LVII. A STUDY OF THE OXIDATION OF THE AMMONIUM SALTS OF NORMAL SATURATED FATTY ACIDS AND ITS BIOLOGICAL SIGNIFICANCE.

BY PERCIVAL WALTER CLUTTERBUCK
AND HENRY STANLEY RAPER.

From the Department of Physiology, The University, Manchester.

(Received March 18th, 1925.)

IN a number of papers Dakin [1908, 1, 2, 3; 1910] described the production of β -keto-acids and the ketones arising from them by loss of carbon dioxide, in a series of experiments on the oxidation of the ammonium salts of normal saturated fatty acids with hydrogen peroxide. The reaction is a general one and at the time it was discovered was of great significance because it afforded great support to the evidence for the theory of β oxidation of fatty acids propounded by Knoop [1904]. It is a good instance of a process taking place *in vitro* which has helped to throw light on a similar process occurring in the living body. The reactions which occur when fatty acids and certain of their phenyl derivatives undergo oxidation in the body are not simple. Phenyl-propionic acid has been shown by Dakin [1908, 4, 5] to yield, in addition to the corresponding β -keto-acid, the β -hydroxy-acid and the α , β -unsaturated acid. It seemed worth while therefore to find out whether Dakin's observations on the oxidation of fatty acids with hydrogen peroxide could be extended to include these other oxidation products, and especially the unsaturated acids which are sometimes formed during oxidation in living animals.

Using conditions similar to but not identical with those of Dakin, attempts were made in the first instance to find out whether stearic acid yielded unsaturated acids on oxidation.

At first it was thought that this was so, an acid giving a lead salt soluble in ether being isolated from the products of oxidation. This acid absorbed iodine, but not in amount sufficient to account for one double linkage, and in other ways was different from the unsaturated acids of the oleic series. The investigation of this substance has not been completed but in addition to this acid (or mixture of acids) another new acid was isolated which proved on further investigation to be γ -keto-stearic acid. This showed therefore that besides β oxidation, γ oxidation also takes place. The γ -keto-acid could not be isolated quantitatively so that it is not possible to compare accurately the extent of oxidation at the β and γ carbon atoms. The β -keto-acid, under the

conditions of the oxidations, will decompose practically quantitatively to yield its corresponding ketone and this can be easily estimated. The amount obtained in several experiments indicated that β oxidation of the stearic acid had occurred to the extent of about 6 %. The amount of γ -keto-acid isolated corresponds to about 1 %, but this must be regarded as a minimum figure. In these experiments a large part of the stearic acid is recovered unchanged. It seems probable that other keto-acids are also produced. This certainly happens when the lower fatty acids are oxidised and will be referred to presently, but with the higher acids, their isolation from the large amount of unchanged fatty acid has not been achieved.

In order to see whether other homologues of stearic acid yielded a γ -keto-acid on oxidation, experiments were carried out on similar lines with palmitic and myristic acid. From both these acids γ -keto-acids were obtained and their identity has been confirmed by synthesis¹. These results with the higher fatty acids suggested the extension of the enquiry to some of the lower members of the series, and the oxidation of hexoic, heptoic and octoic acids has been investigated. The separation of the products of oxidation yielded by these acids proved somewhat troublesome and so far is probably incomplete, but it has been satisfactorily established that each of them, besides undergoing β oxidation, as was shown by Dakin, also undergoes γ and δ oxidation. This has been proved by the isolation of the γ - and δ -keto-acids themselves, or their derivatives, from the products of oxidation and by their identity with specimens obtained by synthesis from substances of known structure. So far as can be determined from the results with these acids, β , γ and δ oxidations take place to about the same extent. Oxidation of fatty acids at the γ carbon atom has been previously observed with α -methylbutyric acid [Raper, 1914] and butyric acid [Cahen and Hurtle, 1917] when hydrogen peroxide was the oxidising agent. In both these instances it seemed likely that the terminal methyl group was oxidised because of its exposed position at the end of the carbon chain, especially as terminally oxidised fatty acids have been found in nature: *e.g.* hydroxypalmitic (juniperic) acid $\text{CH}_2\text{OH}(\text{CH}_2)_{14}\text{COOH}$ and hydroxylauric (sabinic) acid $\text{CH}_2\text{OH}(\text{CH}_2)_{10}\text{COOH}$ have been isolated from the waxes of certain coniferae [Bougault, 1910]; but the results described in the present paper suggest that oxidation of the γ carbon atom is a general feature when fatty acids are oxidised, and these two instances, therefore, are not exceptional. It is also of interest to note that Bödtker [1912] isolated γ -keto-sebacic acid in small amount when sebacic acid was oxidised with strong nitric acid.

