

ON THE UTILIZATION OF ACETIC ACID FOR CHOLESTEROL FORMATION*

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The information at present available as to the metabolism of cholesterol and its relationship to the other biologically important sterols is meager. Balance experiments (1) have demonstrated that cholesterol can be synthesized and destroyed in the animal but have given no convincing evidence as to the nature of specific precursors of cholesterol.

In general, balance experiments fail to differentiate between two alternatives, direct utilization of the dietary component or stimulation of a specific process of metabolism. It cannot, for example, be decided whether the increase of cholesterol observed in some experiments after the feeding of fatty substances is the result of conversion of these substances to cholesterol or whether it merely reflects an increased metabolic activity of the organism. An experiment (2) on the rate of formation of cholesterol, carried out with the aid of deuterium, indicated that about half of all the hydrogen atoms of the newly formed cholesterol was derived from the hydrogen of water; the other half must have originated from the carbon-bound hydrogen atoms of some dietary constituent. This evidence eliminated large molecules as immediate precursors, and suggested that "cholesterol... is formed by the coupling of smaller molecules, possibly those which have been postulated to be intermediates in the fat and carbohydrate metabolism" (2).

The recent observation (3) that the ingestion of deuterio acetic acid, CD_3COOH , leads to the formation of deuterio cholesterol supports this view. On the other hand, deuterio cholesterol is not formed after α,β -dideuterio propionic acid and deuterio succinic acid are fed. α,β -Dideuterio butyric acid and β,γ -dideuterio butyric acid were only slightly effective. The deuterium concentrations in the total cholesterol of the experimental animals after the feeding of deuterio acetate for 8 days are given in Table I. Positive evidence for the utilization of acetate for cholesterol formation was found for mice, growing rats, and adult rats. In each case the concentration of deuterium in the cholesterol was over 3 times that in the body water. The figures in the seventh column give the proportion

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of all the hydrogen in the cholesterol which was derived from the dietary acetate during the experimental period. These values must of necessity be small, if only for the reason that the rate of synthesis of cholesterol is slow.

In considering possible steps in the transformation of acetate to sterol, a mechanism must be postulated in which the acetate molecules are reductively coupled to form larger units. Intermediates containing only labile hydrogen, *i.e.* readily exchangeable hydrogen, can be ruled out. For example, a pathway via oxalacetic acid cannot be considered, as it would result in the elimination of deuterium by enolization. We have tested some compounds into which acetic acid is known to be converted *in vivo* in order to determine whether any of these reactions is involved in sterol synthesis.

TABLE I
Feeding of Deuterio Acetic Acid

Experiment	Animals used	Deuterio acetic acid fed per day per 100 gm. body weight	Deuterium in			Hydrogen in cholesterol derived from acetate	Deuterium in total fatty acids
			Sodium acetate	Body water	Isolated cholesterol		
		mg.	atom per cent excess	atom per cent excess	atom per cent excess	per cent	atom per cent excess
A	Adult mice	372	9.9	0.04	0.13	1.3	0.02
B	Young rats	82	68.0	0.08	0.27	0.4	0.04
C	Adult "	97	27.6	0.05	0.21*	0.8	

* In this experiment the cholesterol was converted to cholesteryl chloride, which was analyzed.

Yeast dehydrogenases rapidly convert acetate to succinate (4). As the results obtained on feeding deuterio succinate were negative, the pathway from acetate to cholesterol does not go through succinic acid.

A conceivable explanation for the failure of succinate to generate deuterio cholesterol might be that the succinic-fumaric-malic equilibrium causes a rapid loss of deuterium to the body fluids and therefore renders detection of cholesterol formation impossible. However, this cannot be the reason. Assuming that acetate were converted to succinate and that the latter lost deuterium by exchange, then deuterio acetate should not be more effective in forming deuterio cholesterol than deuterio succinate. Succinate does not represent an intermediate step.

