CHOLESTEROL SYNTHESIS FROM C^{14}—ACETATE IN MAN [1]

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INTRODUCTION

Bloch and Rittenberg (1) first demonstrated that acetate could be utilized for cholesterol synthesis by animals. In these studies and others (2–4), cholesterol isolated after the administration of isotopically labeled acetate was shown to contain the administered isotope. However, there is no information concerning either the dynamic aspects of this process or the rate of disappearance of labeled cholesterol after its synthesis from acetate in man.[3]

In order to study cholesterol synthesis in this manner, radioactive carbon (C^{14})-labeled acetate was administered to patients, free and ester cholesterol were separately isolated from the plasma, and the radioactivity of these products was measured at frequent intervals over a prolonged period of time.

EXPERIMENTAL METHODS

Subjects

The patients used in this study were hospitalized in the James Ewing Hospital Unit of Memorial Center. A summary of pertinent clinical facts appears in Table I; all had normal plasma cholesterol levels.

Radioactive materials

The use of approximately 200 microcuries (μc) of C^{14}-labeled acetate in patients with limited life expectancy was authorized for this study by the Atomic Energy Commission. Thirteen and seven-tenths microcuries C^{14} per kilogram of body weight (or 960 μc per 70 kilo human) would deliver the tolerance dose of radiation or 0.3 rep. per week if completely retained and equally distributed throughout the body. Therefore, the dose of 200 μc of C^{14} administered to the patients, even if completely retained, would deliver only 20 per cent of tolerance radiation (7). Inasmuch as over 80 per cent of administered C^{14} is rapidly eliminated from the body (5, 6), the amount of radiation the patient receives is far below tolerance levels. If any concentration of the C^{14} isotope above equal distribution had occurred, larger amounts of radiation would be delivered at the points of concentration. However, radioautograph studies of tissues obtained at post mortem examination from patients who had received 200 μc of C^{14} acetate have thus far failed to disclose any areas of concentration of the isotope. In view of these considerations, the use of C^{14} in the manner to be described certainly appears to be a permissible procedure. Until more detailed information is available concerning intracellular distribution and long term effects of the radioactive isotope, caution should be exercised in extending the use of C^{14} labeled materials to human subjects other than those with limited life expectancy.

TABLE I

Clinical data on patients

Patient	Sex	Age	Weight	Diagnosis	Plasma cholesterol (*mg./100 ml.*) Total	Free	Number of microcuries given
AC–3	F	32	102	Non-functioning adrenal cortical carcinoma	243	71	200
AC–4	M	42	142	Refractory aplastic anemia	107	34	200
AC–9A AC–9B	F	57	118	Lymphosarcoma	162	54	212 200
AC–11	F	45	98	Breast carcinoma	216	61	200
AC–13	F	31	106	Functioning adrenal cortical carcinoma	218	58	336
AC–14	M	59	128	Bladder carcinoma	223	56	231

[1] This investigation was carried out under contract AT(30-1)-910 with the United States Atomic Energy Commission and supported in part by a research grant (C-440) from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

[2] This work was begun during tenure of an Atomic Energy Commission Postdoctoral Fellowship in the Medical Sciences of the National Research Council.

[3] Preliminary reports of the studies described in this paper have appeared (5, 6).

