.0	52.6	13.6	6.0
48	.3	1.7	31.5	66.5	6.7	6.6

total blood (assuming 67 ml blood per kg rat). Maximum activity in the blood, 22.8% of absorbed activity, is found 16 hours after the feeding. In another series of experiments in which 10 mg cholesterol were fed in the same test meal, maximal blood activity was not reached until the second day.

Fig. 1 includes the value for total activity present in the blood at the different time intervals and the calculated figures for activity found in small intestinal wall plus liver and plus blood. These later figures show that up to 12 hours after feeding these organs contain more than 90% of the absorbed activity and thus less than 10% was distributed outside these compartments. After this time period there is rapid distribution of the cholesterol over the body.

The per cent activity found in the cholesterol ester fraction of the small intestinal wall during absorption and distribution of the cholesterol shows a linear increase up to 24 hours after feeding when 75% of the labelled cholesterol of the intestinal wall is in the ester form (Fig. 2).

Thoracic duct lymph studies. The thoracic duct of male adult rats was cannulated according to standard procedures and the rats fed the test meal by intubation the day after operation. Distribution of the radioactivity recovered in the lymph over the different time periods is seen in Fig. 3. The largest amount of activity was found in the lymph sample collected between 6 and 9 hours after feeding. The lymph lipid fractions were separated on silicic acid columns, cholesterol ester and free cholesterol being eluted with 5 and 20% ethyl ether in light petroleum. Of the total activity recovered in the lymph during the 24-hour period following the test meal 64% was in the ester form. Of the total amount of cholesterol during the same period 81.3% was esterified. Relative specific activity of the lymph total cholesterol reached its peak about 9 hours after feeding at a level of 2.6%.

Discussion. The results obtained demonstrate that dietary cholesterol is rapidly absorbed from the intestinal content, absorption being almost completed 6 hours after feeding. The absorbed cholesterol is then held up in the cells of the small intestinal mucosa from which it is slowly discharged. Earlier reports of a slow absorption of dietary labelled cholesterol are due to the procedure of combining the intestinal content and intestinal wall and consider the labelled cholesterol present as not absorbed(6).

It has been firmly established that absorbed cholesterol enters the systemic circulation *via* the thoracic duct lymph contained mainly in the chylomicrons of the lymph, the larger amount of the active cholesterol being in the esterified fraction(2). The findings in this investigation are consistent with this pattern. The increasing ratio of ester to free active cholesterol in the intestinal wall during the distribution period is an indicaton that the cholesterol is mainly absorbed in the free form and is esterified during passage through these cells.

Our results indicate that in the rat newly absorbed cholesterol that reaches the systemic

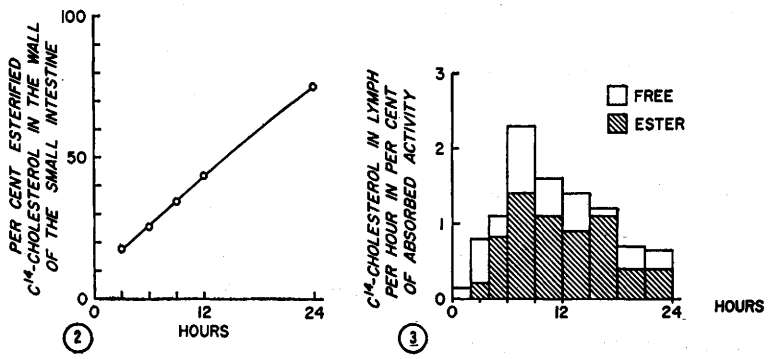

FIG. 2. Per cent of total activity of small intestinal wall present in cholesterol ester fraction at different times after feeding 0.3 mg 4-C^{14}-cholesterol to rats.

FIG. 3. Activity-time relationship for 4-C^{14}-cholesterol in lymph of a thoracic duct cannulated rat fed a test meal containing 0.3 mg 4-C^{14}-cholesterol.

circulation *via* the thoracic duct lymph does not mix or exchange to any appreciable extent with the cholesterol of serum or of other organs before being taken up by the liver. Labeled cholesterol given off from the liver is then distributed over the body.

This course of the metabolism of newly absorbed cholesterol derived from experiments on the whole animal is consistent with the finding of Biggs(2) that labeled cholesterol of thoracic duct lymph when intravenously injected into the rat is selectively taken up by the liver, and with the suggestion of Friedman *et al.*(7,8) that the liver Kupffer cells are responsible for removal of newly arrived cholesterol. On the other hand it has been reported that the cholesterol of chylomicrons from dog lymph exchanges with the cholesterol of serum high density lipoproteins during incubation *in vitro* and also on injection into dogs(9).

From the results obtained it is obvious that the late blood cholesterol activity maximum in the rat is caused by the hold up of the newly absorbed cholesterol first in the intestinal wall and later in the liver before its reappearance in the circulation. By increasing the dose of cholesterol fed to 10 mg, the maximum in the blood activity is further delayed and occurs during the second day in accord with the finding of Gould *et al.*(4). The shape of the blood activity curve thus is dependent on amount of cholesterol absorbed.

Summary. 1) A trace dose of cholesterol-4-C^{14} incorporated into a well-balanced test meal has been fed to rats. The labeled cholesterol is rapidly absorbed *i.e.* 50 to 70%. The newly absorbed cholesterol is then held up by the cells of the small intestinal mucosa, the halflife of the newly absorbed cholesterol in these cells being approximately 12 hours. From the intestinal wall the cholesterol enters the chyle, the peak specific activity of which is reached about 9 hours after the feeding. 2) The newly absorbed cholesterol of the thoracic duct lymph in the intact animal is selectively taken up by the liver and is only subsequently mixed in a metabolic sense with the cholesterol of blood and other tissues. 3) As a consequence of the delay of the absorbed cholesterol in the intestinal wall and the liver, blood cholesterol activity time curve reaches its maximum about 16 hours after the feeding. This delay in blood activity peak is prolonged to the second day if amount of cholesterol fed is increased to 10 mg.

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Serum Lipids in Hypercholesterolemic Rabbits. (24353)

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Kellner, Correll and Ladd(1) showed that intravenous injection of the surface active agent Triton A20 into rabbits fed a high cholesterol diet reduced incidence and severity of the resulting atherosclerotic lesions in spite of the fact that the serum cholesterol levels were, in most instances, higher than the levels found in the controls on the cholesterol diet itself.