The true cholinesterase of the nervous system of the rat has been shown to recover quickly at first, thereafter only slowly. It is particularly interesting that the true cholinesterase of brain shows a similar recovery pattern *in vivo* and *in vitro*, for it would be expected that recovery would be exponential. There seem to be two possible explanations of the findings. It may be that part of the inhibited enzyme (about 40%) is removed as a foreign protein, and that all the remaining inhibited enzyme has recovered its activity after 4 days. The further slow rise in activity would then be due to enzyme resynthesis. Since the recovery rate is the same *in vitro* one must assume that the inhibited enzyme is also removed *in vitro*. A second possibility is that two enzymes are present in the true cholinesterase, one (60% of the whole) which recovers rapidly and a second enzyme whose rate of recovery is slow. In view of the demonstration of the difference between the pseudo cholinesterase of brain and spinal cord and that of other tissues this must be considered.

SUMMARY

1. After inhibition of rat pseudo cholinesterase of heart, serum, jejunum and diaphragm by diethyl *p*-nitrophenyl phosphate (E 600) the total recovery of enzyme activity occurs in about a day both *in vivo* and *in vitro*.

2. A difference in recovery of activity (*in vitro* and *in vivo*) between the pseudo cholinesterase of brain and the other pseudo cholinesterases examined has been shown.

3. The recovery of true cholinesterase after inhibition with E 600 occurs slowly (60% in 4 days) both *in vivo* and *in vitro*.

My thanks are due to Dr W. N. Aldridge for advice and to Miss J. I. Wheatley for technical assistance.

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Studies of Cholesterol Biosynthesis

1. A NEW CHEMICAL DEGRADATION OF CHOLESTEROL

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The study of cholesterol biosynthesis from precursors labelled with isotopic carbon would be greatly assisted if methods were available for isolating each of the twenty-seven carbon atoms of the sterol in a form suitable for isotopic assay. The constituent atoms in the side chain can be separated (Wüersch, Huang & Bloch, 1952) by adapting known procedures originally designed to determine the structure of cholesterol, but known degradations of the alicyclic ring structure do not easily lend themselves to the separation of individual atoms. New procedures were therefore devised; the first of these, aimed at rings *A* and *B*, has been worked out in detail.

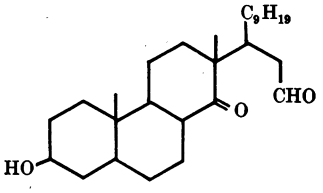
Our point of departure was the observation of Achtermann (1934) and of Laucht (1935) that the keto aldehyde (I), from ergosterol, on pyrolysis gives the ketone (II) and the $\alpha\beta$-unsaturated

aldehyde (III). The process may be viewed as the reversal of a Michael addition reaction (II + III = I).

This suggested that if ring *B* of cholesterol were opened by oxidation, further manipulation might afford the keto aldehyde (IV), which on pyrolysis should give ring *A* as 2-methyl*cyclo*hexanone (V). Separation of the seven carbon atoms in 2-methyl-*cyclo*hexanone could then be undertaken.

and its 2:4-dinitrophenylhydrazone ($\lambda_{max.} = 363 m\mu.$; $\epsilon = 18\,300$) showed the expected light absorption.

A smooth dehydration of the aldehyde to the Δ^3-unsaturated ketone could not be effected. Ethanolic hydrogen chloride gave a complex mixture, and ethanolic sulphuric acid led by a double dehydration to 6-formyl-*B*-norcholesta-3:5-diene (VIII), isolated by chromatography as a viscous yellow oil. The



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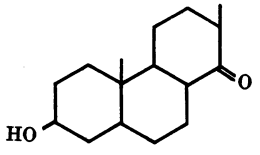

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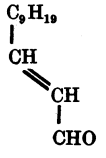

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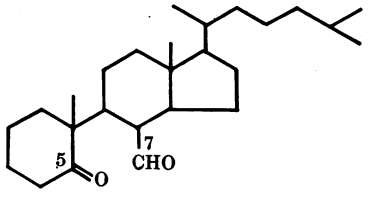

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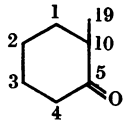


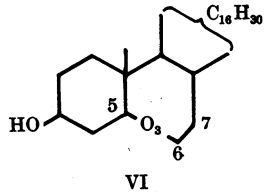
V

The steroid nomenclature used throughout this paper follows the recommendations given in the report of the 1951 Ciba Foundation Conference (*J. chem. Soc.* 1951, p. 3526). It is to be noted that, after removal of C-6, the original C-7 becomes C-6 in the names of the *B*-nor derivatives.

METHODS AND DISCUSSION

The ozonolysis of cholesterol was first studied. Cholesterol ozonide (VI) was originally made by Harries (1912), and optimum conditions for its formation were examined by Berenstein, Georg & Briner (1946). The latter authors attempted, with small success, to reduce the ozonide; however, they did not try the action of zinc dust in cold acetic acid, which we find to give an almost quantitative yield of 5:6-*seco*cholestan-6-al-3-ol-5-one (VII; R = OH). This crystallized with a firmly bound molecule of ether; it was characterized as a 3:5-dinitrobenzoate and as a 2:4-dinitrophenylhydrazone. The aldehyde structure (VIII) is indicated by the ultraviolet light absorption of the substance ($\lambda_{max.} = 293 m\mu.$; $\epsilon = 18\,150$) and of its 2:4-dinitrophenylhydrazone ($\lambda_{max.} = 402 m\mu.$; $\epsilon > 35\,500$). The ultraviolet light absorption data obtained throughout this work conformed closely with the generalizations of Woodward (1941, 1942) and of Evans & Gillam (1941, 1943, 1945) for unsaturated carbonyl compounds, and with those of Roberts & Green (1946) for the 2:4-dinitrophenylhydrazones.

