4. The other acidic fraction contains peptides (other than glutathione) built up from glutamic acid, cystine (or cysteine) and glycine. Some of these peptides contain a strongly acidic group (so far unidentified), so that the molecule has zero net charge at pH 2. One of these peptides is a tripeptide containing an *N*-terminal glutamic acid residue.

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The Metabolism of Propionic Acid

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One pathway of propionic acid metabolism has been shown by Flavin, Ortiz & Ochoa (1956) to be: propionic acid $\rightarrow$ methylmalonic acid $\rightarrow$ succinic acid $\rightarrow$ citric acid cycle. That this is not the only pathway is indicated by the work of Gray, Pilgrim, Rodda & Weller (1951) who demonstrated the synthesis of *n*-valeric acid from propionate and acetate in the rumen. Recently *n*-undecanoic, *n*-tridecanoic *n*-

pentadecanoic and *n*-heptadecanoic acids have been isolated from some natural fats by Hansen, Shorland & Cooke (1954*a*, *b*, 1955) and by Shorland, Gerson & Hansen (1955). James & Martin (1956), James & Wheatley (1956) and Wheatley & James (1957) have demonstrated by means of gas-liquid chromatography the widespread occurrence of these straight-chain odd-numbered acids in natural

fats. Such odd-numbered acids may well be derived from an odd-numbered precursor, propionic acid being the most likely.

In the work to be described fatty acid fractions covering the range C_2 – C_{18} from a cow udder perfused with $[1-^{14}C]$ propionate were analysed by gas-liquid chromatography. The short-chain C_2 , C_4 and C_6 acids were separated as the free acids (James & Martin, 1952) and the longer-chain acids as methyl esters (James & Martin, 1956). The separated substances were collected, plated out and counted in the conventional manner. The same technique was also used with a sample of fatty acids from goat milk isolated after injection of $[1-^{14}C]$ acetate (Popják, French & Folley, 1951a).

EXPERIMENTAL

(1) One-half of an udder removed from lactating cow at the slaughter-house was perfused with 9 l. of heparinized oxygenated blood for 2 hr. by the technique of Peeters & Massart (1952). The udder had previously been emptied by milking. At the beginning of the perfusion 50 mg. of $[1-^{14}C]$ propionate (0.5 mc) dissolved in 20 ml. of water was added to the perfusion blood. At the end of the experiment milk was collected after intra-arterial injection of posterior pituitary extract (10 i.u.). Fatty acid fractions from milk and udder tissue were isolated by the methods developed by Popják *et al.* (1951a) and Popják, French, Hunter & Martin (1951b) and divided into steam-volatile water-soluble, steam-volatile water-insoluble and non-volatile acids. The latter were further fractionated by the lead-salt technique to give a long-chain saturated-acid fraction. All except the steam-volatile soluble acids were converted into methyl esters by treatment with HCl in methanol.

(2) *Gas-liquid chromatography.* The steam-volatile water-soluble acids were separated on the gas-liquid chromatogram described by James & Martin (1952) and estimated by automatic titration. The contents of the cell were removed after each acid had emerged to give samples of sodium salts of the acids contaminated only with phenol red indicator.

Methyl esters of the acids from the other fractions were separated in the manner described by James & Martin (1956). The samples of fatty acid esters were applied to the column with a calibrated micropipette. The separated substances emerged from the gas-density meter (Martin & James, 1956) as vapours in the nitrogen stream at 197° and were collected by attaching with a short length of silicone tubing a calcium chloride tube loosely packed with cotton wool (defatted with ether and acetone) that had been wetted with methanol. This collection technique was found necessary as simple cooling of the gas stream containing vapours of high molecular weight resulted in the formation of a mist of small droplets that could not be condensed. After each peak had emerged (as indicated on the chart from the recording galvanometer) the collecting tubes were changed. Each tube was then washed through with three 5 ml. portions of ether into a boiling tube. The ether was removed by careful distillation, the sides of the tube being cooled frequently to wash down any adherent material with condensed solvent. When only a few μ l. remained the solution was transferred with a micropipette to a polythene planchet of area 2 cm.² (of the type used by

Popják, 1950) containing two pieces of lens tissue paper. The tube was washed twice with 10–20 μ l. of ether to remove the last traces of material, and the washings also were transferred to the planchet. The disks were then counted with an end-window counter for 30 min. only, to minimize any losses by evaporation. No counting corrections were made for sample thickness since the amounts of material handled were usually so small (0.04–2 mg.) and because of contamination with variable amounts of stationary phase from the column. Results were reproducible to within 10%.

