The Relation in Man between Cholesterol Levels in the Diet and in the Blood[1]

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It is widely assumed that the amount of cholesterol consumed in the diet is reflected in the concentration of cholesterol in the blood serum and, eventually, in the tendency to development of atherosclerosis (*3*). It is true that when certain herbivorous animals like the rabbit are fed enormous amounts of cholesterol the blood level rises and atherosclerosis develops, but the situation may well be very different in man when subsisting on more natural diets. In any case, it is by no means certain that moderate differences in the serum cholesterol level in man have any influence on development of atherosclerosis. The following preliminary report deals exclusively with the more immediate question of the relation in man between cholesterol levels in the diet and in the blood.

Single doses of ingested cholesterol, even if these are very large, produce only trivial and transient changes in the serum cholesterol of man. In about 60 experiments on young men who received 10 g of cholesterol in a meal with ample fat, we seldom accounted for as much as 5% of the cholesterol as a very temporary addition to the blood. Much the same result with older men has been reported by others (*2*). In studies on two men with marked familial hypercholesteremia of the lipemic type, we found no difference from normal men in their response to single large doses of cholesterol. But these experiments have very limited significance. The effect of differences in the habitual intake of cholesterol is of more interest.

Our primary study on the habitual diet has been made on 482 clinically ''normal'' men who are volunteer subjects in a long-range research program in the Laboratory of Physiological Hygiene (*6*). For the most part, these men are in a relatively favored economic bracket and few if any of them are restricted in diet because of income. All of them have been studied with care for over 3 years. Their habitual cholesterol intakes have been estimated from repeated interviews and questionnaires, mainly concerning the current intake and that for the preceding 6 months; in most cases estimates apply to a period of several years but may not be valid descriptions of lifelong habits.

Cholesterol occurs only in foods of animal origin, and in these it is relatively stable. Habits with regard to the consumption of eggs, butter, milk, cheese, ice cream, and meat (lean or fat) tend to be well fixed in most American men who are economically comfortable. The man who regularly takes two eggs for breakfast, has several pats of butter at every meal, and does not remove all separable fat from his twice-a-day meat is in a very different category from the man who dislikes milk, cheese, and eggs as such, is sparing of butter, and refuses all but lean meat. Regardless of the rest of the diet, this man can scarcely get more than some 200–300 mg of cholesterol a day, whereas the former must get at least three times that amount. We believe segregation of our subjects into five classes of relative cholesterol intake, where the mean intake of the top class is about 2½ times that of the bottom class, can seldom be in error by more than one class. Serum cholesterol measurements for these men have been made on blood drawn under basal conditions; all analyses were made in duplicate, with suitable standards in every batch. The Liebermann-Burchard method used has been checked with the digitonin precipitation method of Sperry and Schoenheimer.

TABLE 1

MEANS AND STANDARD ERRORS FOR THE SERUM TOTAL CHOLESTEROL CONCENTRATION IN CLINICALLY "HEALTHY" MEN*

	Age 18–25 yr		Age 45–54 yr		Age 46–55 yr	
	low	high	low	high	low	high
No. of men	62	66	81	80	53	51
Mean, serum cholesterol	99.9	100.1	99.9	100.1	100.2	99.8
S.E., serum cholesterol	1.70	2.09	2.09	1.98	2.28	2.39

* All within ± 15% of standard weight for height, habitually subsisting on diets providing cholesterol intakes classed as "low" (not over about 2.0 g/week) and "high" (not less than about 3 g/week). Serum cholesterol expressed as % of the mean for all men in the age group. The results for men in the 46–55-year group include new estimates on some men who were studied, a year earlier, in the 45–54-year group.

Elsewhere we have mentioned, without discussion, some of the results of this analysis (*7*); the mean values for 312 men in five cholesterol-intake classes were essentially constant within each age group. Another analysis, based on new data, is given in Table 1; the men who had ''medium'' cholesterol intakes were discarded for this purpose.

Such findings make it reasonably clear that the serum cholesterol level of ''normal'' men as represented here is not significantly related to differences in the habitual cholesterol intake over a range of something like 250–800 mg per day. As a corollary, it may be inferred that efforts to control the serum cholesterol by alterations of

[1] The work reported here was aided, in part, by grants from the National Dairy Council, acting on behalf of the American Dairy Association, and from the U. S. Public Health Service, Research Grants Division, on recommendation of the Cardiovascular Study Section.

[2] Now at the Division of Chronic Diseases, U.S.P.H.S., Washington, D. C. Dr. Mickelsen collected dietary data and supervised cholesterol analyses (1946–47). Miss Erma v. O. Miller made almost all the cholesterol analyses. Dr. Carleton B. Chapman and the Division of Internal Medicine, Minneapolis Veterans' Hospital, made arrangements for the hypertensive patients and their supervision.

the intake within this range are unlikely to succeed with such men. More direct evidence to this effect is provided by the findings on 41 middle-aged normal men who were studied before and after reducing their cholesterol intakes by 50% or more. On the first occasion they had a mean total cholesterol of 250.12 (standard error = ± 7.06) mg/100 ml of serum. A year later, and after at least several months on the reduced cholesterol intake, the mean, corrected for the normal age trend, was 250.25 (S.E. = ± 6.68) mg/100 ml. The correction for the normal increase in cholesterol level in 1 year was made from a 2-year study on 168 normal men of similar ages whose cholesterol intakes were unchanged from one year to the next.