Although hydroxy-acids are almost insoluble in light petroleum and should therefore be separable easily, it was never found possible to detect even traces of these acids; it was therefore concluded that the first step in the oxidation of a saturated acid is probably the production of a series of keto-acids and not hydroxy-acids.

¹ For these syntheses we are greatly indebted to Professor and Mrs Robinson.

The further stages in the oxidation of γ - and δ -keto-fatty acids are at present under investigation; but one significant fact has already been discovered, namely, that both yield succinic acid as one of their oxidation products. This means that the carbon chain in these acids may break between the γ and δ carbon atoms. Four carbon atoms are thus split off as succinic acid.

These results are of interest from the biological point of view. They suggest that in the saturated acids, the β , γ and δ carbon atoms, and possibly also the α , are equally susceptible to oxidation, and that there is no marked difference in polarity between them as regards this property, though there certainly appears to be a marked one when ease of substitution by halogens is the criterion of polarity.

Whether all these forms of oxidation take place in the body we do not yet know. β oxidation certainly does, but there is practically no evidence in favour of α oxidation [Dakin, 1922]. If we accept the view that a substance placed in an environment with a suitably high oxidation potential is oxidised in a certain way which depends upon the structure of the substance and not upon the method by which the oxidation potential is obtained, then results obtained *in vitro* may be provisionally accepted as likely to take place in the body unless modified by some selective agency. That some such agency prohibits α oxidation seems very likely in view of the results of *in vivo* experiments, but the same cannot yet be said of γ and δ oxidation. If either of these processes occurred and were followed by rupture of the fatty acid chain between the γ and δ carbon atoms with the production of succinic acid, then four carbon atoms would be removed at a time instead of the usual two which are postulated by the β oxidation theory of breakdown. Such a process, associated also with β oxidation, would be quite compatible with the well-known results obtained by Embden and his co-workers [1906, 1908] with the surviving liver perfused with blood containing salts of the lower fatty acids. It would also be compatible with the results of Knoop [1904] and Dakin [1909] who showed that the oxidation of phenylvaleric acid in the body yielded benzoic acid. That a γ -keto-acid may lose four carbon atoms at a time on oxidation in the body is suggested very strongly by the results of Knoop and Ocker [1914], who determined the fate in the body of benzyl-aevulinic acid. When 15 g. of this acid were administered to a dog, 3.6 g. of phenaceturic acid and 0.8 g. phenyl- α -hydroxybutyric acid were isolated from the urine. No hippuric acid was found. A study of the formula of benzyl-aevulinic acid

shows that β oxidation would yield benzylpyruvic acid,

which on reduction would give the phenyl- α -hydroxybutyric acid obtained in this experiment. That the fatty acid side chain did not split between the β and γ carbon atoms and so yield phenylpropionic acid is shown by the

absence of benzoic (or hippuric) acid among the products recovered from the urine, since phenylpropionic acid yields benzoic acid when oxidised in the body. The main product isolated, namely, phenylacetic acid (excreted as phenaceturic acid) seems undoubtedly to have been produced by splitting of the chain between the γ and δ carbon atoms, the δ carbon being oxidised to carboxyl. Presumably the remaining four carbon atoms would be split off as succinic acid which is known to be readily oxidised in the body.