Oxidation of n-pentadecanoic acid

The peak corresponding to *n*-pentadecanoic acid from isolated perfused cow udder was collected, washed into a boiling tube with ether as described, the ether evaporated and 0.1 ml. of acetic acid and 20 mg. of crystalline $KMnO_4$ were added. The solution was heated on the water-bath for 4 hr., the acetic acid being replenished occasionally. Excess acetic acid was removed *in vacuo* (water-pump) and a solution of potassium metabisulphite added to remove manganese dioxide. The solution was extracted three times with ether, dried with anhydrous Na_2SO_4 , and diazomethane in ether added. The mixture was evaporated and the residue dissolved in ethanol and applied to the gas-liquid chromatogram.

Quantitative estimations

The areas under the peaks were measured by triangulation as described previously (James & Wheatley, 1956) and the areas corrected to methyl palmitate as standard (1 cm.² = 0.067 mg. of methyl palmitate).

RESULTS AND DISCUSSION

The occurrence of the odd-numbered saturated fatty acids in many animal fats may be due either to diet or to biosynthesis in the animal organism. The existence of these acids in goat milk obtained from an animal metabolizing $[1-^{14}C]$ acetate had already been demonstrated (James & Martin, 1956). In Table 1 column 3 is given a quantitative analysis of

Table 1. *Quantitative analysis of long-chain saturated acids from cow milk and udder and goat milk*

Acid	Percentage present		
	Cow		Goat milk
	In milk	In udder	
<i>n</i> -Hexanoic	—	Trace	Trace
<i>n</i> -Octanoic	Trace	Trace	Trace
<i>n</i> -Decanoic	0.17	0.14	Trace
<i>n</i> -Undecanoic	Trace	Trace	Trace
<i>n</i> -Dodecanoic	1.60	0.61	0.73
Branched tridecanoic	0.04	Trace	Trace
<i>n</i> -Tridecanoic	0.04	Trace	Trace
Branched tetradecanoic	0.035	0.07	Trace
<i>n</i> -Tetradecanoic	11.2	6.2	9.2
Branched pentadecanoic	0.59	0.56	0.43
<i>n</i> -Pentadecanoic	1.7	1.57	1.54
Branched hexadecanoic	0.56	0.45	0.41
<i>n</i> -Hexadecanoic	44.0	36.8	43.0
Branched heptadecanoic	2.8	2.3	1.87
<i>n</i> -Heptadecanoic	2.2	1.65	1.75
Branched octadecanoic	9.0	13.3	0.73
<i>n</i> -Octadecanoic	26.4	36.2	40.0

Table 2. *Relative activities of fatty acids isolated from goat-milk fat after administration of [1-¹⁴C]acetate*

Activities are expressed as counts in 30 min./cm.² of corrected area under peak. Average values from six determinations.

<i>n</i> -Tetradecanoic	1009
Branched pentadecanoic	740
<i>n</i> -Pentadecanoic	305
<i>n</i> -Hexadecanoic	1023
Branched heptadecanoic	206
<i>n</i> -Heptadecanoic	262
<i>n</i> -Octadecanoic	60

Table 3. *Quantitative analysis of water-insoluble acids from milk fat and udder fat (cow)*

Acid	Percentage present	
	In milk	In udder
Branched C ₇	—	1.0
<i>n</i> -C ₇	Trace only	Overlapped by branched acid
<i>n</i> -C ₈	1.75	1.0
Branched C ₉	0.08	1.3
<i>n</i> -C ₉	0.26	
Branched C ₁₀	0.03	1.0
<i>n</i> -C ₁₀	8.0	11.3
Branched C ₁₁	0.65	1.7
<i>n</i> -C ₁₁	0.52	0.6
<i>n</i> -Branched C ₁₂	0.12	0.46
Branched C ₁₂	0.10	0.7
Mono-unsaturated C ₁₂	0.38	
<i>n</i> -C ₁₂	10.3	15.8
Branched C ₁₃	0.95	0.87
<i>n</i> -C ₁₃	0.75	0.60
Di-unsaturated C ₁₄	0.27	0.19
Mono-unsaturated C ₁₄	2.2	1.02
Branched C ₁₄	2.2	
<i>n</i> -C ₁₄	15.2	18.0
Branched C ₁₅	1.74	2.9
<i>n</i> -C ₁₅	1.15	1.8
Di-unsaturated C ₁₆	1.17	0.4
Mono-unsaturated C ₁₆	3.12	3.1
Branched C ₁₆	0.28	
<i>n</i> -C ₁₆	21.0	17.7
Branched C ₁₇	2.46	—
<i>n</i> -C ₁₇	1.3	—
Mono-unsaturated C ₁₈	11.6	9.6
Branched C ₁₈	6.8	
<i>n</i> -C ₁₈	7.2	5.3

the main components of the long-chain saturated acid fraction from this goat milk. The main components were isolated by the gas-liquid chromatogram, and in Table 2 is shown a list of their relative activities. The relative activities of *n*-tetradecanoic, *n*-hexadecanoic and *n*-octadecanoic acids are very similar to the values reported by Popják *et al.* (1951*b*) for the same material. The odd-numbered straight-chain acids are more radioactive than stearic acid, showing that they are synthesized in part from acetate and are not derived from the diet.