TABLE II

Radioactivity of plasma cholesterol

AC-3A		AC-4		AC-9			AC-11			AC-13			AC-14		
Time*	Free†	Time	Free	Time*	Free†	Ester†	Time	Free	Ester	Time	Free	Ester	Time	Free	Ester
0.25	830	0.25	718	A.			0.13	1,540	183	0.21	1,605	280	0.26	254	86
0.75	463	0.75	416	0.08	396	26	0.42	856	370	0.35	1,230	417	0.53	224	110
1.25	395	1.25	314	0.33	382	81	0.85	660	413	0.50	1,090	546	0.81	179	130
1.75	360	1.75	258	0.88	300	150	1.33	570	476	0.67	—	572	1.29	197	151
2.25	320	2.25	243	1.88	221	199	1.85	452	524	1.23	795	606	1.67	167	154
2.75	300	2.75	227	8.00	122	156	2.33	458	480	1.67	694	607	2.23	172	161
3.25	326	3.75	200	17.00	81	93	2.85	407	447	2.33	583	650	3.20	159	158
3.75	306	4.75	195	27.00	59	68	4.00	350	364	3.25	549	611	5.20	117	131
7.75	151	6.75	154	34.00	49	51	6.00	283	313	5.25	400	499	7.00	98	111
12.75	110	9.75	141				9.00	191	236	7.40	324	372	9.00	72	86
14.75	96	10.75	143	B.			11.00	169	222	9.00	278	323	12.00	53	67
18.75	88	11.75	120	0.10	654	100	16.00	125	148	12.00	226	265	15.00	55	62
25.75	81	17.75	95	0.31	446	156	23.00	79	98	15.00	182	220	19.00	—	53
28.75	57	23.75	70	0.85	284	179	30.00	64	84	19.00	127	166	22.00	35	47
35.75	54	27.75	45	1.85	226	212				22.00	115	159			
39.75	43	31.75	40	8.00	115	152				29.00	98	128			
42.75	38			18.00	55	81				33.00	85	116			

* Elapsed time in days after administration of acetate.
† DPM × 10^3/mM cholesterol.

Sodium acetate (acetate-2-C^{14}) was obtained from a commercial source[4] and had a specific activity of 1 millicurie per millimole. A solution of 16 mg. of sodium acetate, equivalent to approximately 200 microcuries of C^{14}, dissolved in 100 ml. of tap water, was administered orally to each patient. The container was rinsed with an additional 100 ml. of tap water which was also given to the patient. For the isolation of free and ester cholesterol, 30 to 40 ml. samples of blood were collected in either heparinized or oxalated tubes. Collections of urine, feces, and expired air were made over a period of several weeks for the purpose of determining the fate of other substances synthesized from the administered acetate (5, 6).

The quantity of sodium acetate administered to humans in this investigation was well within physiological limits since Rittenberg and Bloch (8) and others have shown that as much as 130 mg. of this compound per 100 Gm. weight of mouse or rat can be given with no apparent effects. Our dosages of approximately 16 mg. of acetate per patient (0.02 mg. per 100 Gm. weight) represent 0.05 per cent of the amounts used in animal experiments.

Isolation of cholesterol

Cholesterol was usually separated from 15 to 20 ml. of plasma. A filtrate was made by a modification of the procedure described by Sperry and Webb (9). One volume of plasma was added to 20 volumes of 1:1 acetone-alcohol mixture, and this solution was brought to the boiling point in a water bath. Precipitated plasma proteins were then removed by filtration. Two volumes of a 0.4 per cent aqueous solution of digitonin were added to each three volumes of acetone-alcohol filtrate of the plasma, and the solution was allowed to stand for 24 hours in 1 liter graduated cylinders while the free cholesterol digitonide precipitated. The supernatant solution was then siphoned off and the precipitate was washed twice with acetone-alcohol, once with acetone-ether 1:1, and three times with ether, centrifuging after each wash. The ester cholesterol and other plasma lipids were adsorbed on the initial precipitate of free cholesterol digitonide, and they were removed by the subsequent washing with organic solvents. The washings were saved for the later isolation of ester cholesterol. The cholesterol digitonide was dissociated in pyridine, the digitonin was precipitated with ether, and the recovered cholesterol was redissolved in 1:1 acetone-alcohol and was reprecipitated with three volumes of 0.4 per cent digitonin solution in 85 per cent ethanol. This second free cholesterol digitonide was washed three times with hot water, in order to remove excess digitonin, and then once with acetone-alcohol, once with acetone-ether, and three times with ether. After this last wash, the cholesterol digitonide was measured for radioactivity.