Little is known of acetate metabolism in normal animals. It has apparently been believed, but not proved, that acetic acid is directly burned to carbon dioxide and water at a rapid rate. Under certain conditions, such as in fasting animals (5) or in liver slices (6), formation of aceto-

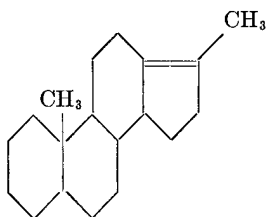
acetic acid and β -hydroxybutyric acid from acetic acid has been demonstrated and ascribed to condensation of acetic acid with either pyruvic acid (7) or another molecule of acetic acid (8).

Morehouse (9) has isolated deuterio β -hydroxybutyric acid from the urine of fasting rats which had been given β,γ -dideuterio butyric acid, but found normal β -hydroxybutyric acid after α,β -dideuterio butyric acid was fed. This demonstrates that, while the α - and β -hydrogen atoms are rapidly replaced, the γ -hydrogen atoms are relatively stable. β,γ -Dideuterio butyric acid can be expected to give rise to deuterio acetoacetic acid in normal animals also. When its sodium salt was fed to rats, the isotopic content of the cholesterol was only slightly higher than that of the body fluids, in contrast to a comparable feeding of deuterio acetate, when the cholesterol formed contained 3 to 4 times more deuterium than the surrounding body water. In view of the known relationship of butyric, acetoacetic, and β -hydroxybutyric acids these compounds may be ruled out as intermediates in sterol formation from acetic acid.

In another experiment our animals received a corresponding amount of α,β -dideuterio butyric acid. This compound was expected to exchange its deuterium by enolization after being oxidized to acetoacetic acid, and the cholesterol isolated should not contain significant concentrations of deuterium. The concentration of deuterium in the isolated cholesterol was slightly higher than that of the body fluids.

Propionic acid was tested as a possible cholesterol precursor, because of its close biological relationship to pyruvic acid. Sodium α,β -dideuterio propionate was fed to rats as a source of deuterio pyruvic acid, but failed to produce a cholesterol with a significant deuterium content. If this failure were due to removal of deuterium by enolization, the same loss of isotope should occur with acetate if it were converted to cholesterol via pyruvate. Our experimental results indicate that propionic acid and presumably pyruvic acid are not intermediates in the synthesis of cholesterol from acetate.

We have attempted, by chemical degradation of the deuterio cholesterol, to obtain some more detailed information on the mechanism of sterol synthesis. Unfortunately the yields of cholesterol fragments which could be obtained by oxidative breakdown of the available deuterio cholesterol samples would be too small to permit deuterium analysis. The only reaction found to be suitable for our purpose was the thermal degradation of cholesterol chloride described by Mauthner (10). When this compound is heated to about 300° in a nitrogen atmosphere, hydrochloric acid is eliminated; at about 400° fission occurs between carbon atoms 17 and 20, yielding isooctane, isooctene, and a high boiling oil to which the structure shown in the accompanying formula has been assigned by Bergmann and Bergmann (11).



By converting the deuterio cholesterol isolated from our animals into cholesteryl chloride and subjecting the latter to the thermal degradation, we have obtained two fractions, representing the side chain and the sterol nucleus respectively. The concentrations of deuterium in the isooctane-isooctene mixture and in the hydrocarbon $C_{19}H_{30}$, which may be regarded as the primary degradation products, are in good agreement with that in the cholesteryl chloride. The occurrence of side reactions leading to a loss of fractions with high deuterium concentrations is therefore unlikely. Small errors in the calculation may arise from two sources. The hydrochloric acid split off in the initial phase of the degradation was not analyzed. However, unless the deuterium concentration at carbon atom 2 or 4 of the sterol nucleus was very much higher than that at other carbon atoms the analytical figure for the nucleus could not have been influenced profoundly. Secondly, the high boiling hydrocarbon $C_{19}H_{30}$ contains only one double bond instead of the two or three expected. The drastic treatment of the cholesteryl chloride evidently involves hydrogen shifts, which, however, should not greatly change the isotopic concentration of the fragments unless the deuterium concentration at any particular carbon is of a different order of magnitude from that at others.

