EFFECT OF DIETARY CHOLESTEROL ON ITS BIOSYNTHESIS IN PARABIOTIC RATS*

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The importance of dietary cholesterol for the cholesterol balance in the mammalian body household is an accepted fact. [Compare Cook (1958) and Kritchevsky (1958).] The influence of an increase in the dietary cholesterol has been investigated therefore especially in its correlation to the biosynthesis of this substance in the intact animal. It has been shown that the state of dynamic equilibrium which normally is prevalent for it in tissues of the mammalian body, except the brain of the adult animal, undergoes a considerable change when cholesterol or a diet rich in this substance is given for any length of time. It was observed, for instance, in experiments of Chaikoff's group (Morris *et al.*, 1957, 1959), that biosynthesis, which is the source of about 75% of the endogenous contribution to plasma cholesterol, was considerably decreased, though not completely suppressed, when rats received for six weeks a diet containing 2% cholesterol.

It was assumed that some more information on this action may be gained from a study of the effect of dietary cholesterol on its biosynthesis in tissues of parabiotic rats after feeding it to one or to both partners for relatively short time intervals.

EXPERIMENTAL

Fifty-six male Sprague-Dawley rats underwent parabiotic operation under slight ether anesthesia when they were 34 days (Exp. I and II) or 29 days (Exp. III) of age. For 9 days following the operation they were given a basic diet (Purina Laboratory Chow). On the 10th day the parabiotic rats were separated in 3 groups as follows:

Experiment I. (Age at operation 34 days). Nine pairs were used as control and continued for 8 days on the basic diet, with water *ad libitum*.

Experiment II. (Age at operation 34 days). Nine pairs were fed a cholesterol diet, containing 2 per cent commercial cholesterol (Wilson) and 5 per cent cottonseed oil in the basic diet, and water for 8 days *ad libitum*.

Experiment III. (Age at operation 29 days). Ten pairs were used. A suspension of 200 mg purified cholesterol- H^3 (1.76×10^4 cpm/mg) in 2 cc water was administered by stomach tube

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daily in one batch to one partner of each pair for 7 days. After each force-feeding they were allowed free access to basic diet and water *ad libitum*. Cholesterol- H^3 was prepared by Wilzbach's method and purified by five times repeated bromination and debromination (Schwenk *et al.*, 1961).

Body weight change and food consumption of each pair were determined daily. At the end of the feeding period, 0.25 mc/kg body weight of aqueous sodium acetate- $1-C^{14}$ was injected intraperitoneally to each animal of a pair. Ten, 20, 40, 80 and 240 mins. after the injection the rats were sacrificed by neck-break, and the animals of each pair were separated. Liver, gastrointestinal tract (G.I. tract) including contents were dissected and all remaining tissues were combined as carcass for further work. The wet tissues were weighed. Two pairs of rats were used for each time interval except the 240 minute groups of Experiment I and II where only one pair was used. The procedures employed for tissue decomposition and separation of cholesterol and its purification have been reported elsewhere (Schwenk *et al.*, 1955). Cholesterol was weighed after isolation from the digitonide but was not further purified by bromination before counting. Tissue cholesterol was determined gravimetrically and radioassayed with a Packard automatic liquid scintillation counter.

RESULTS AND DISCUSSION

1) Body weight change and food consumption

At necropsy remainders of the diet were found in G. I. tracts of all experimental animals. As shown in Figure 1, Exp. I (control group) and Exp. II (2 per cent cholesterol-diet fed group), both groups of animals (operated 34 days after birth) presented no significant differences in weights and had consumed in the average about 23 g diet daily. In Exp. III (200 mg cholesterol- H^3 force-fed group, operated on the 29th days of age) body weight gains were a little less than with the two other groups. Part of this may be due to the age difference, and especially at the end of the experiment, also to the stress of force-feeding to which

Fig. 1. Body weight and food consumption of parabolic rats

the animals of this group had been submitted.

2) Tissue weight

No significant differences were found in tissue weights between the groups of Exp. I and Exp. II (Table 1). Slightly higher percentage values in Exp. III for liver and G. I. tract are due to the lower average body weight and are not statistically significant. No significant differences were found between the two partners of each pair when they were killed at the end of the experiments and all values were within the range found in normal animals as examined elsewhere (Tyler *et al.*).

