Observations on the Mechanism of Cholesterol Absorption[1, 2]

C. R. TREADWELL, LEON SWELL, G. V. VAHOUNY, and HENRY FIELD JR.,
Department of Biochemistry, School of Medicine, The George Washington University,
Washington, D. C., and General Medical Research Laboratories,
Veterans Administration Center, Martinsburg, West Virginia

IN A REVIEW of the literature directly related to cholesterol absorption we are impressed by the few points on which there is substantial agreement and the numerous points on which the available data appear to give support to one or another of several views. Most workers are in agreement that 90% or more of the absorbed cholesterol passes by way of the lymph and the thoracic duct (1, 2); that one-half or more of the absorbed cholesterol is esterified with fatty acid (2, 3); and that bile acids (4) and fatty acids (5) stimulate the absorption of cholesterol.

Recently Siperstein and co-workers have demonstrated an obligatory requirement for bile salts in cholesterol absorption (6); however Kim and Ivy (5) and Phil (7) from data obtained in balance experiments have questioned this requirement for bile salts. It has been previously suggested (8) that bile salts may act in at least two ways: first, in the formation of a bile salt cholesterol complex, which is part of the substrate for esterification of cholesterol by cholesterol esterase (9); secondly, in the passage of cholesterol from the lumen into the mucosa. Here also there may be formed a (water-soluble) complex (10).

Recent work has demonstrated that dietary fat is not obligatory for cholesterol absorption (3, 5, 9, 11). In the rat when cholesterol is absorbed from a fat-free meal, a major part of it still appears in the lymph as ester (12). This fatty acid must come from endogenous sources. Dietary unsaturated fatty acids stimulate absorption to a greater degree than an equivalent amount of comparable triglycerides (5, 8, 13) while glycerol is without effect (5). Thus the stimulating effect of fat is attributable to fatty acids liberated in digestion. The explanation commonly offered for the stimulating effect of fatty acids is that they increase the esterification of cholesterol (14). This explanation has been questioned by Friedman *et al.* (15), Pihl (16), and Metzger and Favager (17) on the basis that administered cholesterol esters have not been shown to be absorbed to a greater extent than free cholesterol under comparable conditions and that conditions in the lumen of the intestinal tract favor hydrolysis rather than esterification. There are also unpublished data which indicate extensive hydrolysis of fed-cholesterol esters in the lumen. However available evidence suggests that the esterification of cholesterol which leads to the appearance of its esters in the lymph occurs in the mucosa.

The cholesterol esterase activity of the mucosa closely resembles that of the pancreas and in 95%-depancreatized rats the cholesterol esterase activity of the mucosa falls to a very low level (18). Hernandez *et al.* have shown that, in rats with pancreatic duct fistula, in 24 hrs. after diverting the pancreatic juice the mucosa has completely lost the capacity to absorb cholesterol, also that after 24 hrs. the ability of the intestinal mucosa to esterify cholesterol is lost (19). However Byers and Friedman (20) have reported that considerable absorption of cholesterol occurs after occlusion of the pancreatic duct or pancreatectomy.

In the fasted lymph fistula rat or in one administered a cholesterol- and fat-free meal the total amount of lymph cholesterol ranges from 8 to 12 mg. for 24 hrs., and the percentage of esters in the total ranges from 60 to 70% (12). This lymph cholesterol in the fasted rat is derived from the absorption of endogenous cholesterol, from the liver by way of the liver lymph (21), and presumably from the lower extremities. In the rat the administration of small amounts of cholesterol of the order of 1–5 mg. dissolved in fat does not produce a chemical increase in the amount of lymph cholesterol above the control level (22) even under the most favorable conditions for absorption (23). However the administration of these amounts of cholesterol-4-C^{14} is followed by the appearance of the labelled compound in the lymph over periods of several days (1). These observations suggested that lymph data obtained after administration of small amounts of cholesterol-4-C^{14} actually described the absorption of endogenous cholesterol. It also seemed clear that the 60 to 70% of esterified cholesterol in lymph under these conditions was an average figure for the cholesterol derived from the several sources mentioned above. Several corollaries of this interpretation of earlier data were apparent. First, the "extra" lymph cholesterol above the fasting level or above the level in an animal fed a cholesterol-free meal would be a measure of the absorption of dietary cholesterol. Secondly, the percentage esterification of the "extra" or absorbed cholesterol in lymph could be calculated from the control and experimental levels of free and esterified cholesterol. Thirdly, the differences between these levels in bile-fistula, lymph fistula rats and in normal lymph fistula rats during fasting would give the amount and degree of esterification of absorbed endogenous cholesterol in lymph.