The solution of organic solvents obtained, as described above, by washing the original free digitonide precipitate was evaporated to dryness as the first step in isolating ester cholesterol digitonide. This residue was extracted five times with small volumes of boiling petroleum ether, (40° to 60° C.), in order to separate the ester cholesterol and plasma lipids from free cholesterol digitonide and excess digitonin. The petroleum ether extract was evaporated to dryness and the residue was saponified for ten minutes in a boiling water bath with 2 ml. of 2 N potassium hydroxide in 90 per cent ethanol. This solution was acidified with glacial acetic acid using phenolphthalein as an indicator, and 2 ml. of acetone was added in order to reconstitute a 1:1 acetone-alcohol solution. Twelve ml. of 0.4 per cent alcoholic digitonin was intro-

[4] Tracerlab Inc., Boston, Mass.

duced in this mixture, and the precipitate of ester cholesterol digitonide was isolated and washed as described above for the second precipitation of free cholesterol digitonide.

The cholesterol digitonide separated in this manner had constant composition and was free of any radioactive contaminant that would alter its specific activity. The free cholesterol digitonide had been dissociated and reprecipitated using alcoholic digitonin solution, in order that the ester and free cholesterol digitonides might have the same physical properties, since it had been observed that cholesterol digitonide precipitated from aqueous digitonin had somewhat different physical characteristics. This procedure for the isolation of free and ester cholesterol digitonide as well as the checks for the constancy of the digitonide composition and specific activity will be reported in detail elsewhere.

Radioactivity measurements

The measurements of the radioactivity of the carbon-14 content of the cholesterol digitonide samples were made in windowless flow-gas counters (Tracerlab, Inc.). A stainless steel planchet with a 1.63 $cm.^2$ sample area was used. The free and ester cholesterol digitonides were plated by transferring the precipitate obtained after the last washing onto the planchet in an acetone-alcohol slurry. This was air-dried until most of the solvent had evaporated and was then dried for one hour in an oven at 60° C. This procedure resulted in a uniform distribution of material on the planchets. The planchets were counted for a sufficient period of time to insure a probable counting error not greater than 3.5 per cent. The actual counts per minute, which were always at least three times greater than background, were corrected to infinite thickness and, by use of suitable calibration factors, were converted to the unit of "disintegrations per minute per millimole" (DPM per mM) of cholesterol. This unit was selected for convenience in comparing these and similar results with information to be reported in future publications concerning *in vivo* transformations of the steroid nucleus. The instruments and all steps in the radioactivity measurements were calibrated against a sodium carbonate-C^{14} standard obtained from the National Bureau of Standards.

RESULTS

Six patients have been studied in the manner described. Patient AC-9 was the subject of two separate studies. In four patients the free and ester cholesterol of plasma were isolated, while in two other subjects only the free cholesterol was obtained. The earliest blood samples taken were at about three hours, and thereafter samples were withdrawn at appropriate intervals for periods in excess of one month. The serial specific activities of free and ester cholesterol are listed in Table II. The data from a typical case (Patient AC-13) in-

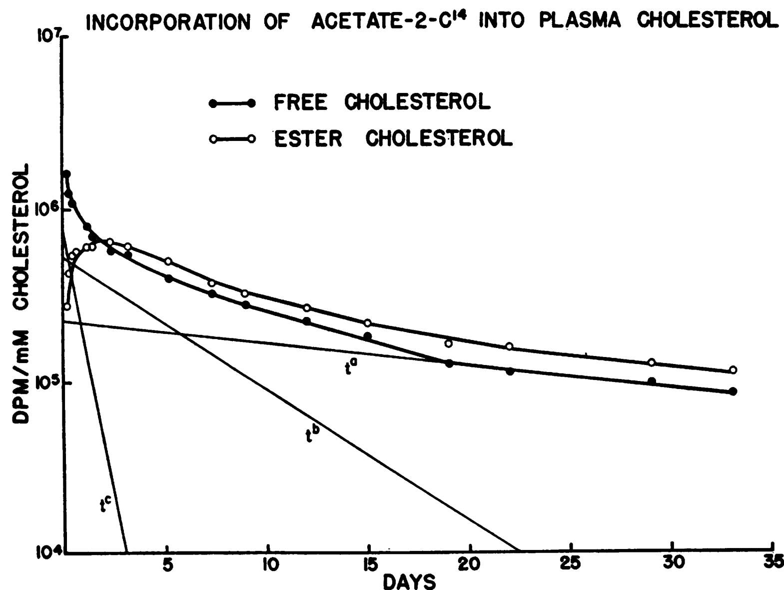

FIG. 1. INCORPORATION OF ACETATE-2-C^{14} INTO PLASMA CHOLESTEROL

Semi-logarithmic plot showing build-up and decline of radiocarbon activity in plasma free and ester cholesterol after oral administration of acetate-2-C^{14}. The lines marked t^a, t^b, and t^c represent the exponential rate processes by which the free cholesterol activity declines.