From the analytical values obtained for the two breakdown products, it becomes evident that acetate had been utilized for the biosynthesis of the side chain as well as the steroid nucleus. Indeed the deuterium concentration found in the side chain exceeds that of the nucleus by approximately 50 per cent. The difference may arise from the fact that the methyl groups of the cholesterol at positions 18, 19, 21, 26, and 27 may originate directly from the acetic acid with the retention of all 3 hydrogen atoms, while the other carbon atoms probably retain only 1 or 2 of the original hydrogen atoms. Since the methyl groups account for 50 per cent of all the hydrogen in the side chain, and for only 20 per cent of the total hydrogen in the nucleus, more of the deuterium could be expected to be incorporated into the side chain.¹

¹ The foregoing considerations ignore the possibility that intramolecular hydrogen shift had occurred between the side chain and the nucleus during the pyrolysis. This, however, cannot be excluded at present. As the deuterium content of the isooctane is found to be higher than that of the ring hydrocarbon, the isotope must

EXPERIMENTAL

Preparation of Deuterio Compounds

Deuterio Acetic Acid—Deuterio malonic acid was prepared by exchange with heavy water and decarboxylated to deuterio acetic acid (12). For feeding experiments the sodium salt was used.

α,β -Dideuterio propionic acid was prepared from methyl acrylate as previously described (13). The sodium salt of the acid contained 34.4 atom per cent excess deuterium.

α,β -Dideuterio butyric acid was prepared by hydrogenating ethyl crotonate with deuterium gas. The sodium butyrate contained 23.1 atom per cent excess deuterium.

β,γ -Dideuterio butyric acid was similarly prepared from vinylacetic acid. The sodium salt of the β,γ -dideuterio butyric acid contained 16.0 atom per cent excess deuterium. As the β,γ double bond in vinylacetic acid is known to shift easily towards the α,β position (14), it was felt necessary to prove that the isotope was actually located at the β - and γ -carbon atoms. To 0.398 gm. of the sodium butyrate (3.57 mm) in water, 10.34 gm. (117.5 mm) of ordinary butyric acid and an excess of sulfuric acid were added. The butyric acid mixture was extracted from the aqueous solution by ether and the ether dried and distilled off. The butyric acid thus obtained was brominated according to the method of Fischer (15). The α -bromobutyric acid obtained distilled at 120–122°, 19 mm. It contained 0.49 atom per cent excess deuterium or 16.6 per cent when calculated for the undiluted acid. This compares with a value of 16.0 per cent for the deuterium analysis of the sodium β,γ -dideuterio butyrate. As the sodium butyrate and the α -bromobutyric acid both contain 7 hydrogen atoms, the agreement between the two values demonstrates that no deuterium had been lost as the result of the bromination; *i.e.*, no shift of double bonds from the β,γ to the α,β positions had occurred during the catalytic hydrogenation of vinylacetic acid.

Isolation of Cholesterol—Cholesterol was obtained in the usual manner from the unsaponifiable fractions of the total pooled carcasses. The samples were converted into the dibromides, debrominated (16), and recrystallized. The melting points of the purified cholesterol samples ranged from 147–149°.

Cholesterol Degradation—1.3 gm. of crude sterol obtained from Experiment C, Table I, were converted into cholesteryl chloride by dissolving in

have been present in the cholesterol side chain prior to the degradation. The same, however, does not hold necessarily for the sterol nucleus. The amounts of deuterio cholesterol available at present do not permit as yet the carrying out of reactions from which the deuterium content at individual carbon atoms could be determined.

chloroform and adding 0.6 gm. of freshly distilled thionyl chloride. The mixture was refluxed for 1 hour, and the solvent and excess reagent were distilled off. The dark brown residue failed to crystallize. Purification was achieved by passing a petroleum ether solution of the crude product through a column of activated alumina. The colorless petroleum ether filtrate was evaporated and the residue crystallized from a small volume of acetone. There was obtained 0.75 gm. of cholesteryl chloride, m.p. 94–95°, containing 0.21 atom per cent excess deuterium.