The production of succinic acid by the oxidation of fatty acids in the body would provide a means of explaining how fatty acids might give rise to carbohydrates. Succinic acid is known to be easily converted into fumaric and malic acids both in muscle and liver and the latter acid in phlorhizin diabetes is converted into glucose. Whether this takes place *via* lactic acid is uncertain, but if it does, then a process of oxidation of fatty acids can be visualised which, by yielding in turn succinic acid, malic acid and either lactic acid or glucose, would provide a scheme whereby fat could supply the energy for muscular exercise. There is no experimental evidence which shows that muscle can use fat directly and it appears that it must undergo some preliminary oxidative processes elsewhere in the body in order that it may be prepared for use in the muscle mechanism. The oxidative breakdown of fatty acids must therefore follow some scheme which provides substances which will be utilisable by muscle, *e.g.* lactic acid or carbohydrate. The scheme of β oxidation cannot be said to do this. It does not throw any light on the mechanism whereby fat can be transformed into carbohydrate, as it apparently must be if the current ideas on the working of the muscle mechanism are correct. The very common occurrence in the body of the enzyme succinoxidase, discovered by Batelli and Stern [1911], and its rapid action certainly seem to suggest that there is a greater use for it than the oxidation only of glutamic acid after this has undergone previous oxidative deamination to succinic acid.

Some of these problems and speculations which have necessarily arisen as a result of the experiments described in this paper are being investigated in the hope that they will throw light on some aspects of the metabolism of fatty acids which are at present obscure.

EXPERIMENTAL.

Oxidation of stearic acid. Two samples of pure acid (M.P. 69° , M.W. by titration in neutral alcohol 284, iodine number 0.1), obtained from Messrs Kahlbaum and Poulenc Frères, were oxidised and gave the same result. Stearic acid (10 g.), water (250 cc.), and ammonia (4 cc. 23 %) were heated under reflux in a 3 litre flask to 60° in a water-bath. Hydrogen peroxide (21 cc. 30 %) was then added and the temperature gradually raised to 90° (there is considerable frothing). Heating was continued for 4 hours. The liquid was acidified with phosphoric acid, cooled and the precipitated acids and ketone filtered off. The precipitate was converted into soaps with potash in 50 %

alcohol and the solution extracted five times with light petroleum, thus removing the β oxidation product, methylquindecyl ketone. The alcoholic solution of the soaps was then acidified with dilute sulphuric acid, diluted, cooled and filtered. The resulting acid was recrystallised once from a small quantity of methylated spirit when 6–7 g. of nearly pure stearic acid separated. The acid in the mother liquid was neutralised with soda, poured into water and the lead salts precipitated by adding 20 % lead acetate solution. The ether-soluble lead salts have not been fully dealt with. The lead salts insoluble in ether were converted into acids and the latter crystallised from light petroleum. The first fraction was shown to be a new acid which after three recrystallisations from light petroleum melted at 97° . This acid was also obtained directly by crystallisation of the mother liquor acids from light petroleum without the precipitation of the lead salts. A number of these oxidations were carried out and the high melting fraction accumulated until sufficient was obtained for complete analysis and identification, when it was shown to be γ -ketostearic acid. This acid was synthesised from stearylactone and melts at 97° and on mixture with the acid obtained by oxidation did not depress the melting point. Both acids gave an oxime, m.p. 84° , and the analysis and molecular weight of the acid produced by oxidation corresponded with the theoretical.

(Found C = 72.59, 72.63, H = 11.61, 11.59 %, $C_{18}H_{34}O_3$ requires C = 72.48, H = 11.4 %.)

A trace of another acid (possibly δ -ketostearic acid) was obtained, but not in sufficient quantity for identification. The amount of β and γ oxidation products actually separated and weighed represented about 6 % and 1 % respectively of the stearic acid used. The β figure is probably maximal whereas the γ figure is low, due to the difficulty of separation and to the fact that γ -ketostearic acid is readily further oxidised by peroxide. This latter oxidation is now being investigated. The constitution of γ -ketostearic acid has already been proved by the Beckmann reaction on its oxime [Shukov and Schestakov, 1903].

Oxidation of palmitic acid. Following the above method, an acid melting at 91 – 92° was isolated. This acid was more soluble in alcohol and less soluble in light petroleum than palmitic acid and corresponded in properties with γ -ketopalmitic acid which was kindly synthesised for us by Mrs Robinson [1925]. The mixed melting point was 91 – 92° .

