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# Fatty Acids and Intestinal Metabolism

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Long-chain fatty acids have inhibitory effects on many metabolic processes *in vitro*. Munoz & Leloir (1943) observed that decanoic acid, lauric acid, palmitic acid, stearic acid or oleic acid at a concentration of 3 mM inhibited the respiration of a particle preparation from guinea-pig liver. Fluck & Pritham (1961) found that, at a concentration of 1·5 mM, decanoic acid, lauric acid or myristic acid inhibited the conversion of mevalonic acid into cholesterol by rat-liver homogenates by over 90 %. Ahmed & Scholefield (1961*a*, *b*) observed that decanoic acid at concentrations above 1·34 mM inhibited the respiration of rat-liver, -kidney and -brain slices. The incorporation of inorganic phosphate into ATP and ADP was also inhibited.

Since the intestinal mucosa probably absorbs 60 % of the dietary fatty acids in the form of free fatty acids the effect of a series of fatty acids on rat intestine has been studied. An inhibition of respiration was observed that depended on the chain length of the fatty acid and its concentration. A study was also carried out on the effect of fatty acids on the uptake and metabolism of glucose by intestine *in vitro* to elucidate the nature of the respiratory inhibition.

## EXPERIMENTAL

### *Materials*

*Animals.* Wistar strain rats, approx. 200 g. each, fed *ad lib.* on a diet of rat cubes (Oxo Ltd., London) were starved for 24 hr. before use.

*Chemicals and enzymes.* All solvents were of analytical grade. Fatty acids were obtained from L. Light and Co. Ltd., Colnbrook, Bucks., and the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. [U-$^{14}$C]-Glucose and [1-$^{14}$C]glutamic acid were obtained from The Radiochemical Centre, Amersham, Bucks. 2,5-Diphenyloxazole was obtained from L. Light and Co. Ltd. and from Nash and Thompson, Tolworth, Surrey. Lactate dehydrogenase and purified peroxidase were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, West Germany, and glucose oxidase was obtained from George T. Gurr Ltd., London.

Fatty acid solutions were prepared by dissolving the requisite amount of fatty acid in ethanol–ether (1:1, v/v). The calculated equivalent of aqueous NaOH was added and the mixture evaporated to dryness at 60° on a rotary evaporator. The solid was then taken up in the appropriate saline medium, pH 6·8, with heating on a water bath if necessary to disperse the material. The fatty acids are referred to below as undissociated acids since these form the major component of the mixture at this pH for fatty acids with 12 or more carbon atoms (Schmidt-Nielsen, 1946).

## Methods

*Analytical procedures.* Dry weights were determined by drying the tissue overnight at 110°. Respiration was measured by following the oxygen uptake by standard manometric techniques at 37° in an atmosphere of $O_2$. Glucose was determined by the glucose-oxidase method (Huggett & Nixon, 1957) and by the anthrone method (Roe, 1955). Lactic acid was determined spectrophotometrically in the presence of lactate dehydrogenase and $NAD^+$ by the method of Hohorst, Kreutz & Bücher (1959).

*Tissue preparations.* Rats were killed by stunning and decapitation. The abdomen was opened and the small intestine severed at both ends. It was washed out *in situ* by inserting a cannula into the duodenum and passing 50 ml. of oxygenated buffered saline at 30–38° through it under gentle pressure from a syringe. It was then stripped from the mesentery and cut into 2 mm. segments. The segments were washed in oxygenated buffered saline, blotted and weighed on a torsion balance. A tissue wet weight of 300–500 mg. was used per manometer vessel. This was 10–30 segments from random positions in the intestine of two rats. Cannulated everted intestinal sacs on glass cannulae were prepared and incubated by the method of Crane & Wilson (1958).

Tissue was incubated either in bicarbonate-buffered saline (Krebs & Henseleit, 1932) gassed with $O_2+CO_2$ (95+5), or in phosphate-buffered saline (Krebs, 1933) gassed with $O_2$, both at pH 6·8. Calcium and magnesium salts were omitted from both media to avoid the precipitation of the fatty acids. This had no effect on the respiration of intestinal rings.