$C_{27}H_{46}Cl$. Calculated, C 80.1, H 11.1; found, C 79.7, H 11.4

For the thermal degradation, 0.65 gm. of the deuterio cholesteryl chloride, diluted with an equal amount of non-isotopic cholesteryl chloride, was slowly heated to 300° in an atmosphere of nitrogen. Hydrochloric acid was evolved. The temperature was kept constant until the HCl evolution ceased (4 hours). The bath temperature was then slowly raised to about 400°. At this temperature a volatile product distilled over very slowly and was collected in a trap cooled by dry ice. About 250 mg. of a mobile distillate were obtained; it was redistilled into a second trap. The liquid was presumed to be a mixture of isooctane and isooctene. Its boiling range was 115–120°; *i.e.*, roughly the same range as that observed by Bergmann and Bergmann (11). For deuterium analysis the liquid was volatilized directly into the combustion furnace by a slow oxygen stream. The resulting water contained 0.128 atom per cent excess deuterium. Since the deuterio cholesteryl chloride had been diluted 1:1, the actual value for the isooctane-isooctene mixture is 0.256 atom per cent excess deuterium.

The residue which remained after the pyrolysis was heated over a free flame, and a viscous yellow oil distilled over between 380–400°. An attempt to obtain a crystalline hydrocarbon from this fraction was unsuccessful. 0.50 gm. of this high boiling oil was dissolved in petroleum ether and the solution passed through a column of activated alumina. Only 10 per cent of the fraction was adsorbed. The combined filtrate and petroleum ether washings were free of pigments. The colorless oil remaining after removal of the solvent had the following composition.

$C_{19}H_{30}$. Calculated, C 88.4, H 11.6; found, C 88.3, H 11.7
 $[\alpha]_D^{25} = +30.7^\circ$ (2% in benzene)

Bergmann and Bergmann reported C 87.9, H 11.9, $[\alpha]_D = +31.4^\circ$. The hydrocarbon contained 0.089 atom per cent excess deuterium, or 0.178 per cent calculated for the undiluted starting material.

On the basis of data obtained by Bergmann and Bergmann, we assume that the composition of the volatile hydrocarbon corresponds closely to that of isooctane, C_8H_{18} . The average deuterium content of the hydrogen

in a compound composed of the fragments C_8H_{18} and $C_{19}H_{30}$, as calculated from their isotope content, would be $(18 \times 0.26 + 30 \times 0.18)/48 = 0.21$ per cent. This compares well with the value 0.21 per cent found for the deuterium content of the hydrogen in the cholesteryl chloride which had been used as a starting material.

The catalytic hydrogenation of the hydrocarbon $C_{19}H_{30}$ with platinum in acetic acid consumed only one-third of the amount of hydrogen required for one double bond. The resistance to hydrogenation is in agreement with the view of Bergmann and Bergmann that the double bond in the hydrocarbon $C_{19}H_{30}$ is located at quaternary carbon atoms.

Animal Experiments

Feeding of Deuterio Acetic Acid. Experiment A—Seven adult mice received the following diet (Stock Diet I): 50 per cent casein, 20 per cent Wesson oil, 14 per cent salt mixture (17), 16 per cent yeast. The high salt content of the diet resulted in a large water consumption and urine excretion, thereby favoring the "washing out" of heavy water from the body fluids. In addition to the stock diet, each mouse received 110 mg. of sodium deuterio acetate (9.9 atom per cent excess deuterium) per day for 8 days. At the end of the experimental period, each mouse had gained an average of 4 gm. The animals were killed, body water was distilled from the tissues, and total fatty acids and cholesterol were isolated from the animal carcasses after removal of the gastrointestinal tracts.

Experiment B—Two growing rats, weighing 102 and 110 gm. respectively, were given the carbohydrate-free Stock Diet I and in addition 137 mg. of sodium deuterio acetate (68 atom per cent excess deuterium) per animal per day over a period of 8 days. Each animal gained about 30 gm. during the feeding period.