TABLE III

Percentage utilization of acetate for cholesterol synthesis and decay rates of plasma cholesterol

	% Dose/mMole cholesterol*			Total % dose† in plasma		Inter-section‡	Decay rates		
Patient	Free 0.3 day	Ester 0.3 day	Inter-section	0.3 day	Inter-section	Days	a $t_{1/2}$	b $t_{1/2}$	c $t_{1/2}$
AC-3	0.188	—	—	—	—	—	23.0	2.3	0.20
AC-4	0.163	—	—	—	—	—	15.5	1.6	0.30
AC-9a	0.081	0.017	0.042	0.38	0.42	2.2	23.0	2.3	0.45
AC-9b	0.101	0.035	0.050	0.58	0.50	2.3	—	—	—
AC-11	0.216	0.068	0.118	1.22	1.32	2.0	24.8	3.4	0.35
AC-13	0.166	0.056	0.089	1.03	1.09	2.0	23.0	4.0	0.47
AC-14	0.050	0.017	0.032	0.40	0.37	2.2	—	—	—

* Per cent administered radioactivity per millimole of plasma cholesterol.
† Per cent administered radioactivity present as cholesterol in total circulating plasma.
‡ Intersection = time at which free and ester specific activities are equal; *i.e.*, intersection of peak of ester curve with the free cholesterol curve (see Discussion).

dicating the changes in the specific activities of free and ester cholesterol with time are shown in a semi-logarithmic plot in Figure 1.

It will be seen in Figure 1, that the maximal value of the specific activity of free cholesterol had been reached by the time of the initial sample, and this was followed by a rapid decline. Blood samples were not withdrawn early enough after administering the acetate to follow the rapid increase in specific activity preceding the maximal value. The curves describing the decline of radioactivity of the free cholesterol can be resolved into a series of exponential rates, and these are listed in Table III. The ester specific activity is at its minimal value with the earliest sample of blood, taken at three to eight hours. It then rises to a peak, at about 2.1 days, at which point it intersects the specific activity curve of the free cholesterol. After this point of intersection, the ester cholesterol radioactivity declines at a similar rate to that of the free cholesterol, and the specific activity of the ester cholesterol continues to exceed slightly that of the free cholesterol for the duration of the period of observation.

At 0.3 day after the administration of acetate, a point closely approximating the maximum value of the specific activity of free cholesterol, from 0.050 to 0.216 per cent of the administered radioactive acetate had been incorporated per millimole of plasma free cholesterol. Corresponding values for plasma ester cholesterol at this point were lower, ranging from 0.017 to 0.068 per cent. At the crossover point of free and ester cholesterol (about two days) where both fractions possess the same specific activity, from 0.032 to 0.118 per cent of the administered dose was present per millimole of sterol. The data concerning the percentage conversion of acetate to cholesterol are summarized in Table III.

DISCUSSION

The specific activity of free cholesterol is at its peak with the earliest sample, since the administered labeled acetate is rapidly dissipated by many competitive metabolic processes, such as oxidation to carbon dioxide, conversion to fatty acids, etc., within one to two hours. Therefore, the maximal specific activity is attained within that period chiefly because the labeled precursor has disappeared as a result of metabolic degradation. This is borne out by the fact that the exhaled CO_2 reached a maximum specific activity two hours after administration of radioactive carbon-labeled acetate (6). Available data indicate that acetate is converted to an "active form," which is probably acetyl co-enzyme A, before it can enter any metabolic pathway (10). There is no unequivocal evidence concerning the subsequent steps in the transformation of acetate to cholesterol but four- and five-carbon condensation products derived from acetate (11, 12), as well as the triterpenoid compound squalene (13), have been implicated in this process. No detectable conversion of acetate to cholesterol occurs in plasma or whole blood (14).

