one amino acid to another as compared with those of PTH derivatives at 270 m μ , being almost entirely a property of the naphthyl group.

The splitting off of naphthylthiazolinones in the stepwise degradation of peptides proceeds more slowly, 45 min. being required for completion of the reaction instead of 15 min. for the corresponding reaction with phenylthiocarbamoyl-peptides. Otherwise, the procedure of Edman's new principle as published by Doolittle (1965) could be followed.

It was found that naphthyl isothiocyanate worked well in the determination of *N*-terminal amino acids of A and B chains of bovine insulin and of yeast cytochrome *c*. The reaction with naphthyl isothiocyanate was even more efficient than that with phenyl isothiocyanate. Thus from yeast cytochrome *c* 0.80 mol. of NTH-threonine/mol. of protein could be recovered in the eluate from the paper of the final chromatogram, whereas 0.70 mol./mol. was found with the original Edman procedure (Yaoi, Titani & Narita, 1964). In this analysis, the greater part of threonine was identified as NTH-dehydrothreonine. Similar results were obtained for threonine and also for serine in other polypeptides.

In the reactions for the sequential amino acid analysis of peptides, we have found no disadvantage in the NTH method compared with the PTH method.

With a dipeptide, glycylthreonine, the NTH method worked perfectly. With the *N*-ethylmaleimide derivative of cytochrome *c* from yeast, the *N*-terminal sequence, Thr-Glu-Phe-Lys-Ala-Gly-Ser, was confirmed. The NTH of each step appeared as a single spot, which made the identification unequivocal. The yield of NTH fell as the number of steps increased and the fifth amino acid, alanine, could be recovered only with a yield of 0.2 mol./mol.

of protein. However, if (Doolittle, 1965) yields are expressed as percentages found in the preceding step, then the average yield, exclusive of the first step, was 72%. This is somewhat lower than that reported by Doolittle (1965) (88%) for lamprey fibrinopeptide. This low yield is probably mainly due to insolubility of the protein, since during the sequential analysis this protein turned out to be insoluble from the second step onwards.

In the analysis of A and B chains of oxidized bovine insulin, insolubility of these polypeptides was not encountered. For the A chain, we followed the sequence up to nine, and the average yield was 85%. For the B chain, in the analysis up to six, the average yield was 81%. These values are comparable with those reported by Doolittle (1965). As with cytochrome *c*, the NTH derivative appeared as a single spot on paper chromatography in each step of the sequential analysis.

In conclusion, the NTH method is as effective as the PTH method in the reactions for sequential analysis of peptides, and is much superior to the latter in sensitivity of spectrophotometric analysis.

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Biochem. J. (1965) **97**, 260

Evidence that the Oxidation of the Side Chain of Cholesterol by Liver Mitochondria is Stereospecific, and that the Immediate Product of Cleavage is Propionate

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When chemically synthesized [26 (or 27)-¹⁴C]-cholesterol is incubated with rat-liver mitochondria under conditions in which ¹⁴CO₂ is formed, [¹⁴C]-

propionic acid can be extracted from the incubation mixture by steam-distillation (Mitropoulos & Myant, 1965). This provides confirmatory evidence

for the suggestion of Suld, Staple & Gurin (1962), who isolated [^{14}C]propionate after incubating mitochondria with trihydroxy[26- ^{14}C]coprostane, that propionate is the immediate product of the cleavage of the side chain of cholesterol by liver mitochondria. We now describe two experiments that give further support to this suggestion. Our results also show that the initial step in the oxidation of the C-26 or C-27 atom of cholesterol is stereospecific.

In the first experiment, [1,7,15,22,27- ^{14}C]cholesterol was prepared by incubating the S_{10} homogenate (Popják, Gosselin, Gore & Gould, 1958) from 1g. of rat liver with 100 μC of [2- ^{14}C]mevalonate (specific activity 5 $\mu\text{C}/\mu\text{mole}$). After incubation, the mixture was hydrolysed in ethanolic NaOH. The cholesterol was extracted with light petroleum and purified by thin-layer chromatography on Kieselgel H plates. After running with benzene-ethyl acetate (5:1, v/v) as solvent, the radioactive bands were located by radioautography and the band corresponding to the cholesterol marker was scraped from the plate and eluted with chloroform. Radioactive impurities in the cholesterol were removed by re-running it twice, first with the same solvent and then with benzene-ethyl acetate (1:2, v/v). About 0.5 μmole of the purified cholesterol (specific activity 2 $\mu\text{C}/\mu\text{mole}$) was incubated with the mitochondria from 60g. of rat liver in the standard conditions described by Mitropoulos & Myant (1965), with the addition of potassium propionate (5mm) to enhance the accumulation of [^{14}C]propionate during the incubation. At the end of the incubation the [^{14}C]propionate was extracted by steam-distillation and purified by column chromatography (Swim & Utter, 1957). The fractions from the column were titrated with 0.02N-NaOH and a portion from each fraction was assayed for radioactivity in a Tri-Carb liquid-scintillation spectrometer (Packard Instrument Co., La Grange, Ill., U.S.A.). A sample (0.5m-mole) of the propionic acid fraction, representing 4.2% of the activity in the C-27 carbon of the radioactive cholesterol incubated, was submitted to stepwise degradation (Phares, 1951), and the carbon from the 1-, 2- and 3-positions was collected as BaCO_3 . The BaCO_3 was weighed and assayed for radioactivity by suspending it in a scintillation gel made by mixing 4g. of Cab-O-Sil (Packard Instrument Co.) with 100ml. of a solution of 2,5-diphenyloxazole in toluene (3g./l.) and counting in the scintillation spectrometer. The results are shown in Table 1. Almost all the ^{14}C in the propionic acid was recovered in the CO_2 derived from the methyl carbon.