Oxidation of myristic acid. The oxidation of this acid was carried out as above but owing to the lower melting point of the acid and the greater solubility in alcohol, the method of separation had to be modified. After separation of the ketone in the usual way, the fatty acids were crystallised from methyl alcohol, the liquid being cooled in ice whereby 60–70 % of unchanged myristic acid was recovered. The acid recovered from the mother liquor was crystallised from 80 % methyl alcohol and yielded a further amount of myristic acid. The acids remaining in the mother liquor were now crystallised

from light petroleum and gave an acid, M.P. 87° , M.W. by titration 244. Calculated for $C_{14}H_{26}O_3$, 242. This acid has been synthesised by Mrs Robinson and from the M.P. and mixed M.P. has been proved to be γ -ketomyristic acid.

Oxidation of n-hexoic (caproic), n-heptoic (oenanthic) and n-octoic (caprylic) acids. The oxidations were carried out as before, the quantities employed being: acid (10 g.), water (250 cc.), ammonia (20 cc. 23 %), and hydrogen peroxide (50 cc. 30 %). As these acids are liquid at the ordinary temperature the fractional crystallisation method of separation had to be abandoned. In each case, the product of oxidation was extracted five times with light petroleum to remove the *n*-propyl-, *n*-butyl-, *n*-amyl-, methyl ketones formed by β oxidation. The liquid was acidified and again extracted five times with light petroleum. This removed the unchanged acid almost completely together with a trace of keto-acid. The resulting liquid was then extracted in a continuous ether extractor for 5-6 hours and the ethereal solution dried and fractionated. Some acetic acid was obtained in each case and also a main fraction boiling from $170-190^{\circ}$ (20 mm.). This was shown to contain the γ - and δ -keto-acids. These were converted into the semicarbazones by adding to the oily fraction a little water, a considerable amount of crystalline sodium acetate and finally semicarbazide hydrochloride. The semicarbazones separated, were filtered off, dried and weighed. From the weight of ketones and of semicarbazones it was calculated that roughly 6 % of the acid had undergone β oxidation and 12 % $\gamma + \delta$ oxidation, and from the fractionation of the semicarbazones it appears probable that the amounts of β , γ and δ oxidation are roughly equal. The melting points (with decomposition) of semicarbazones are known to vary with the rate of heating, but by standardising the latter quite consistent results could be obtained [cf. Vorländer, 1897]. In the case of *hexoic* acid, the keto-acids obtained by oxidation gave a very strong iodoform reaction indicating the presence of δ -keto*hexoic* acid. The semicarbazones were separated by a long and tedious series of fractional crystallisations from methylated spirit into a least soluble portion, melting at 173° , shown to be δ -keto*hexoic* acid semicarbazone, a large number of intermediate fractions melting from $144-153^{\circ}$ and a most soluble portion, melting at 170° , shown to be γ -keto*hexoic* acid semicarbazone. The following melting points and mixed melting points show very definitely that these acids were isolated as semicarbazones in practically pure condition:

Keto <i>hexoic</i> acid semicarbazone	M.P.
γ (synth.), γ (oxidn.)	170°
γ (synth.) + γ (oxidn.)	170
δ (synth.), δ (oxidn.)	173
δ (synth.) + δ (oxidn.)	173
δ (synth.) + γ (synth.)	145-148
δ (synth.) + γ (oxidn.)	145-148
γ (synth.) + δ (oxidn.)	145-148
γ (oxidn.) + δ (oxidn.)	145-148

Dumas nitrogen estimations on the semicarbazones of the oxidation γ - and δ -ketoheptic acids thus separated, gave the following results:

(a) γ : found N = 22.3 %; $C_7H_{13}O_3N_3$ requires N = 22.4 %.

(b) δ : found N = 22.2 %; $C_7H_{13}O_3N_3$ requires N = 22.4 %.