On treatment with sodium ethoxide in ethanol, the *seco*aldehyde (VII; R=OH) passed smoothly into 6-formyl-*B*-norcholest-5-en-3-ol (IX; R=OH). This substance formed a hydrate from which water could not be expelled without loss of crystalline form, but the 2:4-dinitrophenylhydrazone was anhydrous. The noraldehyde (IX; R=OH) was a sensitive substance: on exposure of a hexane solution to air two new products, one neutral and one acidic, were formed. Since these readily liberated iodine from potassium iodide in acetic acid, they are



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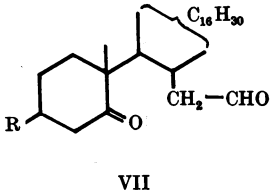

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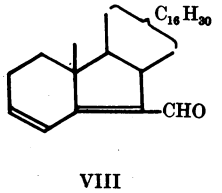
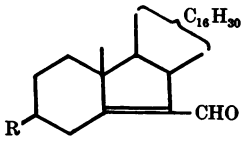


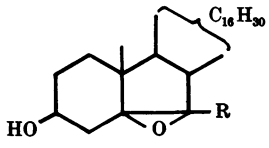
VIII

formulated tentatively as 6-formyl- and 6-carboxy-5:6-epoxy-*B*-norcholestan-3-ol (X; R=CHO and CO_2H) respectively. Attempts to oxidize the noraldehyde (IX; R=OH) to the corresponding carboxylic acid by means of permanganate or silver oxide (Pearl, 1946*a*, *b*) gave unsatisfactory products.

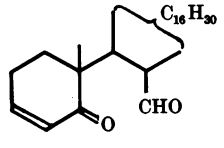
Preliminary attempts were now made to split the diketo acid (XII; R=OH) to give 2-methyl*cyclo*hexenone or 2-methyl*cyclo*hexanolone. Even in a drastic pyrolytic experiment, however, only a mixture of *iso*octane and *iso*octene was isolated. It was accordingly decided to replace the 3-hydroxyl



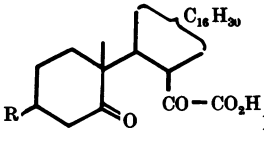
IX



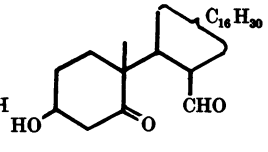
X



XI



XII



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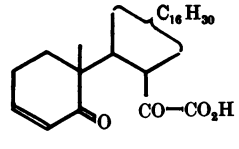
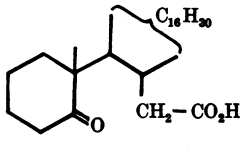


XIV

Ozonization of the noraldehyde (IX; R=OH), followed by reduction with zinc and acetic acid, afforded a crystalline acidic product along with a much smaller amount of neutral material. From the latter, a very small amount of a 2:4-dinitrophenylhydrazone, probably that of 5:6-*seco*-*B*-norcholest-3-en-6-al-5-one (XI) was obtained. The acid proved to be 5:6-*seco*cholest-3-ol-5:7-dion-6-oic acid (XII; R=OH). It lost carbon dioxide readily, not only on heating with aniline, a reaction typical of α-keto acids, but also on attempted preparation of the 3:5-dinitrobenzoate and 2:4-dinitrophenylhydrazone, the derivatives actually isolated being those of the decarboxylated product, 5:7-*seco*-*B*-norcholestan-6-al-3-ol-5-one (XIII). No rearrangement had occurred in the formation of the diketo acid (XII; R=OH), for, by dehydration with ethereal sulphuric acid, it was smoothly convertible into the corresponding unsaturated diketo acid (XIV). This compound had the characteristic ultraviolet light absorption of an αβ-unsaturated ketone. If a shift of the double bond in IX (R=OH) had led to the formation of a compound with the α-keto acid side chain attached at carbon 5, no such dehydration product would have been obtainable.

group by a hydrogen atom before carrying out further experiments. The required 5:6-*seco*cholestan-5:7-dion-6-oic acid (XII; R=H) was soon obtained from the unsaturated diketo acid (XIV) by catalytic reduction with palladized charcoal and hydrogen. The over-all yield was very moderate, and it was found that the same compound was more easily obtainable and in higher yield by carrying out a variation of the series of reactions described above, involving the substitution of cholest-5-ene for cholesterol.