In the second experiment, [26(or 27)- ^{14}C]cholesterol (obtained from The Radiochemical Centre, Amersham, Bucks.) was incubated with rat-liver mitochondria. The [^{14}C]propionate was extracted by steam-distillation, and was purified and de-

Table 1. *Distribution of radioactivity in the carbon atoms of propionic acid formed during incubation with rat-liver mitochondria of [1,7,15,22,27- ^{14}C]cholesterol biosynthesized from [2- ^{14}C]mevalonic acid, and of [26 (or 27)- ^{14}C]cholesterol*

Source of ^{14}C	Specific activity (disintegrations/min./m-mole)	
	From [1,7,15,22,27- ^{14}C]cholesterol	From [26 (or 27)- ^{14}C]cholesterol
$\text{CH}_3\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$	32800	144845
CO_2 from C-1	1200	75865
CO_2 from C-2	152	295
CO_2 from C-3	29211	66720

graded as described above. The results are shown in Table 1. The specific activity of the CO_2 from C-1 and C-3 was about half that of the propionic acid from which it was derived, and the CO_2 from C-2 was not significantly radioactive.

The results from the first experiment show that the initial step in the enzymic oxidation of the isopropyl unit of the side chain of cholesterol is stereospecific, and that the carbon atom which is oxidized is not the one derived from C-2 of mevalonic acid. Cornforth, Cornforth, Popják & Youhtsky-Gore (1957) have shown that the two terminal carbon atoms of squalene, which become the two terminal carbon atoms of the cholesterol side chain, are derived from the β -methyl carbon atom and C-2 of mevalonic acid. It follows therefore that the β -methyl carbon atom of mevalonic acid becomes the terminal carbon atom of cholesterol which is oxidized. Since this carbon atom is conventionally referred to as C-26, as in 26-hydroxycholesterol (Danielsson, 1963), radioactive cholesterol biosynthesized from [2- ^{14}C]mevalonic acid should be referred to as [1,7,15,22,27- ^{14}C]cholesterol, as above. While this work was in progress, Berséus (1965) reported experiments showing that, when [^{14}C]cholesterol biosynthesized from [2- ^{14}C]mevalonic acid is oxidized to 26-hydroxycholesterol by liver mitochondria, the carbon atom which is oxidized is not the radioactive one. He concluded that the initial step in the oxidation of the cholesterol side chain is stereospecific. On this point our results are in complete agreement.

Popják (1965) has shown that the introduction of the double bond between what were C-3 and C-4 of mevalonic acid, during the formation of a dimethylallyl unit, is stereospecific. The double bond from one dimethylallyl unit eventually becomes the Δ^{24} -double bond of desmosterol, which is then reduced to give cholesterol. The finding that propionic acid formed from cholesterol synthesized

from [2-¹⁴C]mevalonic acid is labelled only in the methyl carbon shows that the reduction of the Δ^{24} -double bond of desmosterol to form cholesterol, as well as the oxidation of the terminal carbon atom of the side chain of cholesterol, is stereo-specific. If this were not the case, the C-26 and C-27 would become equivalent, and the [¹⁴C]propionic acid removed enzymically from the side chain would be labelled equally in the methyl and carboxyl carbon atoms.

The results from the two experiments, in both of which the pattern of labelling in the cholesterol side chain was retained in the [¹⁴C]propionate, are difficult to explain if the [¹⁴C]propionate was formed by recombination of C₁ and C₂ fragments derived from the side chain, but would be expected if the carbon skeleton of the isopropyl unit was

removed intact as a C₃ fragment. This could be propionic acid itself or, as suggested by Suld *et al.* (1962), the CoA ester of propionic acid.

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A Mechanism for the Biosynthesis of α -Carotene

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In the biosynthesis of terpenoids from the basic C₅ precursor isopentenyl pyrophosphate, the first step is the isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate and this involves the expulsion of a proton from what was originally C-4 of mevalonic acid (eqn. 1). Chain elongation begins by the condensation of a molecule of dimethylallyl pyrophosphate with a second molecule of isopentenyl pyrophosphate to form geranyl pyrophosphate (C₁₀); this also involves the loss of a proton from the carbon atom originating from C-4 of mevalonic acid (eqn. 2); further chain

elongation to farnesyl pyrophosphate (C₁₅) and geranylgeranyl pyrophosphate (C₂₀) involves the same mechanism. In an elegant series of investigations Cornforth and Popják and their colleagues (Cornforth, Cornforth, Donninger & Popják, 1965a; Cornforth, Cornforth, Donninger, Popják & Ryback, 1965b) have synthesized [2-¹⁴C,4*R*-³H]mevalonic acid and [2-¹⁴C,4*S*-³H]mevalonic acid and shown that, in the biosynthesis of squalene (the C₃₀ steroid precursor formed by the dimerization of farnesyl pyrophosphate) in liver, the elimination of each proton arising from C-4 of mevalonic acid is