The oximes of the synthetic γ -ketoheptic and δ -ketoheptic acids and of the oxidation mixture of these two acids were made by dissolving hydroxylamine hydrochloride (5 g.) in a small amount of water, adding the keto-acid (6 g.) and, after cooling, mixing with potash (7 g. in a little water) and allowing to stand overnight. The liquid was then acidified, saturated with ammonium sulphate and extracted in a constant ether extractor for 2 or 3 hours. The ethereal solution was dried over calcium chloride and the ether evaporated off. In the case of δ -ketoheptic acid, the oxime readily solidified and was recrystallised from benzene; m.p. 104° . In the case of γ -ketoheptic acid a syrup was obtained which, after standing 3 days over sulphuric acid *in vacuo*; on scratching with a glass rod gradually set to a solid. This was pressed on a porous plate and a little oily substance thus removed. The oxime was dissolved in dry ether, the solution filtered and then precipitated with light petroleum. After several purifications it melted at 67° . In the case of the keto-acids from the oxidation products the oxime was a thick syrup. This was dissolved in benzene and the oximes precipitated with light petroleum as an oil which, after inoculating with a very small crystal of δ -ketoxime, was allowed to stand 3 days *in vacuo* over sulphuric acid. Partial solidification took place and the mass was placed on a clean porous plate. The syrup was absorbed and a white solid residue obtained which crystallised readily from benzene, melted at 104° , and, mixed with synthetic δ -ketoxime, showed no depression of melting point. The absorbed syrup was recovered by extraction of the porous plate with benzene and the oxime precipitated with light petroleum and the process repeated. A little more δ -ketoxime was obtained and the syrup recovered after this operation was finally caused partially to solidify after inoculating with a small crystal of synthetic γ -ketoxime and allowing to stand *in vacuo* for several days. This fraction after purification was shown by mixed melting point to be the γ -ketoxime.

Analysis.

(a) γ : found N = 9.4 %; $C_6H_{11}O_3N$ requires N = 9.7 %.

(b) δ : found N = 9.5 %; $C_6H_{11}O_3N$ requires N = 9.7 %.

In the case of *heptic acid*, the semicarbazones of the oxidation keto-acids were separated as above and gave a less soluble first portion, m.p. 194° , identical with the synthetic δ -ketoheptic acid semicarbazone and a much more soluble one, m.p. 152° , identical with γ -ketoheptic semicarbazone. The mixed melting points of oxidation and synthetic semicarbazones were taken and the Dumas

nitrogen analysis of the oxidation acid semicarbazones gave the following results:

(a) γ : found N = 20.8 %; $C_8H_{15}O_3N_3$ requires N = 20.9 %.

(b) δ : found N = 21.1 %; $C_8H_{15}O_3N_3$ requires N = 20.9 %.

The ketoheptonic acids obtained by oxidation gave a slight iodoform reaction suggesting that a little ϵ oxidation had taken place. It was not found possible however to isolate with certainty a pure sample of the ϵ -semicarbazone (M.P. 144°) from the semicarbazone mixture.

In the case of *octoic acid*, similarly, the semicarbazone mass was by a tedious series of fractionations from alcohol separated into a less soluble first fraction of δ -keto-octoic acid semicarbazone, M.P. 187°, and a much more soluble fraction, γ -keto-octoic acid semicarbazone, M.P. 153°, neither of which depressed the M.P. of the corresponding synthetic semicarbazone. The following Dumas nitrogen determinations were carried out on the oxidation semicarbazones:

(a) γ : found N = 19.8 %; $C_9H_{17}O_3N_3$ requires N = 19.5 %.

(b) δ : found N = 19.7 %; $C_9H_{17}O_3N_3$ requires N = 19.5 %.

Synthesis of keto-acids by means of zinc alkyl iodides.

The method adopted was similar to that of Blaise and Kohler [1910]:

(i) *Preparation of the zinc alkyl iodides.*

Pure zinc filings (90 %) and pure copper filings (10 % of total weight) were heated together in a dry flask in the yellow blowpipe flame until the mass fell to a black powder. The alkyl iodide (1 mol.), ethyl acetate ($\frac{1}{3}$ mol., dried for several days over calcium chloride, distilled and first and last fractions neglected), dry redistilled toluene (double the weight of ethyl acetate) and Zn - Cu couple (double the amount required by the iodide) were heated in a dry flask under reflux at 100–110° for 1½ hours. A second equal amount of toluene was added and, after shaking, the flask was cooled. The white fuming liquid was poured into a dry flask and the couple washed with a little dry toluene. In this way were prepared zinc ethyl-, *n*-propyl-, *n*-butyl-, iodides.