*Determination of radioactivity.* After incubation in the presence of $^{14}C$-labelled substrates, the filter papers and the NaOH were removed from the centre well, which was then washed out. The paper and washings were soaked in carrier $Na_2CO_3$ solution which was then plated by the method of Sakami (1955) as $BaCO_3$, all-glass apparatus being used. The plates, of finite thickness, were counted in a gas-flow proportional counter (D. 47; Nuclear Chicago Corp., Des Plaines, Ill., U.S.A.), and the results were corrected for background and calculated for infinite thinness by using a calibration curve. All samples were counted to 10000 counts, giving a reliable error of 1·6%.

Lipid samples labelled with $^{14}C$ were counted in a Ekco liquid-scintillation counter. The lipid was dissolved in sulphur-free toluene, and 1·0 ml. was added to 4·0 ml. of toluene containing 12 mg. of diphenyloxazole in a counting vessel, after determination of the background. All samples were counted to 5000 counts, and quenching was measured by using an internal standard.

*Extraction of lipids.* Since intestinal rings lose lipids into the medium when incubated at temperatures above 25° (Dawson & Isselbacher, 1960) the incubation medium and intestinal rings were freeze-dried after the addition of 0·8 ml. of 10% (w/v) sucrose. The dry material was extracted with two 5·0 ml. portions of chloroform–methanol (2:1, v/v) and the lipid purified by the method of Folch, Lees & Sloane-Stanley (1957) except that during the washing procedures the phases were mixed by shaking vigorously, to ensure the removal of radioactive contaminants. The solution was finally evaporated to dryness at 60° under $N_2$ and the lipid redissolved in toluene for radioactivity determinations.

*Extraction of glucose and lactic acid.* The contents of the manometer vessel were centrifuged and the supernatant was deproteinized with $HClO_4$. The sedimented rings were homogenized with $HClO_4$ and then centrifuged. The $HClO_4$ was removed from the extracts by neutralizing it with KOH and centrifuging the precipitated $KClO_4$ down in the cold (approx. 0–4°). The supernatants were stored frozen to await analysis.

## RESULTS

*Effect of fatty acids on the respiration of rings of small intestine.* The effect of the saturated straight-chain $C_6$–$C_{20}$ fatty acids on the respiration of intestinal rings incubated in phosphate-buffered saline containing glucose (5 mM) is shown in Fig. 1. None of the fatty acids stimulated respiration appreciably but many inhibited respiration, especially at a concentration of 6·0 mM. At 0·6 mM the inhibition of respiration increased, in general, as the chain length of the fatty acid increased, reaching a maximum at myristic acid. With further increase in the chain length the inhibition decreased sharply. Lauric acid, tridecanoic acid and myristic acid decreased respiration to 80, 75 and 66% respectively of the respiration of tissue incubated in the absence of added fatty acids. At a concentration of 6·0 mM all the fatty acids studied except arachidic acid inhibited respiration. Increasing chain length had the same effect as at 0·6 mM except that the maximum inhibition was shown by shorter-chain fatty acids. Hexanoic acid inhibited by 16% and the $C_9$–$C_{13}$ acids all inhibited by more

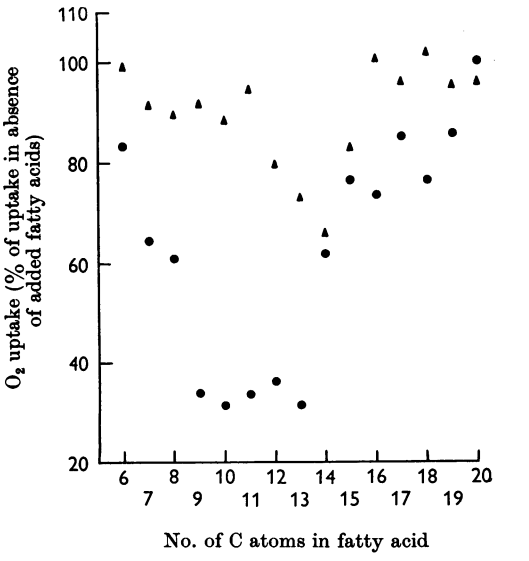


Fig. 1. Effect of $C_6$–$C_{20}$ fatty acids on the oyxgen uptake by rings of intestine incubated in phosphate-buffered saline, pH 6·8, containing glucose (5 mM) at 37° for 1 hr. ▲, 0·6 mM-Fatty acids; ●, 6·0 mM-fatty acids.

than 60 %. Further increase in chain length resulted in a decreased inhibition. The increase in concentration from 0·6 mM to 6·0 mM had least effect on the inhibitory activity of the fatty acids of chain length $C_{14}$ or longer. The inhibition by myristic acid was only 4·6 % more at 6·0 mM than at 0·6 mM.