Glover and Green (24) have suggested that cholesterol enters the intestinal mucosa at a molecular level and is attached to an acceptor lipoproptein. According to this view, endogenous dilution and passage through the mucosa occur because of interchange of the labelled cholesterol with inactive cholesterol on the lipoproteins. Furthermore these workers state that esterification acts in absorption only as an accelerating factor. The mechanism proposed by Glover and Green does not account for the obligatory requirements for bile salt and pancreatic juice or for the appearance of labelled cholesterol in the lymph for 48–72 hrs. after administration.

In recent studies on the mechanism of cholesterol

[1] Presented at annual meeting, American Oil Chemists' Society, Memphis, Tenn., April 21–23, 1958.

[2] These studies were supported by grants from The Life Insurance Medical Research Fund, The American Heart Association, and the National Heart Institute (H-2033, H-1897).

TABLE I

Lipide Fractions of 24-hr. Lymph Samples After Administration of Test Emulsions

Emulsion [a]	Triglyceride	Phospholipide	Cholesterol		Administered cholesterol absorbed	"Extra" cholesterol esterified
			Total	Ester		
	mg.	*mg.*	*mg.*	*%*	*%*	*%*
Control	64	6	10	67		
Chol.	48	9	8	67		
TC + O.A.	159	24	14	72		86
Chol. + O.A.	163	15	15	73	9	90
Chol. + TC	116	18	17	77	13	93
Chol. + TC + O.A.	156	34	28	81	27	93

[a] The control emulsion contained 50 mg. of albumin and saline to 3 ml. Additions: Chol., 50 mg. of cholesterol; TC, 144 mg. of taurocholate; O.A., 109 mg. of oleic acid.

absorption we have utilized the thoracic duct fistula rat prepared by a modification of the technique of Bollman *et al.* (25). The test meals (12) developed for these studies were aqueous emulsions containing albumin, glucose, saline, and combinations of cholesterol or cholesterol esters, fatty acids or triglycerides, and bile salt. They were administered by stomach tube in 3-ml. volumes 24 hrs. after the operation. The data collected have included chemical and radioactivity measurements of the total, free, and esterified cholesterol fractions of lymph, intestinal segments, and the intestinal contents and feces.

Table I shows the levels of the lipide fractions in the lymph after feeding various combinations of cholesterol, taurocholate, and oleic acid in a saline-albumin emulsion. These data demonstrate several points regarding cholesterol absorption. First, there are always increases in triglycerides and phospholipides when endogenous or exogenous cholesterol is absorbed. Secondly, whenever there is a significant increase in lymph cholesterol above the control level, there is a close correlation between this increase and the percentage of ester. Thirdly, the percentage esterification of the "extra" cholesterol absorbed, whether of endogenous or exogenous origin, is approximately 90%; and fourthly, both bile salt and oleic acid promote cholesterol absorption.

In time studies (26) we have shown that under our experimental conditions all lipide fractions of lymph reach a peak in 3–5 hrs. and return to fasting levels in 9–12 hrs. after administration of cholesterol, bile salt, and oleic acid (complete emulsion). Figure 1 shows the total, free, and percentage of esterified cholesterol in consecutive one-hour lymph samples for 9 hrs. after administration of the complete emulsion.

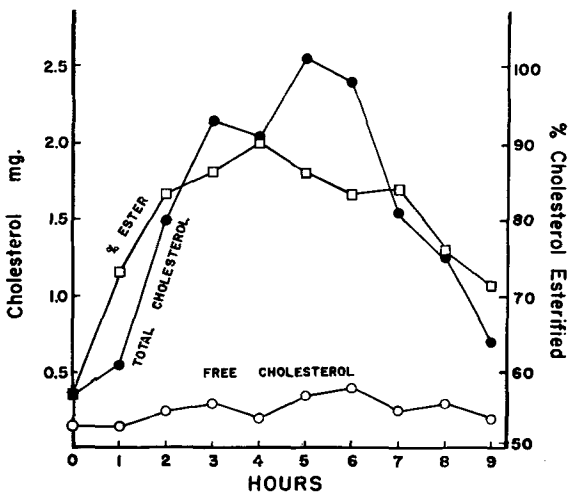

FIG. 1. Cholesterol fractions in successive 1-hr. lymph samples after administration of cholesterol, taurocholate, and oleic acid.