Experiment C—Four adult rats having a combined weight of 1113 gm. received the following Stock Diet II: 86 per cent casein, 3 per cent Wesson oil, 5 per cent yeast, 5 per cent salt mixture (17), 1 per cent cod liver oil. During the 8 day feeding period, each animal received 400 mg. of sodium deuterio acetate (27.6 atom per cent excess deuterium) per day. At the end of the experimental period, the combined weight of the animals was 1196 gm. A total of 1.4 gm. of crude cholesterol was obtained from the animal carcasses.

The results of the deuterium analysis of body water, fatty acids, and cholesterol are given in Table I. The data suggest that variation of dietary composition has had little effect on the formation of deuterio cholesterol. During the experimental period, the feces were collected from the animals of Experiment C, Table I. They were ground up with anhydrous sodium sulfate, extracted with ether and acetone, and the sterols precipitated from

the unsaponifiable fraction by digitonin. If we assume that the fecal sterols were derived from the body sterols and that the deuterium concentration in the body sterols increased linearly during the experiment, then the deuterium concentration of the total fecal sterol should be half of that of the body cholesterol at the end of the experiment. The fecal sterols recovered from the digitonides contained 0.110 atom per cent excess deuterium, as compared to 0.21 atom per cent excess in the body cholesterol.

Feeding of Deuterio Succinic Acid—Nine adult mice received the carbohydrate-free Stock Diet I and a daily addition per mouse of 110 mg. of deuterio succinic acid,² containing 26.9 atom per cent excess deuterium. During the experimental period the combined weight of the mice increased from 180 to 215 gm. Body water and cholesterol from the combined carcasses contained 0.13 and 0.07 atom per cent excess deuterium respectively.

TABLE II

Feeding of Deuterio Fatty Acids to Adult Rats

1.6 mm of the sodium salt of deuterio fatty acid were fed per day per 100 gm. of body weight in each case.

Compound fed (sodium salts)	Deuterium in com- pound fed	Deuterium in		
		Body water	Cholesterol	Fatty acids
	atom per cent excess	atom per cent excess	atom per cent excess	atom per cent excess
Acetic acid.....	27.6	0.05	0.21	
α,β -Dideuterio propionic acid.....	34.4	0.05	0.02	0.01
“ butyric acid.....	23.1	0.03	0.04	
β,γ -Dideuterio “ “.....	16.0	0.05	0.07	0.02

Feeding of Sodium α,β -Dideuterio Propionate—Two rats having a combined weight of 297 gm. received the carbohydrate-free, low fat Stock Diet II. During the experimental period of 8 days, each animal received a daily addition of 231 mg. of sodium deuterio propionate (34.4 atom per cent excess deuterium). On the 3rd day of feeding, the rats developed a slight diarrhea, which disappeared when some bone meal was added to the diet. At the end of the experimental period, the two animals had a total weight of 310 gm. The analytical data are given in Table II.

Feeding of Sodium α,β -Dideuterio Butyrate—Two rats, having a total weight of 294 gm., were given Stock Diet II and in addition 265 mg. of sodium butyrate (23.1 atom per cent excess deuterium) per animal per day. No change of weight occurred during the 8 day feeding period. The deuterium analyses for body water and cholesterol are given in Table II.

² The deuterio succinic acid was kindly supplied by Dr. Abraham Mazur. It had been prepared from diethyl fumarate by catalytic hydrogenation with deuterium gas.

Feeding of Sodium β,γ -Dideuterio Butyrate—Two rats received Stock Diet II and in addition 265 mg. of sodium butyrate (16.0 atom per cent excess deuterium) daily per rat over a period of 8 days. The combined weight of the animals increased from 291 to 329 gm. The analytical results are presented in Table II.