*Effect of fatty acids on the oxidation of glucose.* The inhibition of respiration by fatty acids also resulted in an inhibition of the oxidation of glucose as measured by the production of $^{14}CO_2$ when [U-$^{14}$C]-glucose was present in the medium (Table 1). The inhibition of glucose oxidation followed the same pattern with respect to fatty acid chain length and concentration as the inhibition of respiration. At 0·6 mM myristic acid inhibited by 57 % and at 6·0 mM it inhibited by 55 %. The inhibition of glucose oxidation was usually more extensive than the inhibition of respiration, especially with the most inhibitory fatty acids. Lauric acid at 0·6 mM inhibited respiration by 20·3 % but it inhibited glucose oxidation by 43·6 %, and at 6·0 mM respiration was inhibited by 63·7 % and glucose oxidation by 90·3 %.

*Effect of fatty acids on the incorporation of radioactivity from* [U-$^{14}$C]*glucose into lipids by intestinal rings.* The incubation of many tissues in the presence of fatty acids results in a stimulation of the incorporation of glucose carbon into the glycerol moiety of the tissue glycerides. This process is important during the absorption of fatty acids from the intestine. However, when rat-intestinal rings were incubated with fatty acids very few of the fatty acids stimulated the incorporation of $^{14}$C from [U-$^{14}$C]glucose into the lipids of the tissue and medium (Table 1). At a concentration of 0·6 mM, octanoic acid, nonanoic acid, palmitic acid, margaric acid and stearic acid stimulated the incorporation of glucose carbon into lipids, and at 6·0 mM only margaric acid, nonadecanoic acid and arachidic acid stimulated. The stimulation varied from 3 % with nonanoic acid to 29 % with stearic acid. Of those fatty acids that did not increase the incorporation of glucose, 0·6 mM-hexanoic acid had no effect, and the other fatty acids inhibited, at both concentrations. The pattern of inhibition followed the inhibition of respiration and oxidation of glucose, although it was greater than the inhibition of respiration, but less than the inhibition of glucose oxidation. In the presence of 6·0 mM-undecanoic acid respiration was inhibited by 66 %, the incorporation of glucose carbon into lipid by 82·3 % and the oxidation of glucose by 91·8 %.

*Effect of lauric acid concentration on the respiration of intestinal rings.* Since lauric acid was one of the fatty acids that had the greatest inhibitory effect on the respiration of intestinal rings, its action

Table 1. *Effect of saturated straight-chain $C_6$–$C_{20}$ fatty acids on the oxidation of glucose and on the incorporation of glucose into the lipids of rat intestine* in vitro

Intestinal rings (460–500 mg. wet wt. of tissue) were incubated for 1 hr. in 5 ml. of phosphate-buffered saline containing glucose (5·0 mM), labelled with [U-$^{14}$C]glucose (2·93 × $10^4$ counts/min./μmole), and fatty acid (0·6 or 6·0 mM). Results are expressed as percentages of the controls incubated without added fatty acids. For $^{14}CO_2$, 100 % represents 3·12 μmoles of glucose oxidized/g. wet wt. of tissue/hr. For $^{14}$C in lipid, 100 % represents 0·494 μmole of glucose incorporated into lipid/g. wet wt. of tissue/hr.