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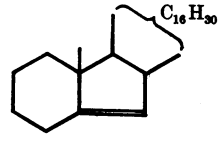
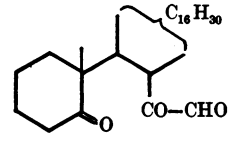


XVI



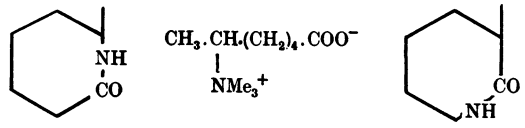
XVII

Cholesterol was converted into cholesteryl chloride by a method similar to that used by Diels & Blumberg (1911). Reduction with sodium and *n*-amyl alcohol (Mauthner & Suida, 1894) then led to the production of cholest-5-ene in 77% over-all yield. Ozonization followed by reduction with zinc and acetic acid afforded 5:6-*seco*cholestan-6-al-5-one (VII; R=H) as a colourless oil which formed a crystalline 2:4-dinitrophenylhydrazone. Fission of the ozonide with aqueous hydrogen peroxide gave the corresponding acid (XV; Lettré, 1933) which was isolated as a crystalline hydrate. Neither of the reported methods (Lettré, 1933) for the conversion of this acid into *B*-norcholest-5-ene (XVI), a possibly

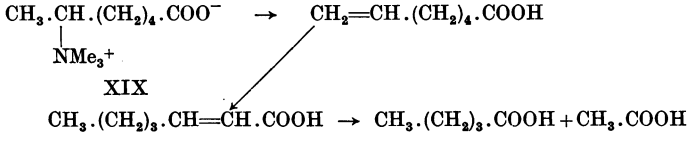
useful intermediate for our purposes, was found to be satisfactory owing to the low yields and difficulties in crystallization. However, the *seco*aldehyde (VII; R=H) was converted easily into the *B*-noraldehyde (IX; R=H), a viscous lemon-yellow oil characterized as its red 2:4-dinitrophenylhydrazone. Ozonization, reduction, and separation of the acidic fraction then gave the desired diketo acid (XII; R=H), identical with the product obtained previously; but in this case an approximately equivalent amount of material was present in the neutral fraction as a viscous pale-yellow oil. Chromatography on alumina (British Drug Houses Ltd., chromatographic grade), washed with methyl formate, separated this oil into two main fractions. (We are indebted to Dr P. A. Robins of this Institute for this unpublished method of treating alumina.) The one which was less strongly adsorbed formed colourless crystals from ethanol, and was apparently 5:6-*seco*cholestan-6-al-5:7-dione (XVII). The other (smaller) fraction failed to crystallize and analytical figures indicated a formula $C_{27}H_{46}O_4$. It was not further investigated.

When the 5:6-*seco*cholestan-5:7-dion-6-oic acid (XII; R=H) was heated with aniline, carbon dioxide was slowly evolved, although we were unable to isolate any product of decarboxylation from the reaction. However, a similar reaction was found to take place when the free diketo acid was heated alone above 200°, carbon dioxide being slowly formed (cf. Darzens & Levy, 1937). In this case, chromatography of the residual neutral fraction from the pyrolysis led to the isolation, in relatively poor yield, of the crystalline *seco*noraldehyde (IV). Thus, our primary objective had been attained. Pyrolysis of this aldehyde with various catalysts did indeed lead to the production of 2-methyl*cyclo*hexanone (V), the best yield being obtained in the presence of potassium carbonate at 450–500°. The identity of the ketonic product was established by conversion to its 2:4-dinitrophenylhydrazone, the melting point of which was undepressed when mixed with the authentic 2-methyl*cyclo*hexanone derivative. Owing to the loss involved in the isolation of the crystalline aldehyde (IV), it was found best to use directly the crude product obtained after pyrolysis of the diketo acid. At the same time, the diketoaldehyde (XVII) was found to yield 2-methyl*cyclo*hexanone under similar conditions; again, the crude product was used for routine work. Although the amount of 2-methyl*cyclo*hexanone isolated was small (yield about 10%), it was sufficient for our purposes as all the other stages proceeded in excellent yield.

All that was now required was a stepwise degradation of 2-methyl*cyclo*hexanone. This ketone readily underwent the Schmidt reaction in aqueous hydrochloric acid solution. The crude ϵ-amino-*n*-heptanoic lactam (XVIII), obtained in almost quantitative yield, had m.p. 70–75°, which rose to 91° after three recrystallizations from light petroleum. Since the loss involved in purification by this method was over 50%, further work was carried out on the crude product. Acid hydrolysis (Eck, 1937) gave ϵ-amino-*n*-heptanoic acid hydrochloride (Ungnade & McLaren, 1945) as a colourless gum. The pure material could readily be isolated by precipitation from ethanolic solution with ether. For our purposes, the gummy material was sufficiently pure for further experiments. Methylation with methyl iodide in methanol in the presence of silver oxide gave *n*-heptanoic ϵ-trimethylbetaine (XIX) in good yield. Although the free betaine proved to be a highly deliquescent substance, the beautifully crystalline hydrochloride could be purified. The method seems to be superior to the one involving methyl sulphate used by Giral (1935) for the preparation of *n*-hexanoic ϵ-trimethylbetaine. Fusion of the crude betaine (XIX) at 350° with potassium hydroxide gave, as expected, *n*-valeric and acetic acids in equivalent quantities, the yield of each being about 44%. This complex reaction involves: (i) elimination of trimethylamine, which occurs freely between 150 and 250°, (ii) migration of the double bond initially formed to the $\alpha\beta$-position, and (iii) fission of the $\alpha\beta$ double bond (Varrentrap, 1840; Edmed, 1898; Hunter & Popják, 1951).