Tissue slice studies have indicated that many organs are capable of transforming acetate into cholesterol (15). In intact animals, the liver (16)

is apparently the principal source of plasma cholesterol. The newly synthesized labeled sterol mixes with the pre-existing pool of unlabeled cholesterol present at site of synthesis in the liver, and this mixed sterol exchanges with plasma cholesterol, which in dogs (3) and in the human (17) is in rapid equilibrium with hepatic cholesterol. From the plasma the labeled cholesterol is distributed throughout the body.

Relation between free and ester cholesterol

The specific activity curves in Figure 1 show that the formation of labeled free cholesterol precedes that of the esterified form, and this finding can be interpreted as suggesting that ester cholesterol is synthesized from the labeled free sterol. The specific activity of ester cholesterol lags behind that of the free cholesterol by an interval related to the time required for the processes of esterification and equilibrium with the plasma. In Figure 1, the peak of the specific activity curve of ester cholesterol (product) intersects the specific activity curve of free cholesterol (precursor). This type of intersection of the two curves is characteristic of substances exhibiting a precursor-product relationship. A mathematical formulation of this relationship has been described (18).

Although the radioactivity of free and ester cholesterol declines at the same rate shortly after their intersection, the specific activities of these two forms of the sterol never again coincide. The finding that the specific activity of ester cholesterol is consistently higher than that of free cholesterol after the cross-over point may be interpreted as suggesting that there is no equilibrium between the two fractions; in other words, ester cholesterol once formed from the free sterol might not be reversibly hydrolyzed back to the free form.

However, since the parallel decline of the specific activities of free and ester cholesterol is compatible with the characteristics of a reversible esterification-hydrolysis relationship with a time lag between the two phases, another mechanism can be considered which would not exclude this reversible esterification-hydrolysis relationship between free and ester cholesterol. Figure 1 shows that it requires 2.0 days for the specific activity of ester cholesterol to come into equilibrium with that of the free cholesterol. This lag period is a function of the time required for the esterification of newly formed free cholesterol. Synthesis of cholesterol from unlabeled sources occurs continuously after dissipation of the labeled precursor, and this endogenously synthesized unlabeled sterol, as well as non-labeled cholesterol derived from the diet, first appears in the plasma in the free form, as demonstrated in this experiment for labeled cholesterol. Since unlabeled cholesterol first appearing in the free fraction continually dilutes the radioactivity of that fraction, the specific activity of free cholesterol could, at any time, be lower than that of ester cholesterol by virtue of the lag period required for esterification. This would occur in spite of the possible existence of a reversible hydrolysis-esterification relationship which could tend to produce identical specific activities in free and ester fractions. Therefore, reversible hydrolysis-esterification reactions would never cause the specific activities of free and ester cholesterol to approach identical values if they proceeded at slower rates than the dilution of free cholesterol by cholesterogenesis and intake from the diet.

From the present data, it is not possible to state whether the difference in specific activities of free and ester cholesterol implies the presence or absence of reversible equilibrium between the two fractions. If the reversible esterification-hydrolysis mechanism does exist, it is unlikely that this process occurs in the plasma, since human plasma esterase activity is known to be low (19, 20); should free and ester cholesterol proceed along independent metabolic routes, the parallelism of the specific activity curves as observed in this experiment can only be regarded as coincidental.

Turnover of plasma cholesterol

Turnover times[5] of 22 to 36 days have been determined for carcass cholesterol in mice (21) by the use of deuterium. Using the method of cholesterol synthesis from acetate, turnover times of 9 and 12 days for liver cholesterol have been reported for rats (22) and dogs (3), respectively. London and Rittenberg (23) have studied the incorporation of deuterium from heavy water into cholesterol in man, and by this method have calculated a turnover time of approximately 12 days for human plasma cholesterol. The rate data obtained

[5] Turnover time ($\overline{T}$) = Half-life ($t_{½}$) × 1.45.

in our laboratory essentially represent the decline of radioactivity in cholesterol that received its label only during the first few hours after the administration of the acetate-C^{14}. In the experiments in which cholesterol was synthesized from heavy water (23), the rate data typify the build-up or incorporation of the deuterium isotope throughout the entire period of observation. Inasmuch as these experimental methods differ in their approach as well as in type of precursors, a direct, simultaneous comparison of the two methods is necessary before comparison can be made of half-life [5] values obtained by each type of study.