Since acetate seemed to be involved in the synthesis of the odd-numbered acids an odd-numbered precursor is necessary, and propionic

acid, as a common component of rumen acids, is the most likely. The fatty acids isolated from a cow udder perfused with [1-¹⁴C]propionate were analysed on the gas chromatogram with the results shown in Tables 1 and 3. In Table 4 are shown the relative radioactivities of the components isolated, high activity being associated only with the peaks assigned to the odd-numbered straight-chain acids.

The results obtained with the fats from goat milk and cow udder can be interpreted only as showing that the odd-numbered straight-chain acids from *n*-heptanoic to *n*-heptadecanoic are synthesized by condensation of acetate with propionic acid.

Degradation of *n*-pentadecanoic acid

The fraction considered to consist of *n*-pentadecanoic acid was oxidized with KMnO₄ and the mixture of acids produced was methylated and separated on the gas chromatogram. A peak in the position of methyl tetradecanoate was found together with small amounts of shorter-chain acids C₁₃, C₁₂ and C₁₁. The tetradecanoate showed radioactivity of the same order as the pentadecanoate. In a repetition of this experiment the degradation was carried further and a small quantity of *n*-heptadecanoic acid was obtained; this too was radioactive. These experiments suggest that the larger part of the radioactivity is concentrated at the methyl end of the chain, and demonstrate that the substance responsible for the peak in the position expected for *n*-pentadecanoic acid is, in fact, that substance, since on oxidative degradation it gave rise to peaks in the positions of *n*-tetradecanoate, *n*-tridecanoate, etc.

Table 4. *Relative activities of fatty acids isolated from cow udder after administration of [1-¹⁴C]-propionic acid*

Counts in 30 min./cm.² of corrected peak area.

Acid	Count/unit of corrected area
<i>n</i> -Octanoic + heptanoic	102
Branched octanoic	0
<i>n</i> -Octanoic	69
Branched nonanoic	90
<i>n</i> -Nonanoic + branched decanoic	1310
<i>n</i> -Decanoic	15
<i>n</i> -Undecanoic	7683
Branched dodecanoic	996
<i>n</i> -Dodecanoic	60
Highly branched tridecanoic	66
Branched tridecanoic	660
<i>n</i> -Tridecanoic	8310
<i>n</i> -Tetradecanoic	10
Branched pentadecanoic	111
<i>n</i> -Pentadecanoic	6200
<i>n</i> -Hexadecanoic	35
Branched heptadecanoic	22
<i>n</i> -Heptadecanoic	438
Branched octadecanoic	12
<i>n</i> -Octadecanoic	2

Short-chain acids

The steam-volatile water-soluble fatty acid fraction from cow udder was separated into its components on the gas-liquid chromatogram and was found to consist of acetic, *n*-butyric and *n*-caproic acids. Neither propionic acid nor *n*-valeric acid could be detected. In Table 5 is shown the activities of the isolated acids. The finding of appreciable activity in these even-numbered acids was surprising, since on the degradative scheme put forward by Flavin *et al.* (1956) [1^{14}C]propionate could not give rise to labelled acetate.

However, it is known that [1^{14}C]acetate can give rise to labelled formate in the rabbit (Popják & James, unpublished work) and similar loss of a methyl carbon from [1^{14}C]propionate would produce [1^{14}C]acetate. Further work is needed to determine the sequence of reactions involved.

From Table 4 it can be seen that both branched-chain acids and straight-chain even-numbered acids show radioactivity. The relatively high activity of the acetate derived from propionate is clearly sufficient to account for these activities.

There are thus at least three distinct pathways of propionate metabolism:

Relative pool sizes

In Table 3 is shown a comparison of the relative amounts of water-insoluble acids in milk and udder. No striking differences between the two fats are indicated.

Table 5. *Relative activities of short-chain acids (as sodium salts) isolated from cow udder and milk after perfusion with [1^{14}C]propionate*

Acid	Specific activity ($\mu\text{mc/mg.}$)	
	Udder	Milk
Acetic	1	0.1
<i>n</i> -Butyric	0.043	0.015
<i>n</i> -Hexanoic	0.067	0.06

A quantitative comparison of long-chain saturated fatty acids from the two fats obtained from cow milk and udder is given in Table 1. The two are very similar, even stearic acid being present in much the same amount in both. The low activity of the stearic acid in milk synthesized from [1^{14}C]acetate cannot, therefore, be explained on the basis of dilution by a large pool of the acid in the udder (Popják *et al.* 1951*a*). Duncombe & Glascock (1954) have, however, shown that milk stearic acid is derived in part from blood so that a complete balance sheet between milk and udder fats cannot therefore be drawn up. The results presented here demonstrate an apparent equilibrium between fatty acids in the udder and in the milk. The similarity in composition of goat milk and cow milk is most striking.