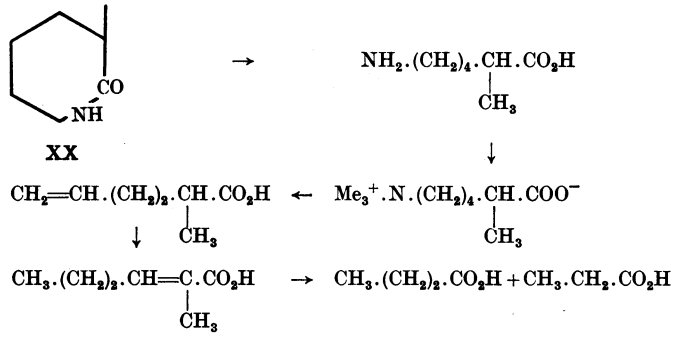
XVIII XIX XX



In preliminary experiments, the acids were identified by chromatographic techniques (Brown, 1950; James & Martin, 1952).

The product proved to be more complex than expected, for, in addition to the *n*-valeric and acetic

acids, about 6% each of *n*-butyric and propionic acids were also detected. However, when the same degradative sequence of reactions was carried out on pure ϵ-amino-*n*-heptanoic lactam, only *n*-valeric and acetic acids appeared in significant quantity in the final acid mixture. On the other hand, material recovered from the mother liquors of the recrystallized lactam gave a very high proportion of *n*-butyric and propionic acids. It was evident that the original lactam preparation had contained also 10–15% of the isomeric ϵ-amino-α-methyl-*n*-hexanoic lactam (XX), from which the *n*-butyric acid (C-4, 3, 2 and 1 of cholesterol) and propionic acid (C-19, 10 and 5 of cholesterol) were ultimately derived.



Attempts to separate the two lactams chromatographically were only partially successful. The main component (XVIII) could be obtained pure, recovery approx. 70%, but the remaining fractions all contained a mixture of the two lactams. Purification could be carried out most efficiently by chromatography of the four acids obtained after the degradation of the mixed lactams. The chromatographic techniques are described in detail in another paper (Cornforth, Hunter & Popják, 1953).

According to Hildebrand & Bogert (1936), Beckmann rearrangement of 2-methyl*cyclo*hexanone oxime gives ϵ-amino-*n*-heptanoic lactam in 97% yield. However, their proof of the structure of the product was based on recrystallized material, and, in fact, we found that the crude product from the Beckmann rearrangement had a composition (cf. Rogovin, Khaĭt, Knunyants & Rymashevskaya, 1947) identical with that of the lactam obtained by the Schmidt reaction. The latter reaction is to be preferred both by virtue of the better and more reproducible yield and also because of the simpler experimental technique required.

The remainder of the degradation, i.e. the degradation of the *n*-carboxylic acids obtained from 2-methyl*cyclo*hexanone, was carried out by standard methods or slight modifications thereof and are considered in detail in the following paper in which the application of the whole process of degradation to [^{14}C]cholesterol is described (Cornforth *et al.* 1953).

EXPERIMENTAL

Products derived from cholesterol ozonide

All melting points are uncorrected.

The elaborate apparatus advocated for the preparation of cholesterol ozonide (Berenstein *et al.* 1946) can be greatly simplified. Yields of 80% were reproducibly obtained by passing excess ozone through a saturated solution of cholesterol in dry *n*-hexane until precipitation appeared to be complete. After centrifugation and washing of the product with light petroleum (b.p. 40–60°), it was dried *in vacuo* at 25° and the glassy ozonide used as such for further work. Cholesterol ozonide (7·0 g.) was heated with stirring on the steam bath with water (30 ml.) and a few ml. of 'Perhydrol', more being added as the reaction proceeded. The solid was gradually replaced by a white glue. After cooling and extraction into ether, acidic material was isolated by extraction into 2N-NaOH. Acidification, re-extraction into ether, drying, and finally evaporation to dryness *in vacuo* at room temperature gave crystalline 5:6-seco*cholestan*-3-*al*-5-*on*-6-*oic acid monohydrate* (5·5 g.), m.p. 75–80°. (Found: C, 72·1; H, 10·6. $C_{27}H_{48}O_5$ requires C, 71·7; H, 10·6%.)

On the other hand, reduction of the ozonide (15·0 g.) yielded the corresponding aldehyde. After shaking at 25° with Zn powder (15 g.) and acetic acid (100 ml.) for 60 hr., I_2 was no longer rapidly liberated from a crystal of KI added to a sample of the solution. Inorganic material was removed by filtration and washed with ether (250 ml.), and the combined filtrates were washed successively with water, 5% aqueous $NaHCO_3$ and water. Evaporation to dryness *in vacuo* at 25° left silvery leaflets of the *etherate* of 5:6-seco-*cholestan*-6-*al*-3-*ol*-5-*one* (13·1 g.), m.p. 55–60°. (Found: C, 76·0, 75·3; H, 11·1, 11·0. $C_{31}H_{56}O_4$ requires C, 75·6; H, 11·4%.) There was no significant absorption in ethanol at $\lambda > 220$ mμ. The aldehyde (0·5 g.) was allowed to stand for 20 hr. in dry benzene (15 ml.) with dry pyridine (2 ml.) and 3:5-dinitrobenzoyl chloride (1·0 g.). The crude product (0·65 g.) was obtained in the usual way, and after crystallization from ethyl acetate-aqueous methanol, it formed white microprisms of the 3:5-*dinitrobenzoate*, m.p. 88–90°. (Found: N, 5·0. $C_{34}H_{48}O_8N_2$ requires N, 4·6%.) On heating the *seco*aldehyde (0·15 g.) with a slight excess of 2:4-dinitrophenylhydrazine in ethanol (20 ml.) with subsequent