In order to determine the half-life or turnover time of a substance, it must be shown that the material disappears by a single exponential rate process. There are no published experiments in which samples have been obtained over a sufficiently long period of time and at frequent enough intervals to make possible a detailed description of the decline in radioactivity. In most studies with small animals, because of technical difficulties, the frequency of sampling and length of observation are necessarily limited. Figure 1 and Table III indicate that the specific activity curves describing the disappearance of the radioactive cholesterol synthesized from acetate decline by a series of exponential processes rather than by one exponential rate which might be called the half-life of plasma cholesterol.[6] There are many processes, each with a specific rate, involved in the appearance and disappearance of labeled sterol from the plasma. These processes involve mechanical as well as biochemical transformations and include the equilibrium of labeled cholesterol at the site of synthesis with that of the plasma, the distribution of the labeled cholesterol from the plasma to the tissues, the return of labeled cholesterol from the tissues to the plasma, and finally the irreversible loss of labeled cholesterol either by conversion to other products or by excretion from the body. The continual addition of non-labeled cholesterol, synthesized in the body and obtained through the diet, serves to dilute and thereby lower the specific activity of the labeled cholesterol. The rates of all these processes as well as others unmentioned, are reflected in the specific activity curve of plasma cholesterol. Until additional information is available concerning the magnitude of the contribution of each of these processes to the composite series of rates observed in the specific activity curve, no definitive statement can be made about the turnover time or half-life of serum cholesterol studied in this manner. It might be assumed that the rapid rate of $t_{\frac{1}{2}}^{c} = 0.20$ to 0.47 days represents the conversion of acetate to cholesterol while the intermediate rate of $t_{\frac{1}{2}}^{b} = 1.6$ to 4.0 days accounts for the mixing and distribution of the newly synthesized labeled cholesterol and includes such processes as esterification and distribution of the labeled sterol from the plasma to the tissue. The dominant slow rate of $t_{\frac{1}{2}}^{a} = 15$ to 25 days might then describe the turnover of cholesterol after the initial rapid processes have been completed.

However, these assumptions as to the significance of exponential rates, derived from experiments such as this, require confirmation by other methods. In the curves obtained, the rate of decay or disappearance of cholesterol continually decreases with time. Therefore, in attempting to calculate the component rates in any such curve, it is necessary that the curves be of the same duration in order that these rates may be compared. If this precaution is disregarded, experiments carried out for prolonged periods of time will have final components of apparently longer half-life, as compared to short-term experiments where the final components will appear shorter. In the determination of the component rates in these studies, calculations were made from curves that extended approximately 30 days. Patients AC-9B and AC-14 were studied for 18 days and 22 days, respectively, (Table II); these times were not considered sufficient for a reasonable experimental determination of the long component ($t_{\frac{1}{2}}^{a}$) which could be compared with the other studies. The turnover of the tissue pools of cholesterol in the human is under investigation at present since it is likely that a portion of the cholesterol present in plasma represents sterol derived from these pools at rates different from the balance of the plasma cholesterol synthesized in the liver.

Even if it were possible to establish that one of the exponential components was related to the turnover of cholesterol, it should be emphasized

[6] The graphical determination of the component rate processes is recognized to be an approximate procedure; however, it is sufficiently reproducible to provide data which are useful in the interpretation of the curves.

that the cumulative effect of changes as small as five to ten per cent in that exponential rate, maintained for sufficiently long periods, would be adequate to account for elevated or reduced levels of plasma cholesterol or total body cholesterol. Detection of changes of this order of magnitude with the present method of study will continue to be difficult until more information is available about the over-all experimental error inherent in the procedure.