Table 1. Tissue weights of parabiotic rats

(1) Original weights of tissues (g)

Partner I				
Group	Body weight	Liver	G.I. tract	Carcass
Exp. I	256 (212-272)	13.2 (10.1-15.9)	29.6 (28.6-34.9)	202.8 (170.7-221.0)
Exp. II	243 (222-272)	13.0 (10.7-12.8)	30.2 (27.0-34.0)	193.0 (167.2-218.0)
Exp. III	201 (192-224)	12.0* (8.4-14.9)	26.8* (21.4-30.8)	

Partner II				
Group	Body weight	Liver	G.I. tract	Carcass
Exp. I	248 (234-270)	13.4 (10.4-16.4)	28.6 (22.4-32.8)	197.8 (177.2-213.1)
Exp. II	264 (234-296)	15.0 (11.6-16.7)	31.4 (25.0-35.5)	209.4 (190.1-242.3)
Exp. III	175 (172-185)	10.2 (9.5-10.9)	24.4 (20.9-28.5)	

(2) Percentage of body weights

Partner I				Partner II		
Group	Liver	G.I. tract	Carcass	Liver	G.I. tract	Carcass
Exp. I	5.2 (4.9-5.7)	11.6 (10.5-12.6)	80.4 (79.4-81.3)	5.5 (4.3-7.1)	11.5 (9.3-13.5)	80.0 (76.3-82.5)
Exp. II	5.3 (4.8-5.7)	12.3 (11.3-15.3)	79.3 (74.6-81.2)	5.5 (4.4-6.6)	11.6 (9.5-14.1)	79.1 (75.4-83.6)
Exp. III	6.0* (4.3-6.8)	13.4* (11.0-16.0)		5.8 (5.1-6.4)	14.0 (12.7-16.6)	

* Force-fed partner

3) *Cholesterol content in tissues*

After feeding of 2% cholesterol in a diet containing 5% added cottonseed oil for eight days as in Exp. II the cholesterol content per gram of wet livers was nearly twice that of the controls in Exp. I (Table 2). Values for G. I. tracts

Table 2. Cholesterol content of tissues of parabiotic rats
(mg cholesterol per g wet tissue)

	Partner I			Partner II		
	Liver	G.I. tract	Carcass	Liver	G.I. tract	Carcass
Exp. I	2.13 (2.02-2.27)	2.22 (1.47-2.79)	1.88 (1.62-2.07)	2.17 (1.86-2.34)	2.44 (1.70-3.09)	1.80 (1.55-2.16)
Exp. II	4.14 (2.66-5.20)	2.43 (1.44-3.06)	2.03 (1.76-2.33)	3.83 (2.67-7.20)	2.29 (1.87-2.80)	1.91 (1.62-2.10)
Exp. III	1.85* (1.61-2.27)	1.66* (1.57-1.79)		1.95 (1.79-2.22)	1.44 (1.10-1.74)	

* Force-fed partner

and carcasses, however, did not show statistically significant differences between the two experiments and did also not differ from normal values reported, showing that the cholesterol fed is predominantly deposited in the livers. In Exp. III with animals which were 5 days younger than in Exp. I and II the amounts of cholesterol per gram wet livers are almost the same for both partners and slightly

Table 3. Cholesterol-H³ in tissues of parabiotic rats
force-fed to one partner

	Cholesterol-H ³ fed Partner I		Partner II	
	Liver	G.I. tract	Liver	G.I. tract
*Specific activity	1.12	1.38	0.85	0.65
**% Deposition	0.196	0.505	0.136	0.182
***Total count	2.04	2.26	1.64	0.91

Cholesterol-C¹⁴ in tissues of parabiotic rats
after force-feeding one partner with cholesterol-H³

	Cholesterol-H ³ fed Partner I		Partner II	
	Liver	G.I. tract	Liver	G.I. tract
*Specific activity	2.46	1.38	3.84	2.06
**% Incorporation	0.254	0.264	0.344	0.338
***Total count	4.45	2.34	7.61	3.00

* cpm/mg × 10⁴

** total count in tissue/total count administered to the host

*** count/g tissue × 10⁴

lower than in the control animals of Exp. I but statistical analysis proves that this difference is not significant. This is somewhat unexpected because partner I of each pair of the animals in Exp. III received cholesterol- H^3 by force-feeding. However the amounts fed were smaller—only 200 mg in Exp. III against about 500 mg in Exp. II—and besides were administered in aqueous suspension and without oil added as in Exp. II. It is striking that there is also no difference between the force-fed partner and the companion; the amounts fed were evidently not large enough to show up in the deposition of cholesterol in the liver but as will be seen in the following there was nevertheless transfer of cholesterol- H^3 from the force-fed partner to the companion (Table 3), and also incorporation of C^{14} from acetate in the force-fed partner was lower than in its companion (Table 3) (compare Section 5).