addition of a few drops of conc. HCl at the boiling point and cooling to 0°, an orange precipitate appeared. Filtration and crystallization from aqueous ethanol gave 5:6-seco*cholestan*-6-*al*-3-*ol*-5-*one* 2:4-*dinitrophenylhydrazone*, m.p. 196° decomp. (Found: C, 66·2; H, 8·3; N, 9·3. $C_{33}H_{50}O_6N_4$ requires C, 66·2; H, 8·4; N, 9·4%.) $\lambda_{max.} = 363$ mμ.; $\epsilon_{max.} = 18300$ in ethanol.

5:6-*seco*Cholestan-6-al-3-ol-5-one (5·0 g.) in dry ether (50 ml.) was allowed to stand for 24 hr. at room temperature with 10% (v/v) ethereal H_2SO_4 (50 ml.). The solution was poured into water and the ether layer separated. After washing and drying, the solvent was evaporated, leaving a yellow gum which was dissolved in a little benzene. Chromatography on Al_2O_3 (British Drug Houses Ltd., chromatographic grade) gave in the first benzene eluate 6-*formyl*-B-*norcholesta*-3:5-*diene* (3·2 g.) as a viscous yellow oil. (Found: C, 84·5; H, 10·8. $C_{27}H_{42}O$ requires C, 84·8; H, 11·0%.) $\lambda_{max.} = 293$ mμ.; $\epsilon = 18150$ in ethanol. A bright red 2:4-*dinitrophenylhydrazone*, m.p. 267° decomp., was readily obtainable and crystallized from ethyl acetate in felted needles. (Found: C, 69·9; H, 8·1; N, 9·9. $C_{33}H_{46}O_4N_4$ requires C, 70·4; H, 8·2; N, 10·0%.) $\lambda_{max.} = 402$ mμ.; $\epsilon > 35500$ in ethanol.

The same *seco*aldehyde (VII; R=OH; 13·1 g.) in ethanol (175 ml.) was allowed to stand overnight with 4% ethanolic sodium ethoxide (25 ml.). After the solution was poured into a large volume of dilute acetic acid and was extracted into ether, the product was isolated by washing, drying, and evaporation to dryness *in vacuo* at 25°. 6-*Formyl*-B-*norcholest*-5-*en*-3-*ol* (10·5 g.) formed yellow leaflets of the *monohydrate*, m.p. 60–65°. (Found: C, 77·4; H, 11·0. $C_{27}H_{46}O_3$ requires C, 77·5; H, 11·0%.) $\lambda_{max.} = 251$ mμ.; $\epsilon = 12275$ in ethanol. The corresponding 2:4-*dinitrophenylhydrazone* separated from ethyl acetate in clusters of orange needles, m.p. 248–249° decomp. (Found: C, 68·4; H, 8·3; N, 9·8. $C_{33}H_{48}O_5N_4$ requires C, 68·3; H, 8·3; N, 9·7%.) $\lambda_{max.} = 390$ mμ.; $\epsilon = 21700$ in ethanol.

When the noraldehyde (1·8 g.) was allowed to stand in *n*-hexane (25 ml.) for 7 days, a crystalline deposit (0·2 g.) appeared. Recrystallization from $CHCl_3$:*n*-hexane produced spherical clusters of fine needles, m.p. 224°, of (?) 5:6-*epoxy*-B-*norcholestan*-3-*ol*-6-*carboxylic acid hemihydrate*. (Found: C, 72·9; H, 10·2. $C_{27}H_{44}O_4 \cdot \frac{1}{2}H_2O$ requires C, 73·5; H, 10·2%.) Evaporation of the *n*-hexane filtrate and washings gave a yellow crystalline solid (1·5 g.). Chromatography on Al_2O_3 in acetone removed impurities, and the main product was then eluted with $CHCl_3$:ethanol (1:1). Crystallization from ethanol gave (?) 5:6-*epoxy*-6-*formyl*-B-*norcholestan*-3-*ol hemihydrate* as colourless prisms, m.p. 160–165°. (Found: C, 76·1; H, 10·5. $C_{27}H_{44}O_3 \cdot \frac{1}{2}H_2O$ requires C, 76·2; H, 10·6%.) The formyl-*B*-norcholestenol could also be dehydrated with ethereal H_2SO_4 to give the doubly unsaturated noraldehyde (VIII), previously obtained in like manner from the *seco*aldehyde (VII; R=OH).