DISCUSSION

In all experiments the test substance was supplied with the diet for 8 days. During this period the animals were slowly synthesizing and destroying cholesterol. The rate of these reactions for the adult mouse is about 2.5 per cent per day (2); so that at the end of the 8 day period the total cholesterol, *i.e.* the cholesterol actually analyzed, should represent a mixture of about 1 part of newly formed and 4 parts of preexisting cholesterol. The rate of cholesterol synthesis in the rat has not been determined but may be assumed to be of similar magnitude. We know from the experiments on the deuterium content of cholesterol of mice whose body fluids contain D₂O that the maximum concentration of deuterium in their cholesterol is about half of that of the body fluids. A level of about one-tenth is reached in 8 days. Whenever in the present experiments the concentration of deuterium in the total cholesterol is appreciably over one-tenth that of the body fluids, there is presumptive evidence for a synthesis utilizing hydrogen from a deuterio dietary constituent and not from water alone.³ The appearance of cholesterol with a concentration greater than half that of the body fluids is conclusive evidence for the utilization of the isotopic test substance in the synthesis of cholesterol, since no assumption need then be made as to the rate of this reaction. The results of the three acetic acid experiments satisfy both criteria for conversion of acetate to cholesterol. In each case the deuterium concentration is over 30 times that which could be expected to enter the cholesterol from the body fluids.

Our experimental results demonstrate clearly that the cyclopentano-perhydrophenanthrene structure can be synthesized from C₂ units. A condensation of numerous small molecules with the intermediate uptake and elimination of the elements of water, with or without reduction of double bonds, is therefore suggested. From our data the exact nature of the unit undergoing condensation cannot be stated. It seems unlikely to be the relatively inert acetic acid itself, but rather a closely related inter-

³ The deuterium concentration of the body fluids was zero at the start of the experiment and at a maximum, given in Tables I and II, at the end. It may be assumed that the major part of the acetic acid was oxidized to water and carbon dioxide. From the amounts of deuterium administered and the fluid intake of the animals it can then be calculated that the deuterium concentration in the body fluids rose for about 3 days and thereafter remained relatively constant.

mediate such as acetaldehyde,⁴ into which acetic acid might readily be converted by the organism.

The available data are not sufficient to enable one to decide whether acetic acid (or a related intermediate) forms the sole precursor for cholesterol, because it is not known to what extent the dietary deuterio acetate was diluted by acetic acid formed in the organism. At present, even the occurrence of acetic acid as such in intermediary metabolism is not definitely established. However, the animal organism is undoubtedly capable of metabolizing acetic acid. The following discussion therefore refers not only to acetic acid itself but also to closely related compounds into which acetic acid might be converted by the animal.

The minimum fraction of cholesterol derived from dietary acetate can be estimated. The deuterium concentration in the cholesterol isolated from Experiment A (Table I) amounted to 1.3 per cent of that of the administered sodium acetate. Since the cholesterol analyzed represented a mixture containing approximately one-fifth of the cholesterol newly formed during the experimental period, the percentage of hydrogen derived from acetate during this period must have been $5 \times 1.3 = 6.5$ per cent. Furthermore, we know from previous findings that one-half of the total hydrogen atoms in cholesterol had, during its synthesis, originated from the body fluids. The body water in this experiment contained only 0.04 per cent deuterium. Since the deuterium concentration of the hydrogen derived from the body fluids could not have had a measurable value (<0.005 atom per cent excess), the deuterium concentration of the cholesterol hydrogen derived from acetate must have been twice as high as 6.5 per cent. We may, therefore, conclude that a minimum of 13 per cent of all the hydrogen in the newly formed cholesterol derived from organic molecules had originated in the dietary deuterio acetic acid.

Data on acetic acid production in the rat are not available. Bernhard (19), working on sulfanilamide acetylation, observed an approximately 10-fold dilution of deuterio acetate in rabbits. It may be inferred that, if acetic acid is formed at comparable rates in the rat and in the rabbit, the deuterio acetate or related compound would have been diluted several times by the ordinary analogue before being used for cholesterol synthesis. On this basis, a substantially higher fraction than the calculated 13 per cent of the cholesterol would have been derived from acetic acid. If acetic acid is the sole precursor of cholesterol, a large daily synthesis of acetic acid to dilute the dietary acetic acid must be postulated.