4) Absorption and distribution of administered cholesterol

Figure 2 and Table 3 indicate that radioactive cholesterol- H^3 force-fed to one partner was absorbed through the intestinal tract and deposited in the tissues not only of the force-fed partner but also in those of the companion. Apparently, however, during the resorption cholesterol does not simply pass through the intestinal wall but is retained there for some time and then deposited in the liver where it again stays for a certain time before moving on with the circulation. This is the reason why in Exp. III liver and G. I. tract of the force-fed partner show a higher deposition of cholesterol- H^3 than in the other partner (Table 3). Slow absorption from the intestine was observed by Favarger *et al.* (1955) and Biggs *et al.* (1951) also reported that in rats dietary cholesterol is absorbed from the intestine and conveyed to the systemic circulation via the thoracic duct. A similar observation was recorded by Chaikoff *et al.* (1952). Probably the most

Fig. 2. Distribution of cholesterol- H^3 force-fed to one partner (called "host") of pairs of parabiotic rats

detailed experiments were made by Borgstroem *et al.* (1958). Their observations as far as cholesterol absorption is concerned, are the same as of Biggs *et al.* In addition however they found that the absorbed cholesterol does not mix or exchange to any appreciable degree with cholesterol in serum or other organs before it is taken up by the liver. Cholesterol thus deposited in the liver began to discharge into the general circulation 12 hrs. after absorption and only then was distributed over the entire body. They also reported that the absorbed cholesterol remains for a considerable period in the intestinal wall so that after 24 hrs. 24% was still present in the mucosa of the small intestine.

The observation in the present study that the tissues of the force-fed partner in Exp. III contain considerably more cholesterol- H^3 than those of its companion are in good agreement with these findings of Borgstroem *et al.*

5) Biosynthesis of cholesterol in tissues

Figures 3~5 present values of specific activities, percent incorporation and total count of tissue cholesterol- C^{14} at 10, 20, 40, 80 and 240 mins. after intraperitoneal injection of acetate- C^{14} and Table 4 shows the average sum totals of these values for both partners. From Figures 3~5 one can see that the incorporation of radioactivity from acetate- C^{14} into cholesterol of the liver is at a maximum at the beginning shortly after injection and then decreases slowly, while in the G. I. tract and the carcass the values increase continuously. A probable explanation for this difference in the incorporation picture of radioactivity between liver and other tissue cholesterol is apparently that biosynthesis of cholesterol- C^{14} from acetate- C^{14} in the liver is fast and immediate, reaching its maximum about 10~20 mins. after injection. The incorporation of C^{14} in cholesterol in the liver then falls off because of its concurrent oxidation to bile acids and possibly also

Fig. 3. Distribution of radioactivity of cholesterol- C^{14} in the tissues of parabiotic rats after injection of acetate- C^{14}

Fig. 4. Incorporation rate of label into cholesterol of tissues of parabiotic rats after injection of acetate-1-C¹⁴

Fig. 5. Total count of cholesterol-C¹⁴ of parabiotic rats after injection of acetate-1-C¹⁴

because there is no more radioacetate available. Van Bruggen *et al.* (1953) have reported that incorporation of intraperitoneally injected acetate-1-C¹⁴ reached a maximum within 22 mins. after administration and similar observations were made by Schwenk *et al.* (1955). In the G.I. tracts and the carcasses, as pointed out above and according to Borgstroem *et al.*, cholesterol-C¹⁴ arrives only some time after its formation in the liver. The label of cholesterol-C¹⁴ in these tissues there-

fore increases only slowly and there is no maximum at the beginning of the experiment. This dissimilar type of incorporation of the label into liver and other tissue cholesterol seems to support the assumption that in the intact animal extrahepatic biosynthesis of cholesterol is probably only of a minor importance in comparison to the synthetic function of the liver.