These curves show the close correlation between the level of total cholesterol of lymph and the percentage of esterification. Here at 4 hrs. the percentage of ester in the total cholesterol was 90%, which is the highest level that has been reported for lymph. It seems obvious that in 24-hr. samples these high levels of esterification tend to be diluted out by the endogenous cholesterol, which continues to appear in the lymph after absorption of the administered cholesterol is over.

Table II shows the lipide fractions of 24-hr. lymph samples of normal and bile fistula rats. These data show that about one-half of the cholesterol of fasting lymph is caused by absorption of endogenous cholesterol and that this absorbed endogenous cholesterol is esterified to a high degree as is absorbed dietary cholesterol. Moreover the triglyceride data suggest that very little, if any, endogenous fatty acid is absorbed in the bile fistula animal. In the bile fistula rats which received relatively large amounts of cholesterol, fatty acid, and bile salts, the triglyceride and cholesterol levels were no higher than in the fasted rat. This demonstrates the importance of the entero-hepatic circulation of bile salts in absorption.

In experiments in which approximately 3-mg. amounts of cholesterol-4-C^{14} have been administered to bile fistula lymph fistula rats, we have been able to demonstrate one specific function of bile salts in cholesterol absorption. Table III shows the recovery of cholesterol-4-C^{14} in lymph and in the intestinal wall 6 hrs. after administration. There was no cholesterol-4-C^{14} in the lymph and only a trace in the intestinal wall except when taurocholate was included in the test emulsions. These data demonstrate that bile salts are necessary for the transfer of cholesterol from the lumen into the intestinal mucosa. It is apparent that if cholesterol cannot enter the mucosa in the absence of bile salts, it cannot be absorbed into the lymph; therefore these data do not eliminate the possibility that bile salts may also function in the later steps of cholesterol absorption. The explanation for this effect is most likely that a complex of cholesterol and bile salt is formed in the lumen and this enters the intestinal wall. However work by A. L. Smith (27) in this laboratory suggests that the effect may be through some other mechanism than complex formation.

As mentioned above, there are several types of data which suggest that fed cholesterol esters are hydrolyzed in the lumen prior to transfer into the mucosa. One type of data, obtained by feeding a series of cholesterol esters, is shown in Table IV. Free cholesterol and cholesterol butyrate were absorbed about equally well and to a greater extent than any of the other esters. The two saturated acid esters, laurate and stearate, were poorly absorbed while the two unsaturated esters, oleate and linoleate, were absorbed to a greater degree but less than the butyrate ester. These

TABLE II

Lipide Fractions of 24-hr. Lymph Samples in Normal and Bile-Fistula Rats

Type of animal	Emulsion	Triglyceride	Phospholipide	Cholesterol		"Extra" cholesterol	
				Total	Ester	Total	Ester
		mg.	mg.	mg.	%	mg.	%
Bile-fistula	None	7	3	5	50		
Normal	None	81	7	10	67	5	84
Bile-fistula	Cholesterol Oleic acid Taurocholate	64	14	9	60		
Normal	Cholesterol Oleic acid Taurocholate	335	30	37	79	19	85

relationships are the same as those we found earlier for the ease of hydrolysis of different esters by pancreatic cholesterol esterase (28). The finding that cholesterol trimethylacetate was not absorbed is particularly interesting. This ester is completely resistant to the hydrolytic action of pancreatic cholesterol esterase (29) and therefore would not be hydrolyzed in the lumen. The degree of esterification of the absorbed cholesterol in the lymph was the same for the free cholesterol and the various esters, irrespective of the degree of absorption. All of the ester data are in agreement with the concept that cholesterol esters are hydrolyzed in the lumen prior to absorption and that only free cholesterol enters the mucosa.

TABLE III

Recovery of Cholesterol-4-C^{14} from Lymph and Intestinal Wall Six Hours After Administration of Cholesterol-4-C^{14} to Bile-Fistula Lymph-Fistula Rats [a]

Additions to emulsion	Lymph		Intestinal wall	
	Free Chol.-4-C^{14}	Esterified Chol.-4-C^{14}	Free Chol.-4-C^{14}	Esterified Chol.-4-C^{14}
	%	%	%	%
Chol.-4-C^{14}	0.0	0.0	0.7	0.0
Chol.-4-C^{14} Oleic acid	0.0	0.0	0.4	0.0
Chol.-4-C^{14} Taurocholate	0.7	2.9	26.2	2.0
Chol.-4-C^{14} Oleic acid Taurocholate	1.2	3.1	16.0	2.2

[a] There were 5 rats per group. Each rat received 0.5 μc or approximately 3 mg. of cholesterol-4-C^{14}.