⁴ MacLean and Hoffert (18) in their study on the formation of fat and sterols by aerated yeast observed an inhibition of sterol synthesis by sulfite, whereas it had no effect on fat formation. They account for the inhibition by suggesting that an aldehyde is an active metabolite in sterol synthesis.

The deuterium concentrations in cholesterol after both α,β - and β,γ -deuterio butyrate are fed are slightly higher than that of the body fluids; the deuterium of the cholesterol, therefore, cannot have been derived only from the body fluids. The results may be due to hydrolysis of acetoacetate to acetic acid, although the actual value indicates that under our experimental conditions hydrolysis of acetoacetic acid could not have been its main metabolic pathway.

The concentration of deuterium in the cholesterol of animals fed succinate seems too high to have been derived only from the body fluids. A slight conversion to acetic acid may have occurred. The essentially negative results obtained with propionic, succinic, and the butyric acids indicate that the conversion of acetate to cholesterol is a specific reaction scarcely shared by the related lower fatty acids.

The observation that in animals which had formed deuterio cholesterol from deuterio acetate the fatty acids had not taken up stably bound deuterium makes it seem unlikely that fatty acids were utilized. These results do not exclude the possibility that the carbon atoms of acetic acid are utilized for fatty acid formation. The mechanisms for these two syntheses, however, must be fundamentally different.⁴

In this connection it is of interest to consider the data reported by Sonderhoff and Thomas (20) on sterol formation by oxygenated yeast with deuterio acetate as the sole nutrient. Under their experimental conditions the unsaponifiable fraction of yeast contained roughly one-third as high a deuterium concentration as the deuterio acetate used. The acetate hydrogen did not exchange in the yeast culture and acetic acid was not formed by the cells, as the acetate recovered from the culture contained an unchanged isotope concentration. The deuterium content of the sterols was twice as high as that of the yeast fats and 20 times higher than that of the yeast carbohydrates. These authors interpret their data to indicate a rather direct utilization of acetate molecules for sterol synthesis.

A mechanism involving the condensation of a great number of acetic acid or related molecules accompanied by reduction of double bonds and uptake of hydrogen from the surrounding fluids would be in agreement with the results of Sonderhoff and Thomas for yeast as well as ours for the animal tissues. The preformed chains of the higher fatty acids, such as palmitic, stearic, or the corresponding unsaturated acids, have frequently been discussed as probable building stones for the sterol molecule (21). The evidence secured from the study of fat metabolism with the aid of deuterium points strongly against a participation in sterol synthesis of the higher fatty acids *per se*. Fecal sterols of rats which had received for 8 days an addition of deuterio palmitate to a sterol-free diet did not contain an excess of isotope (22). As the excreted sterols must have been derived from cholest-

terol synthesized in the animal, the fatty acid as such could not have served as a precursor. The possibility that fatty acids are the source of acetic acid and thereby supply carbon and hydrogen for cholesterol synthesis still would not permit us to regard them as precursors in the accepted sense.

SUMMARY

1. The feeding of sodium deuterio acetate to mice and rats leads to the formation of deuterio cholesterol. By degradation of the sterol isolated from the animals, isotope was shown to be present in both the side chain and the nucleus of the cholesterol molecule.

2. A minimum of 13 per cent of the hydrogen atoms of cholesterol was derived from the acetate ion. The actual value must be higher, as the dietary acetate must have been diluted either by endogenous acetate or a closely related derivative into which the acetic acid is converted by the organism prior to utilization for sterol synthesis.

3. The experimental results exclude propionic, butyric, and succinic acids directly, and pyruvic and acetoacetic acids indirectly, as intermediates in the acetate-sterol conversion.

4. The absence of deuterium in the fatty acids of animals fed deuterio acetate is additional support for the previously expressed view that fatty acids are not directly involved in cholesterol synthesis.

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