| Fatty acid | $C_6$ | | $C_7$ | | $C_8$ | | $C_9$ | | $C_{10}$ | | $C_{11}$ | | $C_{12}$ | | $C_{13}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (mM) | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 |
| $^{14}CO_2$ produced | 74 | 75 | 89 | 90 | 71 | 62 | 107 | 74 | 74 | 8 | 55 | 8 | 57 | 10 | 59 | 15 |
| $^{14}$C in lipid | 100 | 90 | 95 | 59 | 107 | 25 | 103 | 14 | 90 | 21 | 54 | 18 | 63 | 21 | 68 | 22 |

| Fatty acid | $C_{14}$ | | $C_{15}$ | | $C_{16}$ | | $C_{17}$ | | $C_{18}$ | | $C_{19}$ | | $C_{20}$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (mM) | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 | 0·6 | 6·0 |
| $^{14}CO_2$ produced | 43 | 45 | 58 | 59 | 74 | 57 | 83 | 68 | 89 | 57 | 83 | 65 | 96 | 84 |
| $^{14}$C in lipid | 82 | 66 | 91 | 57 | 111 | 79 | 108 | 107 | 129 | 76 | 97 | 108 | 83 | 112 |

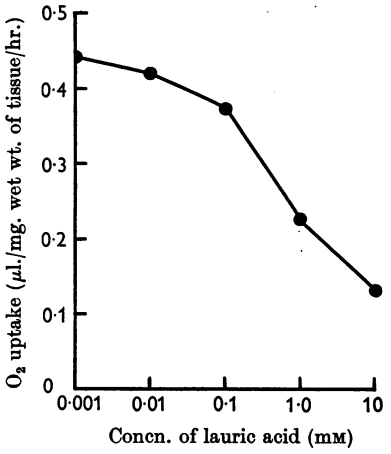


Fig. 2. Effect of lauric acid concentration on the oxygen uptake by rings of intestine incubated in phosphate-buffered saline, pH 6·8, containing glucose (5 mM) at 37° for 1 hr.

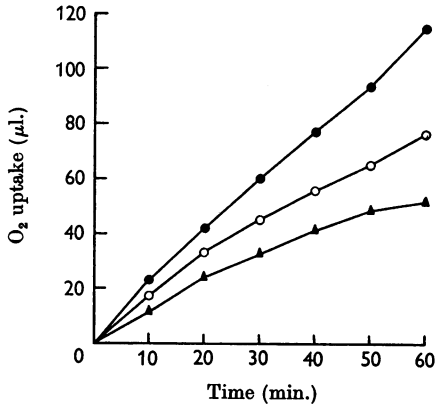


Fig. 3. Time-course of oxygen uptake by rings of intestine incubated in phosphate-buffered saline, pH 6·8, at 37°, containing glucose (5 mM) and lauric acid: ●, 0·001 mM; ○, 0·1 mM; ▲, 10 mM.

was studied further. The effect of increasing concentrations of lauric acid on the respiration of intestinal rings in the presence of 5·56 mM-glucose is shown in Fig. 2. In the absence of lauric acid glucose stimulated respiration by 23 %, and the presence of lauric acid at 0·001 mM did not affect this stimulated respiration. Raising the lauric acid concentration to 10 mM progressively inhibited respiration. Concentrations of lauric acid above 0·01 mM did not stimulate respiration at any stage of the incubation during which the oxygen uptake was determined. Fig 3 shows that the inhibition of respiration by 10 mM- and 0·1 mM-lauric acid was apparent after 10 min. incubation. Varying the glucose concentration from 1·0 mM to 40 mM in the presence of 1·0 mM-lauric acid did not reverse the inhibition of respiration. In the absence of lauric acid the oxidation of glucose was a constant proportion of the total respiration but in the presence of 1·0 mM-lauric acid the rate of glucose oxidation decreased more than respiration. The effect of 1·0 mM-lauric acid was also studied in the presence of 10 mM-succinic acid or -glutamic acid (Table 2). In the presence of either substrate lauric acid inhibited respiration by 30 %. Although neither succinic acid nor glutamic acid alone caused a marked stimulation of respiration, the production of $^{14}CO_2$ from [1-$^{14}$C]glutamic acid indicated that it was entering the tissue. The oxidation of the glutamic acid to $^{14}CO_2$ was inhibited by 73 % in the presence of 1·0 mM-lauric acid. Thus it resembled the inhibition of glucose oxidation.