We have studied the processes occurring in the mucosa after the entrance of free cholesterol from the lumen by feeding lymph fistula rats cholesterol-4-C^{14} and following the changes occurring in the lumen, mucosa, and lymph. In these experiments we have confirmed earlier data (1) showing considerable dilution of labelled cholesterol with inactive cholesterol before its appearance in the lymph (30). Furthermore our data indicate that this dilution must occur in the mucosa. Figure 2 shows the specific activity of the fed cholesterol in the test emulsion and of the cholesterol in the lumen and lymph after 6 hrs. There was an 11% dilution of the fed cholesterol in the rumen. In the lymph there was a further 46% dilution of that in the lumen. This marked dilution between the lumen and the lymph must have occurred by mixing with a pool of endogenous cholesterol in the mucosa. Practically all of the cholesterol in the mucosa is present as the free alcohol. The amount of esterified cholesterol in the wall is not adequate to account for the observed degree of endogenous dilution. Also the appearance of cholesterol-4-C^{14} in the lymph for as long as 72 hrs. suggests a dynamic pool several orders of magnitude greater than can be accounted for by the esterified cholesterol fraction in the mucosa. There is adequate free cholesterol in the mucosa to account for the endogenous dilution of fed cholesterol, and we have found that after the administration of a single dose of cholesterol-4-C^{14} the free cholesterol of the mucosa does contain labelled cholesterol for more than 48 hrs. (31).

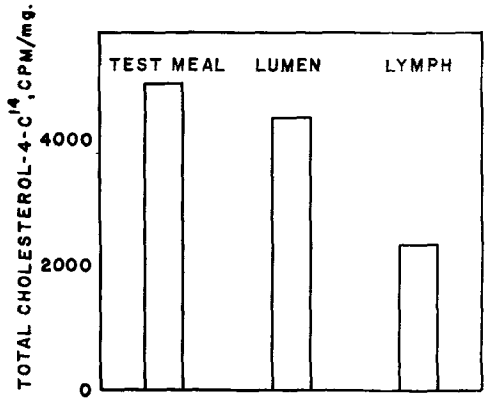

FIG. 2. Endogenous dilution of fed cholesterol-4-C^{14} in lymph and the lumen of the intestine. Levels are the averages for 4 rats sacrificed at 6 hrs. after test meal containing 40 mg. cholesterol-4-C^{14} (0.5 μc.), 292 mg. oleic acid, and 279 mg. taurocholate.

Table V shows data on the lymph and intestine 6 hrs. after the feeding of small and large doses of cholesterol-4-C^{14}. In the animals receiving 3 mg. or a tracer dose of cholesterol-4-C^{14} without bile salt or fat the levels of cholesterol in the lymph and intestine were the same as found in other animals fed the test emulsion without cholesterol. However there was labelled cholesterol in both the lymph and the small intestine. In the lymph 68% of the cholesterol was in the esterified form while in the intestine 91% of the total was in the free form. The same general relationship also held for the radioactive cholesterol except that the percentage of the labelled cholesterol esterified in the lymph was 80%. This would be expected since all of the labelled cholesterol was from the mucosa while part of that determined chemically was from other sources, as mentioned above.

TABLE IV

Cholesterol in 24-hr. Lymph Samples After Administration of Cholesterol Esters [a]

Emulsion	Lymph cholesterol		Cholesterol absorbed	"Extra" cholesterol esterified
	Total	Ester		
	mg.	%	%	%
Control	12	64		
Cholesterol	30	78	36	87
Chol. butyrate	32	82	40	93
Chol. laurate	19	75	13	95
Chol. stearate	20	72	16	88
Chol. oleate	25	79	25	94
Chol. linoleate	25	78	27	91
Chol. trimethylacetate	11	66	0	

[a] The control emulsion contained albumin, taurocholate, oleic acid, and saline to 3 ml. Additions: cholesterol, 50 mg.; esters equivalent to 50 mg. of cholesterol.