(ii) *Preparation of half ester half acid chlorides of*

(a) *Succinic acid.* Succinic anhydride (1 mol.) was boiled with methyl alcohol (2 mols.) for 45 minutes. The excess of alcohol was distilled off and the mass after solidifying was crystallised from a large quantity of carbon disulphide. The monomethyl ester (M.P. 58°) thus obtained was boiled with three times its weight of redistilled thionyl chloride, the excess of thionyl chloride distilled off *in vacuo* and the methyl ester chloride (B.P. 93°/18 mm.) was collected.

(b) *Glutaric acid.* Glutaric acid was prepared by a method, the details of which were kindly provided by Professor C. K. Ingold. Cyclohexanol

(500 g.) was added, drop by drop, with occasional shaking, to 2700 g. conc. nitric acid in a 5 l. flask provided with an efficient reflux condenser and heated in a boiling water-bath. After the reaction was completed (4–5 hours) the liquid was poured into a basin and allowed to stand overnight. The crystals of adipic acid were filtered off on a glass wool pad in a large Buchner funnel and thoroughly dried in the steam oven, powdered and heated in the oven for 48 hours completely to drive off the nitric acid. A little more was obtained from the mother liquors making the total yield of adipic acid 558 g. This experiment was repeated. Dry adipic acid (300 g.) and baryta (50 g.) were heated in a 1.5 l. distilling flask in an air-bath at 290°. The water coming over at first was neglected and the cyclopentanone collected as long as any liquid distilled (3–4 hours). This was shaken with ether, when a little water separated, and the ethereal solution was dried over calcium chloride and distilled, using a long fractionating column. The cyclopentanone collected at 129–133° weighed 118 g. This experiment was repeated. Cyclopentanone (200 g.) was dropped (as in the oxidation of cyclohexanol) into 2000 cc. of dilute nitric acid (sp.g. 1.2). The resulting liquid was evaporated to dryness, thoroughly dried and freed from nitric acid. The powder obtained was placed in a large Soxhlet thimble and suspended in a flask over boiling benzene, the condensed benzene dripping into the thimble. Almost pure glutaric acid, m.p. 95–96°, separated from the benzene solution on cooling. The extraction was continued until only succinic acid remained in the thimble. 1 kg. cyclohexanol gave 390 g. glutaric acid. Glutaric anhydride was prepared by heating dry glutaric acid with five times its weight of thionyl chloride, evaporating off the excess of the latter *in vacuo* and collecting the fraction boiling at 145–165°/20 mm. This fraction readily solidified and was crystallised from boiling ether when it melted at 55°. The anhydride was boiled with double the theoretical amount of methyl alcohol, the excess of alcohol was distilled off and the monomethyl ester collected at 153°/20 mm. This was boiled with three times its weight of redistilled thionyl chloride, excess of the latter was removed *in vacuo* and the methyl ester chloride collected at 110°/20 mm.

(iii) *Preparation of the keto-acids.*

The methyl ester acid chloride dissolved in its own volume of dry toluene was added drop by drop to the zinc alkyl iodide solution, the latter being about 25 % in excess for the acid chloride used. During the whole operation the reaction flask was kept in ice and shaken constantly. After standing, water was added, a little at a time at first, shaking vigorously. A little precipitated zinc hydroxide was dissolved in dilute sulphuric acid. The toluene solution was separated and washed with dilute ammonia, then with dilute sulphuric acid and finally with dilute bicarbonate and thiosulphate. The solution was dried over anhydrous sodium sulphate and fractionated *in vacuo*. The methyl esters of the keto-acids were then hydrolysed by shaking with strong potash, the product acidified and extracted with ether. (In the case of

γ -ketohectic acid continuous ether extraction was necessary.) The ethereal solution was dried and fractionated *in vacuo*. In this way were prepared:

(a) γ -ketohectic acid; using zinc ethyl iodide and the half-ester half-acid chloride of succinic acid. The acid was collected from 150–180°/20 mm. and set to a hard mass on standing *in vacuo*. This was dissolved in benzene, filtered from a trace of succinic acid and the benzene evaporated to a small volume, when the acid crystallised. Purification by precipitation from benzene solution by light petroleum was also tried. γ -Ketohectic acid melts at 32°, boils at 180°/20 mm. and gives a semicarbazone melting with decomposition at 170°, which appears to be identical with that obtained by Campbell and Thorpe [1910]. The oxime melts at 68°. The methyl ester boils at 110°/20 mm.