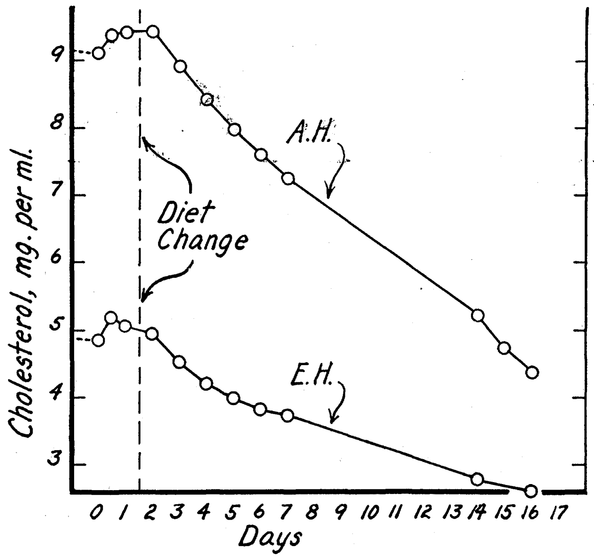

FIG. 1. Concentration of total cholesterol in the blood serum of two men with familial hypercholesteremia before and after changing to a fat-free vegetarian diet totally devoid of cholesterol. Before the diet change these men were subsisting on a moderately low cholesterol intake.

In contrast, there are the results of experiments in which the cholesterol intake is completely eliminated. The rice-fruit diet, currently popular in the treatment of hypertension (*4, 5*), is cholesterol-free if strictly applied. This diet is also substantially fat-free, is sufficient in protein, provides extremely little sodium, and is likely to be calorically deficient as well (*9*). With this diet we have regularly and rapidly produced marked declines in the serum cholesterol of hypertensive patients. In a typical series of four patients, the mean total cholesterol value of 232.5 mg/100 ml of serum fell to an average of 151.8 mg after about 3 weeks on the rice-fruit diet; continuation of the diet for another 2 weeks had apparently little further effect, but a few later observations suggested that a slow rate of decline may continue for some time.

At present we are carrying out similar studies on men with idiopathic hypercholesteremia. The response to complete elimination of cholesterol from the diet is, as shown in Fig. 1, even more dramatic than in normal men. For some months previously these men had been on a diet moderately restricted in cholesterol. At least in the first two weeks following change to the rice-fruit diet, the decline was linear with time in both men; the average daily decrement in serum concentration was 33 mg for A.H. and 14 mg for E.H. These data suggest that the daily decline may be related to the initial (prediet) level of cholesterol in the blood serum but not, as might have been expected on simple mass-action theory, to the cholesterol level on each day. This is in agreement with the only other comparable study in the literature where a similarly linear but slower decline was observed in a patient with idiopathic hypercholesteremia when her diet was completely vegetarian (*8*).

The general picture, then, is that the blood cholesterol level is independent of the intake over a wide range, but that at zero intake it falls at a rate related to the previous level of cholesterol in the blood. Conceivably, there may be a critical intake level above zero but below something like 200 mg per day; if so, it may be suspected that the critical level will differ from individual to individual. It is possible that other factors in the diet besides the absence of cholesterol are important in the cholesterolytic effect on the serum cholesterol level; this point is currently being examined.

In any case, it is doubtful whether most so-called low cholesterol diets in current use reach critical levels or have significant utility for the purpose of their use. Diets that permit ordinary amounts of lean meats and the use of skim milk, and that do not rigidly exclude from every item of cookery and baking all dairy products, eggs, and animal products, will regularly supply more than 100 mg or even as much as 200 mg of cholesterol daily. Unhappily for the proponents of dietary prophylaxis against atherosclerosis, an effectively low cholesterol level in the diet is not easily achieved. If the level of cholesterol in the serum is the criterion, half-way measures may be useless. On the other hand, with a much more rigorous diet the blood cholesterol may be readily and rapidly lowered, both in more or less normal men and in men with extreme hypercholesteremia of the type represented by patients E.H. and A.H.

Addendum. Since the foregoing was submitted for publication, we have obtained interesting new information from prolonged studies on patient A. H. When he was changed from a diet completely free from both cholesterol and all fats to one containing vegetable fats but no trace of cholesterol, his blood cholesterol level returned toward his previous high level almost as rapidly as it had fallen. Judging from the serum alone, he was accumulating cholesterol at a rate of 700 mg daily on a diet devoid of any trace of cholesterol or animal fat but containing moderate amounts of corn oil. After a month of this experience, his diet was again modified to contain minimal amounts of any kind of fat but providing one serving of meat or fish daily. On this diet his serum cholesterol level has been stabilized for several months at 500–600 mg/100 ml, i.e., some 60% below his plateau on an ordinary "low-cholesterol" diet.