TABLE V

Cholesterol Fractions in Lymph and Small Intestine of Rats Six Hours After Feeding Cholesterol-4-C^{14}

Emulsion fed	Free		Esterified		Est. chol.	Est. chol.-4-C^{14}	Chol.-4-C^{14} recovered	Free chol. pool
	Chol.	Chol.-4-C^{14}	Chol.	Chol.-4-C^{14}	Tot. chol.	Tot. Chol.-4-C^{14}		
Lymph								
	mg.	*mg.*	*mg.*	*mg.*	%	%	%	*mg.*
Fasting	0.8		1.4		64			
Chol.-4-C^{14}, 3.3 mg	0.7	0.02	1.5	0.08	68	80	3	
Chol.-4-C^{14}, 3.3 mg Bile salt Oleic acid	1.5	0.09	3.0	0.30	66	77	12	
Chol.-4-C^{14}, 42 mg Bile salt Oleic acid	1.6	0.60	6.1	3.10	79	84	9	
Small intestine								
Fasting	12.1		1.0		8			
Chol.-4-C^{14}, 3.3 mg	12.5	0.34	1.2	0.04	9	10	12	5.5
Chol.-4-C^{14}, 3.3 mg Bile salt Oleic acid	13.1	0.58	1.2	0.09	10	13	21	5.5
Chol.-4-C^{14}, 42 mg Bile salt Oleic acid	14.4	4.30	2.5	1.40	15	25	14	8.5

When bile salt and oleic acid were administered along with the tracer dose of cholesterol-4-C^{14} (third group under lymph and small intestine), the amounts of free and esterified cholesterol in the lymph were approximately double those in animals which did not receive the supplement while the amounts of radioactive cholesterol were four times greater. In the intestinal wall there was no significant increase in the amount of free or esterified cholesterol, but the amount of labelled cholesterol was approximately doubled. From this it appears that the addition of bile salt and oleic acid lead to an increased transfer of cholesterol from the lumen into the free cholesterol pool and an increased passage from the pool into the lymph. An increased passage from a pool in the mucosa into the lymph rather than simply an increased transfer of the labelled cholesterol from lumen into the lymph is also suggested by the fact that the increase in labelled cholesterol of lymph of 0.3 mg. accounts for only 13% of the total increase in cholesterol.

Of course, not all of the free cholesterol of the mucosa is involved in cholesterol absorption, and we have calculated from the specific activity of the cholesterol fractions in the intestine (31) that the labelled cholesterol after entrance into the mucosa was mixed with a pool of free cholesterol of about 5.5 mg. From other data it can also be calculated that this pool turns over once per 24 hrs. in the fasting animal, which would account for the amount of labelled cholesterol in the lymph when a tracer dose is fed, as in this experiment. The fourth lines under lymph and small intestine show the levels when a large dose of radioactive cholesterol was fed. In this case there were chemical increases above the fasting levels of both free and esterified cholesterol in the lymph and intestinal wall. Favager and Metzger (32) have reported that there is no increase in the amount of esterified cholesterol in the mucosa during absorption and that the ester fraction does not become labelled during the absorption of labelled cholesterol. The data in the table do not agree with these findings. In fact, comparison of the amounts of labelled cholesterol in the ester fraction when the two levels of cholesterol-4-C^{14} were fed shows that with the larger amount, while the amount of ester was approximately doubled, the amount of labelled ester was increased 16 times over that when the tracer dose was fed. This would be expected if this fraction were the immediate precursor of the large amount of ester appearing in the lymph. The large dose increased the free cholesterol pool of the mucosa from 5.5 to 8.5 mg.

Based on the data described above and that from other laboratories, the tentative mechanism of cholesterol absorption shown in Figure 3 is proposed. Free and esterified dietary cholesterol and fat enter the small intestine and are mixed with endogenous cholesterol from bile and other secretions, bile salts, lipase, and cholesterol esterase of the pancreatic juice. Cholesterol esters and triglycerides are hydrolyzed, the cholesterol esters to cholesterol and fatty acid, the triglycerides to monoglycerides, diglycerides, and fatty acids; the free cholesterol, perhaps as a complex with bile salt, passes into the mucosa along with fatty acid, bile salt, pancreatic cholesterol esterase, and the products of triglyceride digestion. The free cholesterol becomes mixed with the pool of free cholesterol in the mucosa. In the fasting animal this pool is being constantly turned over by the entrance from the lumen of endogenous cholesterol and by synthesis of cholesterol in the mucosa and by the synthesis of cholesterol esters and their passage with a small amount of free cholesterol into the lacteals. That this occurs in the fasting animal is shown by the constant amount of cholesterol appearing in the lymph for 24 hrs. and the labelling of this cholesterol when a tracer dose is given. The passage of free cholesterol from the lumen into the mucosa in amounts larger than tracer doses tends to increase the size of the free cholesterol pool, and at