Analysis.

Semicarbazone: found N = 22.35 %; $C_7H_{13}O_3N_3$ requires N = 22.4 %.

Oxime: found N = 9.9 %; $C_6H_{11}O_3N$ requires N = 9.7 %.

(b) γ -ketoheptic acid; using zinc propyl iodide and the half-ester half-acid chloride of succinic acid. The acid melts at 48° and gives a semicarbazone, m.p. (decomp.) 152°. The methyl ester boils at 125°/20 mm.

Analysis.

Semicarbazone: found N = 21.1 %; $C_8H_{15}O_3N_3$ requires N = 20.9 %.

(c) γ -keto-octic acid; using zinc butyl iodide and the half-ester half-acid chloride of succinic acid. The acid melts at 53° and gives a semicarbazone, m.p. (decomp.) 153°. The methyl ester boils at 111°/15 mm.

Analysis.

Semicarbazone: found N = 19.8 %; $C_9H_{17}O_3N_3$ requires N = 19.5 %.

(d) δ -ketoheptic acid; using zinc ethyl iodide and the half-ester half-acid chloride of glutaric acid. The acid melts at 50° and gives an oxime m.p. 118° and a semicarbazone, m.p. (decomp.) 194°. The methyl ester boils at 130°/20 mm.

Analysis.

Semicarbazone: found N = 21.2 %; $C_8H_{15}O_3N_3$ requires N = 20.9 %.

(e) δ -keto-octic acid; using zinc propyl iodide and the half-ester half-acid chloride of glutaric acid. The acid gives a semicarbazone, m.p. (decomp.) 187°. The methyl ester boils at 120°/15 mm.

Analysis.

Semicarbazone: found N = 19.7 %; $C_9H_{17}O_3N_3$ requires N = 19.5 %.

Synthesis of δ -ketohectic acid.

The method adopted was essentially that of Bentley and Perkin [1896]. Sodium (3.4 g.) was dissolved in alcohol (45 g.) and to the well-cooled solution acetoacetic ester (19 g.) and then β -iodopropionic ester (33 g.) were added. After standing for half an hour the whole was heated on a water-bath for

2 hours, water added and the oil extracted with ether. The ethereal solution was washed with a little water containing SO_2 , dried over CaCl_2 , evaporated and the oil fractionated *in vacuo*. Acetylglutaric ester was collected at $190\text{--}200^\circ/50$ mm. and boiled under reflux with five times its volume of dilute (1 : 2) HCl for 5 hours. The liquid was saturated with ammonium sulphate and extracted six times with ether. The ethereal solution was dried over CaCl_2 and fractionated, the keto-acid being collected at $195\text{--}200^\circ/65$ mm. The acid is an oil which forms a crystalline monohydrate, M.P. 35° , and very readily gives the iodoform reaction. The semicarbazone melts at 173° and the oxime at 103° .

Analysis.

Semicarbazone: found $\text{N} = 22.6\%$; $\text{C}_7\text{H}_{13}\text{O}_3\text{N}_3$ requires $\text{N} = 22.4\%$.

Oxime: found $\text{N} = 9.6\%$; $\text{C}_6\text{H}_{11}\text{O}_3\text{N}$ requires $\text{N} = 9.7\%$.

Synthesis of γ -ketostearic acid. Stearolactone, M.P. 52° , was prepared by the action of sulphuric acid on oleic acid [Schukov and Schestakov, 1908] and oxidised to γ -ketostearic acid by means of chromic acid in acetic acid solution [Clutterbuck, 1924].

The acid melts at 97° and was converted into its oxime as follows: γ -ketostearic acid (1 mol.), hydroxylamine hydrochloride (1 mol.) and caustic soda (3 mols.) in alcoholic solution were boiled for 2 hours on the water-bath. The alcohol was distilled off, the residue dissolved in water and, after cooling, made just acid. The precipitated acids, after extraction with ether and removal of the latter, were boiled with light petroleum in which the oxime was more soluble than the unchanged keto-acid. The oxime when pure melted at 85° . The reaction was studied under varying conditions but the yield was never theoretical and consequently oxime formation could not be used for the quantitative estimation of γ -ketostearic acid in the oxidation mixture.