Feeding a diet containing 2% cholesterol depressed incorporation of C^{14} -radioactivity into cholesterol in all tissues of rats in Exp. II. This suppression of the incorporation of radioactivity (Table 4) is evidently more efficient in the liver (90% against 25% in the G. I. tract) so that incorporation into the G. I. tract and carcass cholesterol is comparatively higher than in the liver material. It is possible that this difference is caused by the fact that if there is any biosynthesis of cholesterol in extra-hepatic tissues, it is not at all or not as much influenced by deposited dietary cholesterol as is biosynthesis in the liver itself. It should,

Table 4. Data on cholesterol- C^{14} in tissues of parabiotic rats after intraperitoneal injection of acetate-1- C^{14}

(Values presented as sum of values at 10, 20, 40, 80 and 240 mins. of total amount of tissues from Partner I and II)

Group	Exp. I			Exp. II			Exp. III	
Tissue	Liver	G.I. tract	Carcass	Liver	G.I. tract	Carcass	Liver	G.I. tract
*Specific activity	6.92	3.51	0.98	0.74	2.63	0.77	6.30	3.44
**% Incorporation	0.59	0.71	0.39	0.10	0.55	0.31	0.60	0.60
***Total count	14.86	8.20	0.64	2.27	6.16	0.53	12.06	5.34

* cpm/mg $\times 10^4$

** total count in tissue/total count injected

*** cpm/g tissue $\times 10^4$

however, not be overlooked that the same effect of a lower incorporation could be obtained if the dietary cholesterol would cause an increase in the processes of oxidation in or other elimination of cholesterol from the liver.

A different picture is offered when the distribution of cholesterol- C^{14} in Exp. III is considered. Here it is seen (Figs. 3~5 and Table 3) that specific activity, percentage of incorporation and total amount of radioactivity are lower in the force-fed partner than in its companion. This shows that the feeding of the small amount of cholesterol- H^3 , although not influencing the isolatable amount of cholesterol deposited in the tissues of the force-fed partner (compare Table 2), had definitely an influence on the biosynthesis of cholesterol- C^{14} in these animals resulting in a lower amount of C^{14} caused radioactivity in the liver and G. I. tract of the force-fed partner than in its companion.

SUMMARY

The experiments on parabiotic rats, as here reported, confirm observations of other workers that dietary cholesterol is absorbed and deposited in the tissues of rats. They show moreover that in parabiotic pairs of animals cholesterol which is fed to one of the partners is carried evidently by the blood stream to the companion. This work supports the suggestion of Borgstroem *et al.* that dietary cholesterol is retained for a time and to a certain degree in the intestinal wall and subsequently in the liver before it is released into the circulation to be carried to other tissues in the body. When both partners are fed cholesterol an even distribution of this substance is found and both fed animals of a pair show the known effect of cholesterol feeding on the incorporation of radioactivity from injected acetate-1-C¹⁴, in a considerably decreased incorporation of C¹⁴ into the newly synthesized cholesterol of the liver. In other tissues there is however much less decrease of the incorporation into this substance. The experiments suggest that if there is any extrahepatic biosynthesis of cholesterol in the intact animal, it may be only of quite minor importance in the total picture. When only one of the partners of a pair is fed cholesterol, the synthesis-depressing effect is observed only in the partner which received the feeding while the companion shows a normal incorporation of the radioactive label. Even an amount of cholesterol which does not visibly increase the deposition of this material in the liver has a very definite depressing influence on the incorporation of C¹⁴ from acetate-1-C¹⁴ into liver cholesterol.

REFERENCES

- Borgstroem, B., B. Lindle and P. Woldauer (1958). *Proc. Soc. Exptl. Biol. Med.* **99**, 365.
Biggs, M.W., M. Fricken and S.O. Byers (1951). *Ibid.* **78**, 641.
Chaikoff, I.L., B. Bloom, M.D. Siperstein, J.Y. Kyasu, W.O. Reichardt, W.G. Dauben and J.F. Eastham (1952). *J. Biol. Chem.* **194**, 407.
Cook, R.F. Cholesterol. Academic Press, New York, p. 240 and 277 (1958).
Favarger, P., J. Garach and M. Roth (1955). *Helv. Physiol. et Pharmacol. Acta* **13**, 245.
Kritchevsky, D. Cholesterol. John Wiley & Sons Inc., New York, p. 143 (1958).
Morris, M.D., I.L. Chaikoff, J.M. Felts, S. Abraham and N.O. Fansah (1957). *J. Biol. Chem.* **244**, 1039.
Morris, M.D. and I.L. Chaikoff (1959). *Ibid.* **234**, 1059.
Schwenk, E., Y. Omori and E. Joachim (1961). *Proc. Soc. Exptl. Biol. Med.* **107**, 83.
Schwenk, E., G.J. Alexander and C.A. Fish (1955). *Arch. Biochem. Biophys.* **58**, 37.
Tyler, D.B., T.E. Nelson, Jr. and Y. Omori. Unpublished.
Van Bruggen, J.T., T.T. Hutchens, C.K. Claycomb and E.S. West (1953). *J. Biol. Chem.* **200**, 31.