SUMMARY

1. The relative radioactivities of odd- and even-numbered fatty acids obtained from goat milk after injection of [1^{14}C]acetate have been determined after separation of the compounds by gas-liquid chromatography. Perceptible activity was demonstrated in the odd-numbered acids.

2. Fatty acids from a cow udder perfused with [1^{14}C]propionate have been separated on the gas chromatogram and their radioactivity was determined. Highest activity was found in the odd-numbered fatty acid fractions.

3. Oxidative degradation of *n*-pentadecanoic acid isolated from the cow udder by gas chromatography produced a variety of radioactive shorter-chain fatty acids.

4. It is concluded that the naturally occurring odd-numbered fatty acids are synthesized by condensation of acetate with propionate by a mechanism similar to that obtaining for the even-numbered fatty acids.

5. Acetate, *n*-butyrate and *n*-caproate isolated from the udder after perfusion with [1^{14}C]propionate were found to be radioactive. No scheme of degradation yet put forward can account for this. An analogous conversion of [1^{14}C]acetate into [1^{14}C]formate in the rabbit is known to occur.

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Hormones and Liver Cytoplasm

2. ADENOSINE TRIPHOSPHATASE, GLUCOSE 6-PHOSPHATASE AND XANTHINE OXIDASE AS AFFECTED BY HYPOPHYSECTOMY, GROWTH-HORMONE TREATMENT AND ADRENALECTOMY*

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The yield of the mitochondrial fraction obtained from rat liver by differential centrifuging is decreased after adrenalectomy, and also after hypophysectomy unless growth hormone is administered; on the other hand, the yield of the microsomal fraction is increased after hypophysectomy, whether or not growth hormone is given (Reid, 1955, 1956). It cannot be assumed that there are parallel changes in the amounts of the enzymes characteristically present in the different cytoplasmic elements. Data bearing on this question are presented here and in the following paper (Stevens & Reid, 1956).

The enzymes adenosine triphosphatase (ATPase), glucose 6-phosphatase and xanthine oxidase have been selected for study, because of their different locations in normal cytoplasm and also because of their possible intrinsic interest in relation to hormonal control of liver metabolism.

EXPERIMENTAL

Treatment of animals

The experiments were performed with young albino male rats, which were fed on a protein-rich diet in restricted amount and were studied 1½–3 weeks after any operative

procedure. Treatment with the growth-hormone preparation, which was virtually free of thyrotrophin, was given for 8 days at a daily dose of 0.3 mg., as described in a previous paper (Reid, O'Neal, Stevens & Burnop, 1956) in which fuller details of the general procedures are given. Some experiments were performed with rats given thiouracil or thyroxine as in the experiments of Glock & McLean (1955).

To minimize the effect of any day-to-day variations in environment or technique, the values for experimental rats were not directly averaged, but were compared with those for control rats (intact or sham-operated) studied simultaneously, the differences being averaged. At least six untreated hypophysectomized rats, five hypophysectomized rats given growth hormone and six adrenalectomized rats were used in the study of each enzyme.

Isolation of tissue fractions

The procedures for isolation were, unless otherwise stated, as described previously (Reid *et al.* 1956). One modification, which was introduced with the aim of stabilizing glucose 6-phosphatase (Beaufay, Hers, Berthet & Duve, 1954), was the addition of ethylenediaminetetraacetic acid (EDTA; 0.001 M) to the isotonic sucrose solution other than that used for the preliminary perfusion of the liver.

The 'nuclear fraction' was discarded, and the supernatant 'cytoplasmic fraction' was further centrifuged to give a mitochondrial fraction (which was washed once, without removal of the 'fluffy layer'), a microsomal fraction and a supernatant fraction. These centrifugings were performed in an angle-head centrifuge under conditions which have been described elsewhere (Reid, 1955, 1956), the final centrifuging being at 20 000 g for 90 min. The mitochondrial and microsomal fractions were resuspended in the sucrose medium. Portions of each isolated fraction were taken for certain of the enzymic assays, as indicated below; the remainder was frozen by immersion in a mixture of acetone and solid CO₂, and was stored at -30°.

* The paper by Reid, O'Neal, Stevens & Burnop (1956) is to be regarded as Part 1.

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