6-Formyl-*B*-norcholest-5-en-3-ol (10·2 g.) was dissolved in dry *n*-hexane (200 ml.) and ozonized until the solution became colourless. The residue, after evaporation nearly to dryness *in vacuo*, was shaken overnight with Zn (11·0 g.) and acetic acid (100 ml.). After filtration and addition of water (200 ml.) and ether (250 ml.) to the filtrate, the ethereal extract was washed successively with water, 5% aqueous $NaHCO_3$ and water. The acidic material was then extracted into 2N-NaOH. The neutral ethereal extract, after washing, drying and evaporation *in vacuo*, left a gummy residue (about 1·0 g.) which did not crystallize. Treatment with ethanolic 2:4-dinitrophenylhydrazine in the presence of HCl led to the formation of a small quantity of 5:6-seco-B-*norcholest*-3-*en*-6-*al*-5-*one* 2:4-*dinitrophenylhydrazone* in masses of small orange prisms, m.p. 118° decomp. (Found: C, 67·7; H, 8·6; N, 10·3. $C_{32}H_{46}O_5N_4$ requires C, 67·8; H, 8·1; N, 9·9%.) Acidification of the alkaline extract and subsequent working up in the usual way gave 5:6-seco*cholestan*-3-*ol*-5:7-*dion*-6-*oic acid* (5·4 g.) as a colourless flaky glass, softening and melting indefinitely in the range 60–100°. (Found: C, 72·2; H, 10·1. $C_{27}H_{44}O_5$ requires C, 72·3; H, 9·8%.) Absorption at $\lambda > 220$ mμ. was very small. Reaction with boiling ethanolic 2:4-dinitrophenylhydrazine in the presence of HCl led to the formation of 5:6-seco-B-*norcholestan*-6-*al*-3-*ol*-5-*one* 2:4-*dinitrophenylhydrazone*, which crystallized from ethyl acetate-methanol in orange prisms, m.p. 164–166° decomp. (Found: C, 65·9; H, 8·5. $C_{32}H_{48}O_6N_4$ requires C, 65·8; H, 8·2%.) 5:6-seco-B-*Norcholestan*-6-*al*-3-*ol*-5-*one* 3:5-*dinitrobenzoate* was obtained from the keto acid and 3:5-dinitrobenzoyl chloride in dry pyridine-benzene. It formed an amorphous white powder, m.p. 128–129°, when precipitated from ethyl acetate by ethanol at −20°. (Found: C, 66·1; H, 7·7; N, 4·8. $C_{33}H_{46}O_8N_2$ requires C, 66·2; H, 7·7; N, 4·7%.)

The diketo acid (XII; R=OH) (3·0 g.) was allowed to stand in 10% (v/v) ethereal H_2SO_4 (50 ml.) for 20 hr. at room temperature. After washing the H_2SO_4 out with water, evaporation to dryness *in vacuo* gave 5:6-seco-*cholest*-3-*ene*-5:7-*dion*-6-*oic acid* (2·25 g.), yellow crystalline plates, m.p. 72°. (Found: C, 74·9; H, 9·7. $C_{27}H_{42}O_4$ requires C, 75·3; H, 9·8%.) $\lambda_{max.} = 220$ mμ.; $\epsilon = 11500$ in ethanol. Hydrogenation at atmospheric pressure was carried out in ethyl acetate in the presence of 5% $PdCl_2$ on charcoal, when the desired two atoms of hydrogen were taken up in 3 hr. From the alkaline extract of the product, a 30% yield of 5:6-seco*cholestan*-5:7-*dion*-6-*oic acid*, m.p. 57°, was obtained. It formed colourless plates when reprecipitated from dilute NaOH solution with dilute HCl. (Found: C, 74·8; H, 10·0. $C_{27}H_{44}O_4$ requires C, 75·0; H, 10·2%.)

Degradation of cholest-5-ene to 2-methylcyclohexanone

Finely powdered cholesterol (50·0 g.) was placed in a 250 ml. two-necked round-bottomed flask fitted with a calcium chloride guard tube and a small separating funnel. Purified $SOCl_2$ (40 ml.) was added rapidly with shaking, and the dark-green solution allowed to stand at room temperature for 18 hr. Excess $SOCl_2$ was removed at 45°/25 mm. Two crystallizations of the residue from acetone now gave cholesteryl chloride (43·6 g., 84% yield), m.p. 95°, of a high degree of purity. Cholesteryl chloride (40·0 g.) in boiling *n*-amyl alcohol (800 ml., freshly distilled) was treated with Na (approx. 40 g.) added in small lumps over 2 hr. The solution was heated under reflux for a further 1·5 hr. and then allowed to cool to room temperature overnight. After thorough washing with water (formation of an emulsion may be prevented by warming) to remove NaOH, the product was isolated by distillation of the *n*-amyl alcohol *in vacuo*. The white crystalline residue was recrystallized from ethanol, yielding pure cholest-5-ene (33·9 g., 93% yield), m.p. 92°.