*Effect of fatty acids on the uptake of glucose by intestine.* Since the oxidation of added glucose was inhibited by fatty acids to a greater extent than total respiration, the uptake of glucose by the intestine in the presence of fatty acids was investigated. Table 3 shows the effect of lauric acid (10 mM) and palmitic acid (7·8 mM) on the accumulation of glucose against a concentration gradient by everted intestinal sacs. This depends on the accumulation of glucose in the tissue. Initially 5·56 mM-glucose was present on both the serosal and mucosal sides of the tissue. In the absence of

Table 2. *Effect of lauric acid on the metabolism of succinic acid and glutamic acid*

Intestinal rings were incubated in phosphate-buffered saline for 1 hr. at 37° in $O_2$ in the presence of succinic acid, [1-$^{14}$C]glutamic acid or [U-$^{14}$C]glucose. The $O_2$ uptake and $^{14}CO_2$ production were determined in the presence and absence of 1·0 mM-lauric acid.

| | $Q_{O_2}$ | | $^{14}CO_2$ produced (counts/min./mg. wet wt. of tissue/hr.) | |
|---|---|---|---|---|
| Substrate added | Substrate alone | Substrate + 1 mM-lauric acid | Substrate alone | Substrate + 1 mM-lauric acid |
| None | 5·1 | — | — | — |
| Glucose (5 mM) | 6·4 | 4·4 | 7·1 | 3·6 |
| Succinic acid (10 mM) | 5·1 | 3·6 | — | — |
| Glutamic acid (10 mM) | 5·5 | 3·9 | 13·8 | 5·1 |

Table 3. *Accumulation of glucose by everted intestinal sacs in the presence of fatty acids*

Cannulated everted intestinal sacs were incubated in either phosphate-buffered saline or bicarbonate-buffered saline containing glucose (5·56 mM) and fatty acids for 1 hr. at 37°. The sacs were filled with saline containing glucose (5·56 mM).

| Expt. no. | Sac no. | Buffer | Fatty acid | Glucose uptake into serosal fluid (mg./g. wet wt. of tissue) | Glucose loss from serosal fluid (mg./g. wet wt. of tissue) |
|---|---|---|---|---|---|
| 1 | 1 | Bicarbonate | Lauric acid (10 mM) | — | 5·1 |
| | 2 | Bicarbonate | Lauric acid (10 mM) | — | 4·8 |
| | 3 | Bicarbonate | Palmitic acid (7·8 mM) | — | 3·1 |
| | 4 | Bicarbonate | Palmitic acid (7·8 mM) | — | 5·3 |
| | 5 | Bicarbonate | None | 10·2 | — |
| | 6 | Bicarbonate | None | 6·8 | — |
| 2 | 1 | Bicarbonate | None | 3·8 | — |
| | 2 | Phosphate | None | 1·5 | — |
| | 3 | Phosphate | None | 0·9 | — |
| | 4 | Phosphate | Lauric acid (10 mM) | — | 0·6 |
| | 5 | Phosphate | Lauric acid (10 mM) | — | 1·0 |

Table 4. *Effect of fatty acids on the entry of glucose into the serosal fluid of everted intestinal sacs*

Cannulated everted intestinal sacs were incubated in bicarbonate-buffered saline containing glucose (5·56 mM) for 1 hr. at 37° in the presence of 5 mM-fatty acids. Sacs contained saline without glucose and fatty acids.

| Sac no. | Fatty acid | Glucose uptake into serosal fluid (mg./g. wet wt. of tissue) | Inhibition of glucose uptake (%) |
|---|---|---|---|
| 1 | None | 2·02 | — |
| 2 | Octanoic acid | 0·53 | 78·8 |
| 3 | Octanoic acid | 0·25 | 87·6 |
| 4 | Lauric acid | 0·29 | 85·6 |
| 5 | Lauric acid | 0·46 | 67·2 |
| 6 | Palmitic acid | 0·84 | 58·4 |
| 7 | Palmitic acid | 0·94 | 53·5 |
| 8 | Arachidic acid | 0·87 | 57·0 |
| 9 | Arachidic acid | 0·67 | 68·9 |

fatty acid the concentration of glucose on the serosal side of the sacs increased, but in the presence of fatty acids the glucose concentration in the serosal fluid fell. The effect was the same whether the incubation was carried out in phosphate-buffered saline or bicarbonate-buffered saline. To determine the effectiveness of this inhibition of glucose transfer, glucose was omitted from the medium bathing the serosal side of the sacs, which were then incubated with 5 mM-octanoic acid, -lauric acid, -palmitic acid or -arachidic acid. All these acids inhibited the entry of glucose into the serosal medium by 53–88 % (Table 4).