These findings call to mind the animal studies of Abelin (*1*) where vegetable oils in the diet promoted cholesterol accumulation in the body.

References

1. Abelin, I. *Helv. Physiol. Acta,* 1948, **6**, 879.
2. Collen, M. F., de Kruif, D., and Geier, F. *Permanente Found. med. Bull.,* 1949, **7**, 60.
3. Dock, W. *J. A. M. A.,* 1946, **131**, 11.
4. Kempner, W. *N. C. med. J.,* 1945, **6**, 117.
5. ———. *Amer. J. Med.,* 1948, **4**, 545.
6. Keys, A. *Chicago Heart Assoc. Bull.,* 1948, **26**, 3.
7. ———. *Fed. Proc.,* 1949, **8**, 523.
8. Mélon, J., and Dallemagne, M. J. *Bull. Soc. Roy. Sci.*
9. Schwartz, W., and Merlis, J. *J. clin. Invest.,* 1948, **27**, 406.

Effect of Density and Average Atomic Number of the Medium on the Counting Yield of Beta and Gamma Radiation in a Solution-Type Geiger Counter[1]

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In investigations that employ radioactive tracers, it is frequently desirable to determine the quantities of different chemical species containing the tracer by counting them in a solution-type Geiger tube. Often these species are most conveniently introduced into the solution chamber of the tube in media that have different densities and different average atomic numbers. In such cases different counting rates may be obtained when equal amounts of the radioactive species are dissolved in the different solvents, and therefore a correction factor must be applied.

The magnitude and direction of this correction are dependent on a number of variables in addition to the density and average atomic number of the solution. They include: (1) the type of radiation (beta or gamma); (2) the energy of the radiation; (3) the geometrical arrangement of the solution relative to the counting tube; and (4) the thickness of the wall of the counting tube. It is known that these characteristics of the system determine the degree of absorption and scattering of the radiation and the efficiency of secondary electron formation by gamma rays, but knowledge of these phenomena is not sufficient to allow satisfactory calculations of the effect of the solvent medium on the counting efficiencies of different radiations in different solution tubes. Only a few specific examples of such effects are cited in the literature. These include: (*a*) data on the counting yield of K^{40} in water solutions of different density obtained by addition of zinc chloride or sucrose, and in solutions of equal density but different average atomic number obtained by the use of different salts (*2*); (*b*) data on the counting yield of P^{32} as a function of the sulfuric acid concentration of the solution (*8*), and the tricalcium phosphate concentration of the solution (*1*); (*c*) data on the relative counting efficiency of different nuclear species with a dipping counter, using different volumes of solution and with different counters (*15*). In contrast to the data available on solution counting, there is relatively extensive information in the literature on the factors affecting the counting yield from solid samples (for example, *3, 4, 6, 7, 10, 11*).

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TABLE 1

Radioisotopes Tested

Isotope* and half-life	Radiation, max Mev	Max range of β^- in Al, mg/cm²	Absorp. half-thickness in Al (approx.), mg/cm²	Rel. counting probability of β and γ from each species (approx. %)
P^{32}	β^-, 1.7 no γ	790	90	100
$Ce^{144} - Pr^{144}$	β^-, 3	1,500	200	97
275 d parent	β^-, 0.35			0
18 m. dau.	γ, 1.2		13,000	} 3
	γ, 0.3		7,000	
Br^{82}	β^-, 0.5	150	18	50
35 h†	γ, 1.3		13,000	} 50
	γ, 0.8		10,500	
	γ, 0.5		8,500	
Co^{60}	β^-, 0.3	75	7	5
5.3 y	γ, 1.2, 1.3		13,000	95

* Procured from the Oak Ridge National Laboratory of the U. S. Atomic Energy Commission.

† Determinations in this laboratory by A. A. Miller and by W. S. Ginell indicate that 35 h is more nearly correct than the commonly used value of 34 h.

In order to obtain a better understanding of the factors that control the counting yield in solution, we have determined the effect of increasing density and average atomic number of the solvent on the counting efficiency of the radiations from the four sources listed in Table 1. For these tests we have used a Technical Associates TA-B-2 solution-type Geiger counter consisting of a cylindrical glass tube of about 30 mg/cm² wall thickness coated on the inside with a thin silver cathode and surrounded by an annular jacket capable of holding 12 ml of solution. The counting gas was tetramethyl lead. The diameter of the sensitive volume was 2 cm, the length in contact with the solution was 10 cm, and the width of the annulus (thickness of the solution layer) was 0.3 cm.

Solutions of different density and different average atomic number were obtained by using varied concentrations of different salts. The density so obtained was determined by weighing the solution in a volumetric flask. All the solutions of each radioactive species tested contained the same concentration of that species. Portions of these solutions were used in turn to fill the annulus of the Geiger tube to the reference mark, and counts