TENTATIVE MECHANISM OF CHOLESTEROL ABSORPTION

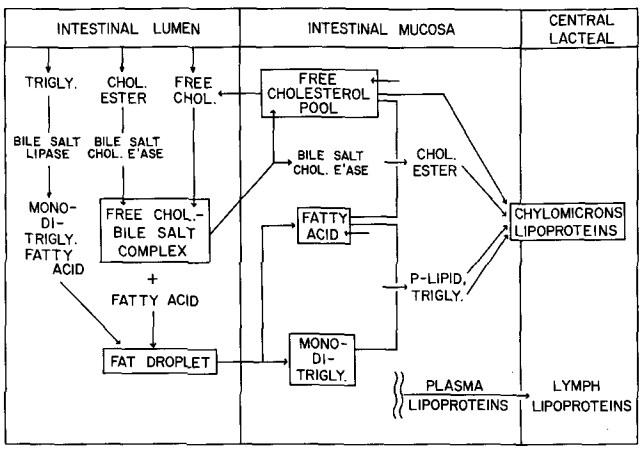

FIG. 3. Tentative mechanism of cholesterol absorption.

the same time conditions are favorable for the esterification of cholesterol caused by the presence of bile salts, fatty acid, and cholesterol esterase also entering from the lumen. There results an increased synthesis of cholesterol esters, which then pass into the central lacteal. The cholesterol esters do not pass into the lymph alone but rather are transferred along with triglyceride, phospholipide, free cholesterol, and protein. All of these substances occur together as the chylomicron when cholesterol is absorbed. The exact site of this chylomicron formation is unknown, but free cholesterol is probably drawn from the free cholesterol pool in the mucosa for this formation. Consistent with this proposed mechanism is the observation that during cholesterol absorption there are increases in the phospholipide and triglyceride contents of lymph. Also when fat alone is fed, there is an increase in the cholesterol of lymph. If the mechanism presented is correct, the appearance of free cholesterol in lymph is a necessary part of the transport mechanism for the esterified cholesterol.

Finally, the appearance of "extra" cholesterol in lymph after the administration of cholesterol results from increases in the rate of processes going on at all times in the mucosa. In a way the appearance of this "extra" cholesterol may be looked upon as the result of a homeostatic mechanism for maintaining the constancy of the cholesterol fractions of the intestinal mucosa.