It can be seen from Table III that for the typical patient, AC-13, 0.166 per cent of the radioactivity originally administered was present in a millimole (386 mg.) of free cholesterol in the plasma after 0.3 days. The corresponding figure for the ester cholesterol at this time was 0.056 per cent. The per cent of the administered dose present as cholesterol in the *total* circulating plasma at 0.3 day may then be calculated to be 1.03 per cent from the plasma free and ester cholesterol levels, weight of the subject (Table I) and the accepted value of 45 ml. of plasma per kilogram of body weight. At the point of intersection of the free and ester cholesterol curves (2.0 days), 0.089 per cent of the administered radioactivity was circulating per millimole of plasma cholesterol, and thus in the total circulating plasma 1.09 per cent of the C^{14} of acetate was present in plasma cholesterol. The values for the percentage of administered radioactivity present in total circulating plasma at 0.3 days and at 2.0 days, 1.03 and 1.09, respectively, are in good agreement. The data for the other patients in Table III reveal the same close correlation between corresponding values as well as a constant time at which free and ester cholesterol possess the same specific activities.

Thus at the time when the specific activities of both free and ester cholesterol of plasma are identical (intersection), the per cent of administered radioactivity present as plasma cholesterol has not appreciably changed from that measured 0.3 day after administration of labeled acetate. This strongly implies that the incorporation of a single dose of acetate into cholesterol is complete after 0.3 day and that the radioactivity that subsequently appears in the ester fraction is derived from the free sterol. This observation is additional evidence for the role of plasma free cholesterol as the precursor of the ester cholesterol fraction.

Since it is known from other data that liver and red cell cholesterol are in isotopic equilibrium with plasma cholesterol (5, 6), a calculation can be made of the amount of activity incorporated as cholesterol in these organs. There are approximately 3 Gm. of free cholesterol present in red cells, 3.8 Gm. of free cholesterol, and 0.7 Gm. of ester cholesterol contained in liver. Using these quantities, approximately three per cent of the activity administered as acetate has been incorporated as cholesterol in plasma, red cells, and liver. This figure represents a minimum value for cholesterol synthesis since there are undoubtedly pools other than those mentioned.

As noted in Table III, 0.050 to 0.216 per cent of the administered acetate had been incorporated per millimole of circulating cholesterol after 0.3 day. Taking into account the differences in dose, the amount of C^{14} incorporated varies as much as fourfold from patient to patient. This may be explained by the fact that cholesterol produced in the body from acetate represents a very small percentage of the two carbon precursor; the major portion of the acetate is metabolized, excreted, or utilized in the biosynthesis of other body constituents. Therefore, the amount of radioactive acetate incorporated into cholesterol depends on the quantity of the administered dose that escapes utilization by metabolic processes other than cholesterogenesis. Since these transformations consume by far the overwhelming majority of the administered acetate, cholesterol synthesis may vary widely, depending on the amount of radioactive acetate metabolized by all the other processes.

Finally, it should be emphasized that none of the subjects in this study were in normal health and that they represented a wide variety of disease processes other than disturbances in cholesterol metabolism. It is not known whether the data reported would necessarily apply to normal subjects. However, in spite of their disease conditions, the data concerning the behavior of cholesterol are constant for all the patients. This suggests that cholesterol metabolism was not disturbed by the particular disease present in any patient, and that the pattern observed in these patients for cholesterol synthesis from acetate might well apply to other diseased, as well as normal, subjects.

CONCLUSIONS

1. Plasma cholesterol synthesized from radioactive carbon-labeled acetate has been studied in six patients with malignant disease.

2. A method for the isolation of plasma free and ester cholesterol has been described.

3. A minimum of 3 per cent of the administered acetate was incorporated into the body cholesterol.

4. Plasma free cholesterol is at its highest specific activity within eight hours after administration of the tracer dose, and then declines. The plasma ester cholesterol radioactivity rises from a minimal value to a peak in two days and then declines at a rate similar to that of free cholesterol. After the peak, the specific activity of ester cholesterol exceeds that of free cholesterol.

5. Because many distribution and metabolic reactions of cholesterol are reflected in the disappearance curve derived from the specific activity values, no statement can be made about the turnover of plasma cholesterol from data derived from this type of study until additional information is available.

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