Analysis. Found $\text{N} = 4.29\%$; $\text{C}_{18}\text{H}_{35}\text{O}_3\text{N}$ requires $\text{N} = 4.49\%$.

γ -Ketostearic acid phenylhydrazone was prepared by dissolving the keto-acid in warm glacial acetic acid and adding a few drops of phenylhydrazine. The hydrazone crystallised out on standing and was recrystallised from light petroleum. It melts with total decomposition at 80° and therefore great care was required in its purification.

Analysis. Found $\text{N} = 7.0\%$; $\text{C}_{24}\text{H}_{40}\text{O}_2\text{N}_2$ requires $\text{N} = 7.2\%$.

Synthesis of γ -ketopalmitic and γ -ketomyristic acids. These were kindly prepared by Mrs Robinson [1925]. The former melts at $91\text{--}92^\circ$ and gives an oxime, M.P. 54° . The latter melts at 87° .

SUMMARY.

(1) The ammonium salts of normal saturated fatty acids when oxidised with hydrogen peroxide yield products which show that oxidation occurs at the γ and δ carbon atoms as well as the β carbon atom (and possibly also the α).

(2) From myristic, palmitic and stearic acids, the corresponding γ -keto-acids have been isolated as products of oxidation.

(3) From *n*-hexoic, *n*-heptoic and *n*-octoic acids the corresponding γ - and δ -keto-acids have been isolated as semicarbazones and in some cases also as oximes. Several of these acids had not been previously prepared and have been synthesised for reference.

(4) The possibility of the occurrence of γ and δ oxidation in the body is discussed and it is pointed out that if further investigation proves that it does occur, some light may be thrown on the processes by which fat may be utilised to supply energy for the contraction process of muscle.

(5) The first step in the oxidation of normal saturated fatty acids appears to be the formation of a series of keto-acids and not of hydroxy-acids. The latter, although almost insoluble in light petroleum, could not be detected. This would support the view that the hydroxy-acids obtained in experiments *in vivo* result from reduction of keto-acids rather than from direct oxidation of saturated acids.

The authors wish to express their indebtedness to the Government Grant Committee of the Royal Society for a grant in aid of the expenses of this investigation.

REFERENCES.

- Batelli and Stern (1911). *Biochem. Z.* **30**, 172.
Bentley and Perkin (1896). *J. Chem. Soc.* **69**, 1511.
Blaise and Kohler (1910). *Bull. Soc. Chim.* **iv**, **7**, 215.
Böttker (1912). *J. prakt. Chem.* [2], **85**, 221.
Bougault (1910). *Compt. Rend. Acad. Sci.* **150**, 874.
Cahen and Hurtley (1917). *Biochem. J.* **11**, 164.
Campbell and Thorpe (1910). *J. Chem. Soc.* **97**, 1315.
Clutterbuck (1924). *J. Chem. Soc.* **125**, 2330.
Dakin (1908, 1, 2, 3, 4). *J. Biol. Chem.* **4**, 77, 221, 227, 419.
— (1908, 5). *J. Biol. Chem.* **5**, 173, 303.
— (1909). *J. Biol. Chem.* **6**, 203, 221.
— (1910). *Amer. Chem. J.* **44**, 41.
— (1922). *Oxidations and Reductions in the Animal Body*. Monograph. Longmans.
Embden, Salomon and Schmidt (1906, 2). *Beitr. chem. Physiol. Path.* **8**, 129.
Embden and Kalberlah (1906, 1). *Beitr. chem. Physiol. Path.* **8**, 121.
Embden and Marx (1908). *Beitr. chem. Physiol. Path.* **11**, 318.
Knoop (1904). *Beitr. chem. Physiol. Path.* **6**, 150.
Knoop and Oeser (1914). *Z. physiol. Chem.* **89**, 141.
Raper (1914). *Biochem. J.* **8**, 320.
Robinson and Robinson (1925). *J. Chem. Soc.* **127**, 180.
Schukov and Schestakov (1903). *J. Russ. Phys. Chem. Soc.* **35**, 1.
— (1908). *J. Russ. Phys. Chem. Soc.* **40**, 830.
Vorländer (1897). *Liebig's Ann.* **294**, 269.