REFERENCES

1. Biggs, M. W., Friedman, M., and Byers, S. O., Proc. Soc. Expt. Biol. and Med., *78*, 641 (1951).
2. Chaikoff, I. L., Bloom, B., Siperstein, M. D., Kiyasu, J. Y., Reinhardt, W. O., Dauben, W. G., and Eastham, J. F., J. Biol. Chem., *194*, 407 (1952).
3. Bollman, J. L., and Flock, E. V., Am. J. Physiol., *164*, 480 (1951).
4. Mueller, J. H., J. Biol. Chem., *22*, 1 (1915).
5. Kim, K. S., and Ivy, A. C., Am. J. Physiol., *171*, 302 (1952).
6. Siperstein, M. D., Chaikoff, I. L., and Reinhardt, W. O., J. Biol. Chem., *198*, 111 (1952).
7. Pihl, A., Acta Physiol. Scand., *34*, 206 (1955).
8. Swell, L., Flick, D. F., Field, H. Jr., and Treadwell, C. R., Am. J. Physiol., *180*, 124 (1955).
9. Swell, L., Field, H. Jr., and Treadwell, C. R., Proc. Soc. Exp. Biol. and Med., *84*, 417 (1953).
10. Swell, L., Trout, E. C. Jr., Hopper, J. R., Field, H. Jr., and Treadwell, C. R., Proc. Soc. Exp. Biol. and Med., *98*, 174 (1958).
11. Pihl, A., Acta Physiol. Scand., *34*, 183 (1955).
12. Vahouny, G. V., Fawal, I., and Treadwell, C. R., Am. J. Physiol., *188*, 342 (1957).
13. Vahouny, G. V., and Treadwell, C. R., unpublished.
14. Swell, L., Boiter, T. A., Field, H. Jr., and Treadwell, C. R., J. Nutrition, *57*, 121 (1955).
15. Friedman, M., Byers, S. O., and St. George, S., Am. Rev. Biochem., *25*, 613 (1956).
16. Pihl, A., Acta Physiol. Scand., *34*, 197 (1955).
17. Metzger, E. F., and Favager, F. A., Helv. Chim. Acta, *35*, 1805 (1952).
18. Swell, L., Byron, J. E., and Treadwell, C. R., J. Biol. Chem., *181*, 543 (1950).
19. Hernandez, H. H., Chaikoff, I. L., and Kiyasu, J. Y., Am. J. Physiol., *181*, 523 (1955).
20. Byers, S. O., and Friedman, M., Am. J. Physiol., *182*, 69 (1955).
21. Friedman, M., Byers, S. O., and Omoto, C., Am. J. Physiol., *184*, 11 (1956).
22. Daskalakis, E. G., and Chaikoff, I. L., Arch. Biochem. and Biophys., *58*, 373 (1955).
23. Swell, L., Field, H. Jr., and Treadwell, C. R., unpublished.
24. Glover, J., and Green, C., Biochem. J., *67*, 308 (1957).
25. Bollman, J. L., Cain, J. C., and Grindlay, J. H., J. Lab. and Clin. Med., *33*, 1349 (1948).
26. Vahouny, G. V., and Treadwell, C. R., Am. J. Physiol., *191*, 179 (1957).
27. Smith, A. L., Hauk, R., and Treadwell, C. R., Am. J. Physiol., in press.
28. Swell, L., and Treadwell, C. R., J. Biol. Chem., *212*, 141 (1955).
29. Stern, H. S., and Treadwell, C. R., Proc. Soc. Exp. Biol. and Med., *97*, 579 (1958).
30. Swell, L., Trout, E. C. Jr., Field, H. Jr., and Treadwell, C. R., J. Biol. Chem., *232*, 1 (1958).
31. Swell, L., Trout, E. C. Jr., Hopper, J. R., Field, H., Jr., and Treadwell, C. R., J. Biol. Chem., *237*, 49 (1958).
32. Favager, F. A., and Metzger, E. F., Helv. Chim. Acta, *35*, 1811 (1952).

[Received April 22, 1958]

Effect of Various Dietary Components on Cholesterol Metabolism

DAVID KRITCHEVSKY, The Wistar Institute, Philadelphia, Pennsylvania

MANY of the experiments purporting to report on cholesterol metabolism are, in fact, concerned only with changes in the serum cholesterol levels. Serum cholesterol is an important biological parameter, but if we are to consider it as the sole measure of the degree of cholesterol metabolism, it might be well to consider the variability of serum cholesterol levels in standard conditions in man and animals.

The wide fluctuations in serum cholesterol levels shown by many individuals make a base-line study mandatory when only changes in serum cholesterol values are measured as the criterion of deeper metabolic changes. Wilkinson (1) has studied the variations in human cholesterol levels over a two-year period. The range of fluctuation is wide and displays no apparent periodicity. Schube (2) observed 10 men over a 16-week period and found only three cases in which the maximum variation was less than 20%. His data are summarized in Table I. More recently Rivin and co-workers (3) studied 10 persons over periods ranging from six months to a year. Here again, the maximum deviation from the main serum cholesterol level ranged from 9 to 29% with most of the deviations between 14 and 18%. In a larger study (30 men), carried out over a shorter period (13 weeks), Gordon and Brock (4) observed similar fluctuations. In animal studies variations in serum cholesterol levels have been observed in monkeys (5, 6) and dogs (7); and a strain of rats, in which the serum cholesterol levels go through a minimum with age, has been described (8). The foregoing has been presented to show that the disposition of cholesterol throughout the animal body should be known for proper assessment of the metabolism of this sterol. In many cases serum cholesterol must serve as the sole indicator of cholesterol metabolism, but, where possible, as many other data as possible should be assembled.

TABLE I
Serum Cholesterol Variability
(Ten males, 16-week study)

Case	Mean cholesterol level	Range
	mg. %	
1	143	100–187
2	167	151–186
3	122	106–148
4	160	142–198
5	132	120–151
6	152	115–185
7	148	135–170
8	154	134–190
9	141	124–170
10	155	116–196

In the ensuing discussion many experiments will