Cholest-5-ene (10·0 g.) in *n*-hexane (100 ml.) was treated with a steady current of ozone until a dilute solution of Br_2 in acetic acid was no longer rapidly decolorized on mixing with a few drops of the *n*-hexane solution. Evaporation to a small volume and fission with H_2O_2 as described for

cholesterol ozonide led to the isolation of 5:6-seco*cholestan*-5-*on*-6-*oic acid* (4·9 g.) as colourless plates of the *monohydrate*, m.p. 61°. (Found: C, 73·8; H, 10·7. $C_{27}H_{48}O_3.H_2O$ requires C, 74·3; H, 11·0%.) In a further experiment on the same scale, the *n*-hexane solution of the ozonide was evaporated *in vacuo* to small bulk, and reduction effected by shaking with Zn (10 g.) and acetic acid (70 ml.) for 12 hr. Normal isolation procedures gave 5:6-seco*cholestan*-6-*al*-5-*one* (10·1 g.) as a thick viscous oil. (Found: C, 76·8; H, 11·5. $C_{27}H_{46}O_2.H_2O$ requires C, 77·0; H, 11·4%.) Treatment with ethanolic 2:4-dinitrophenylhydrazine as before in the presence of HCl gave a 70% yield of the 2:4-*dinitrophenylhydrazone*, which crystallized from ethanol-(N-HCl) in clusters of bright-yellow prisms, m.p. 123°. (Found: C, 68·3; H, 8·8; N, 9·3. $C_{33}H_{50}O_5N_4$ requires C, 68·0; H, 8·6; N, 9·6%.) The same *seco*aldehyde (8·8 g.) in ethanol (90 ml.) was allowed to stand for 24 hr. with 7% ethanolic sodium ethoxide (10 ml.). The product was isolated as described for the analogous compound from cholesterol ozonide: 6-*formyl*-B-*norcholest*-5(6)-*ene* (8·0 g.) formed a lemon-yellow oil. $\lambda_{max.} = 253$ mμ.; $\epsilon = 6230$ in ethanol. The 2:4-*dinitrophenylhydrazone* crystallized from ethyl acetate in spherical clusters of fine orange hair-like needles, m.p. 235°. (Found: C, 70·2; H, 8·9; N, 10·0. $C_{33}H_{48}O_4N_4$ requires C, 70·2; H, 8·9; N, 9·9%.)

Ozonization of the noraldehyde (13·5 g.) was carried out as before, disappearance of the yellow colour being the criterion of a completed reaction. The subsequent working up this time revealed the presence of a large neutral fraction (5·65 g.) in addition to the 5:6-*seco*cholestan-5:7-dion-6-oic acid (6·6 g.), m.p. 57°, identical with the material previously obtained by reduction of the corresponding unsaturated diketo acid. The neutral fraction (6·1 g.) was purified by chromatography on alumina (194 g.) washed with methyl formate. Elution with benzene gave 5:6-seco*cholestan*-6-*al*-5:7-*dione* (3·5 g.) which crystallized from ethanol-water in colourless plates, m.p. 142–143°. (Found: C, 77·9; H, 10·8. $C_{27}H_{44}O_3$ requires C, 77·9; H, 10·6%.) The above diketo acid (5·0 g.) was heated under N_2 at 250° for 1·5 hr. The product was separated into neutral (2·3 g.) and acidic (mainly unchanged starting material) fractions. The neutral material was dissolved in light petroleum (b.p. 80–100°) and chromatographed on alumina (69 g.) washed with methyl formate. Elution with light petroleum (b.p. 80–100°) gave crystalline 5:6-seco-B-*norcholestan*-6-*al*-5-*one* (1·24 g.) which recrystallized from ethanol as colourless needles, m.p. 114°. (Found: C, 80·8; H, 11·0. $C_{26}H_{44}O_2$ requires C, 80·4; H, 11·3%.)

The crude neutral product (0·4 g.) from the ozonolysis of 6-formyl-*B*-norcholest-5-ene was heated at 450–500° with K_2CO_3 (1·5 g.) for 10 min. Neutralization and steam distillation of an aqueous suspension of the combined residue and distillate gave 2-methyl*cyclo*hexanone, isolated as the 2:4-dinitrophenylhydrazone. This derivative, recrystallized from ethanol, had m.p. 127–132°; an authentic specimen had a m.p. of 134°; the mixed m.p. was 131–133°. The yield was 0·028 g. (10%). A similar yield of 2-methyl*cyclo*hexanone was obtained by pyrolysing the diketo acid (X, R=H, 0·5 g.) at 250° for 1·5 hr., and then heating the crude residual product for 10 min. with K_2CO_3 (1·5 g.) at 450–500°. When degradations were being carried out on material labelled with ^{14}C, inactive 2-methyl*cyclo*hexanone was added to the steam distillate (after assay of a small portion by conversion to the 2:4-dinitrophenylhydrazone), and the 'diluted' material extracted into ether.

Degradation of 2-*methyl*cyclo*hexanone*

2-Methyl*cyclo*hexanone (10·15 g.) in conc. HCl (50 ml.) was cooled to 0°. NaN_3 (9·0 g.) was added in small portions with stirring during 5–10 min. Stirring was continued for 30 min., the solution was allowed to come slowly to room temperature, and then evaporated to dryness *in vacuo*. The residue was taken up in the minimum amount of water and made strongly alkaline with 50% KOH. Extraction into $CHCl_3$ (3 × 2 vol.) now removed all the product from the aqueous phase. The $CHCl_3$ solution was washed with a little water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*, leaving a mixture of lactams (10·6 g., 92% yield; m.p. 70–75°). Three crystallizations from light petroleum (b.p. 80–100°) gave pure ϵ-amino-*n*-heptanoic lactam, m.p. 91°. (Found: C, 66·2; H, 10·3; N, 11·0. Calc. for $C_7H_{13}ON$: C, 66·1; H, 10·2; N, 11·0%.) The over-all recovery was only about 30%. A better yield of pure material (60–70%) was obtained by chromatography on alumina washed with methyl formate. The first fraction, eluted with light petroleum (b.p. 80–100°), contained only pure ϵ-amino-*n*-heptanoic lactam, but later fractions all proved to be mixtures. A method was not found for the isolation in a pure state of the other isomer, ϵ-amino-α-methyl-*n*-hexanoic lactam, although it was considerably concentrated both in the mother liquors from the first crystallization and in the later chromatographic fractions.