The uptake of glucose into intestine was also determined by measuring the glucose concentration in intestinal rings that had been incubated with 15 μmoles of glucose in the presence of 2·5 mM- or 0·4 mM-lauric acid. There were only slight differences in the uptake of glucose. After 10 min. 12·8, 13·6 and 11·2 μmoles/500 mg. wet wt. of tissue had been taken up in the absence of lauric acid and in the presence of 0·4 mM- and 2·5 mM-lauric acid respectively. After 20 min. the quantities taken up were 15·6, 16·9 and 15·3 μmoles and after 60 min. 20·0, 19·6 and 19·4 μmoles respectively. Under all conditions the rate of glucose uptake decreased with time of incubation. This was probably the result of the fall in the glucose concentration in the medium which after 10 min. had decreased by 56, 66 and 66 % in the presence of 2·5 mM-lauric acid, 0·4 mM-lauric acid and the control respectively. After 60 min. it had fallen by 89, 96 and 95 % respectively. The concentration of glucose in the tissue water (Table 5) was calculated on the assumption that it was distributed throughout the total tissue water. In the presence of 2·5 mM-lauric acid the concentration of glucose in the tissue after 10 min. was only 0·13 mM, although the glucose removed from the medium was 88 % of that removed by the control that had a tissue glucose concentration of 1·68 mM. In the presence of 2·5 mM-lauric acid the tissue glucose concentration did not exceed the concentration in the medium at any time. In the presence of 0·4 mM-lauric acid or in the absence of lauric acid, the tissue glucose concentration was equal to or exceeded the concentration in the medium at all times studied.

*Effect of lauric acid on the formation of lactic acid by intestinal rings.* The low concentration of glucose found in the tissue incubated with 2·5 mM-lauric acid cannot be explained by the decreased rate of glucose uptake, but, since the oxidation of glucose to carbon dioxide is inhibited at this concentration of lauric acid, a metabolite must have accumulated. Since intestine is known to have a high rate of aerobic glycolysis (Weil-Malherbe, 1938) the lactic acid production was determined in the presence of 2·5 mM- and 0·4 mM-lauric acid (Table 6). After

10 min. incubation the total lactic acid production (tissue plus medium) was 41·4 and 27·2 % higher in the presence of 2·5 mM- and 0·4 mM-lauric acid respectively. After 60 min. the lactic acid production was 5 % higher than the control in the presence of 2·5 mM-lauric acid, and 4 % lower than the control in the presence of 0·4 mM-lauric acid. The similarity was probably caused by the removal of most of the glucose from the medium.

In the presence of 2·5 mM-lauric acid the concentration of lactic acid in the tissue after 10 min. was 22 % less than in the control and it decreased linearly between 10 min. and 60 min. although the total lactic acid production was greater than in the control. In the presence of 0·4 mM-lauric acid and in the control the concentration of lactic acid in the tissue increased from 10 min. to 20 min. but had fallen after 60 min. After 10 min. 19 % more lactic acid was present in the tissue incubated with 0·4 mM-lauric acid compared with the control but the quantities were the same after 60 min. The concentration of lactic acid in the control tissue after 60 min. was higher than the concentration in the presence of 2·5 mM-lauric acid at all times studied.

The ratio of the tissue lactic acid concentration to the medium lactic acid concentration (Table 6) emphasizes the inability of the tissue to maintain a high concentration of lactic acid in the presence of 2·5 mM-lauric acid. In the presence of 2·5 mM-lauric acid after 10 min. 41·1 % more lactic acid had been produced than in the control, but the concentration in the tissue was lower.