The crude lactam (10·0 g.) was boiled with 2N-HCl (80 ml.) for 1·5 hr., and the clear solution evaporated to dryness *in vacuo*. The product (14·25 g., 99%) solidified on standing; m.p. 112–120°. It crystallized from ethanol-ether in beautiful clusters of colourless rods; the pure ϵ-amino-*n*-heptanoic acid hydrochloride had m.p. 130–132°. (Müller & Krauss, 1932, give m.p. 131°. Found: C, 46·4; H, 8·9; N, 7·8. Calc. for $C_7H_{16}O_2NCl$: C, 46·3; H, 8·8; N, 7·7%.)

The crude amino-acid hydrochloride (7·5 g.) from the hydrolysis was dissolved in methanol (100 ml.) and heated under reflux for 12 hr. with methyl iodide (28·0 g.) and freshly prepared Ag_2O (from 45·5 g. $AgNO_3$). Filtration and evaporation to dryness *in vacuo* yielded a crude betaine (about 7 g., very deliquescent). However, after re-evaporation of a portion with dilute HCl, *ϵ-amino*-n-*heptanoic betaine hydrochloride* was obtained, and crystallized from ethanol-ether in clusters of colourless spears, m.p. 146–149°. (Found: C, 53·4; H, 10·1; Cl, 16·2. $C_{10}H_{22}O_2NCl$ requires C, 53·7; H, 9·9; Cl, 15·9%.) The betaine also formed a *picrate* which crystallized from water in magnificent yellow needles, m.p. 273–274° decomp. It was highly explosive, and attempts to obtain analytical figures were abandoned after the destruction of several pieces of micro-analytical apparatus.

One-fifth of the crude betaine obtained above was fused at 350° for 10 min. with solid KOH (10 g.). The residue was taken up in the minimum amount of water and acidified with H_2SO_4, when extraction with ether (3 × 2 vol.) removed all the fatty acids present except negligible amounts of acetic and propionic. The ethereal solution was dried and filtered. Neutralization with ethanolic NaOH, extraction of the salts into water, and evaporation to a small bulk left a concentrated solution of the sodium salts of the fatty acids which were separated by chromatography (see following paper). There were isolated: *n*-valeric (370 mg., 44%) *n*-butyric (47 mg., 6%), propionic (38 mg., 6%) and acetic acids (220 mg., 43%). (The yields are based on the crude amino-acid.)

The same sequence of reactions as that described in the previous paragraph was carried out on pure recrystallized ε-amino-*n*-heptanoic lactam, m.p. 91°. Liquid-vapour partition chromatography (James & Martin, 1952) of the final mixture of fatty acids showed them to be present in the following molar proportions: *n*-valeric and acetic 99% each, *n*-butyric and propionic 1% each. In a similar series, the mother liquor from the first light petroleum crystallization of the Schmidt reaction mixture was taken, and the crude product (m.p. 58–65°), obtained by evaporating to dryness *in vacuo*, was used as starting material. The following molar proportions of fatty acids were finally detected: *n*-valeric and acetic 67% each, *n*-butyric and propionic 33% each.

SUMMARY

1. A new method for the degradation of the ring structure of cholesterol is described. This involves the opening of ring *B* by oxidation and the splitting off of ring *A* as 2-methyl*cyclo*hexanone by pyrolysis.

2. Carbon atom 6 is obtained as carbon dioxide from the decarboxylation of 5:6-*seco*cholestan-5:7-dion-6-oic acid. From the further degradation of 2-methyl*cyclo*hexanone, carbon atoms 4 and 5 of cholesterol are obtained as acetic acid and carbon atoms 19, 10, 1, 2 and 3 as valeric acid; by a side reaction carbons 19, 10 and 5 were isolated as propionic acid accompanied by carbons 4, 3, 2 and 1 as butyric acid.

3. Two different ways are described for carrying out the early stages of the degradation: the first method starts from cholesterol ozonide, the second and more efficient method from cholest-5-ene.

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Studies of Cholesterol Biosynthesis

2. DISTRIBUTION OF ACETATE CARBON IN THE RING STRUCTURE

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It is now firmly established, largely on the basis of the work of Bloch and his collaborators (see Bloch, 1951) that acetic acid forms the primary building unit from which animal tissues synthesize cholesterol. Little & Bloch (1950) showed that both the side chain and the *cyclo*pentenophenanthrene nucleus are formed from acetate, and that of the twenty-seven carbon atoms of cholesterol fifteen originate from the methyl and twelve from the carboxyl carbon of acetate. A knowledge of the pattern according to which these acetate carbons are distributed in the sterol molecule might give valuable information about the intermediary stages of biosynthesis. Wüersch, Huang & Bloch (1952)