The rapid glycolysis in the presence of 2·5 mM-lauric acid could account for the low tissue glucose if the rate of entry of glucose into the tissue was the limiting process. The glucose utilized by the tissue after 10 min. could have formed 16·7 μmoles of lactic acid and 16·4 μmoles of lactic acid were found. In the absence of fatty acid the tissue metabolized glucose equivalent to 18·4 μmoles of lactic acid in 10 min. but only 11·8 μmoles of lactic acid were found.

Table 5. *Effect of lauric acid on the concentration of glucose by intestinal rings*

Intestinal rings were incubated in 3·0 ml. of phosphate-buffered saline containing glucose (15 μmoles) at 37° in $O_2$ with or without lauric acid.

| | Concn. of glucose in tissue (mM) | | |
|---|---|---|---|
| Incubation time (min.)... | 10 | 20 | 60 |
| Conditions | | | |
| Control (no fatty acid) | 1·68 | 2·43 | 0·35 |
| Lauric acid (0·4 mM) | 2·53 | 1·19 | 0·39 |
| Lauric acid (2·5 mM) | 0·13 | 0·32 | 0·32 |
| | Tissue glucose:medium glucose concentration ratio | | |
| Control (no fatty acid) | 1·0 | 1·8 | 1·4 |
| Lauric acid (0·4 mM) | 1·5 | 1·4 | 2·2 |
| Lauric acid (2·5 mM) | 0·05 | 0·3 | 0·6 |

Table 6. *Production and concentration of lactic acid by intestinal rings*

Conditions were as given in Table 5.

| | Total lactic acid produced (μmoles/g. wet wt. of tissue) | | |
|---|---|---|---|
| Incubation time (min.)... | 10 | 20 | 60 |
| Conditions | | | |
| Control (no lauric acid) | 30·9 | 46·9 | 64·1 |
| Lauric acid (0·4 mM) | 39·3 | 53·9 | 61·4 |
| Lauric acid (2·5 mM) | 43·7 | 45·9 | 67·4 |
| | Concn. of lactic acid in tissue (mM) | | |
| Control (no lauric acid) | 20·4 | 23·1 | 19·9 |
| Lauric acid (0·4 mM) | 24·3 | 25·7 | 19·8 |
| Lauric acid (2·5 mM) | 17·8 | 16·1 | 12·9 |
| | Tissue lactic acid:medium lactic acid concentration ratio | | |
| Control (no lauric acid) | 10·7 | 7·0 | 3·5 |
| Lauric acid (0·4 mM) | 9·7 | 6·3 | 3·5 |
| Lauric acid (2·5 mM) | 4·8 | 3·7 | 1·9 |

## DISCUSSION

The results demonstrate that the respiration of rat intestine *in vitro* is inhibited by certain fatty acids in a similar manner to the respiration of rat-liver slices. Ahmed & Scholefield (1961*a*) observed that at 4·0 mM decanoic acid was the most potent inhibitor of respiration of rat-liver slices, closely followed by lauric acid. However, although decanoic acid was one of the most inhibitory fatty acids in the present study at 6·0 mM, together with pelargonic acid, undecanoic acid, lauric acid and tridecanoic acid, at 0·6 mM myristic acid was most inhibitory. Since at 6·0 mM myristic acid and the longer-chain fatty acids were present in the medium mainly as a suspension of free fatty acids their true effectiveness as inhibitors of respiration might have been masked by their inability to penetrate the tissue in this state.

The absence of any stimulation of respiration by non-inhibitory concentrations of fatty acids is in contrast with the results of Ahmed & Scholefield (1961*a*), who observed a stimulation of the respiration of rat-kidney and -liver slices by 1·34 mM-decanoic acid. It is unlikely that the absence of stimulation was caused by the presence of glucose that was competing successfully as a substrate for oxidation since it was also present in the latter study. An alternative explanation is that free fatty acids were already present in the tissue. Nakamura *et al.* (1959) found free fatty acids in freshly prepared rat-intestine homogenate. This is also indicated by the fact that inhibitory concentrations of fatty acids inhibited from the start of the incuba-

tion whereas with rat liver there was an initial stimulation followed by an inhibition.

The inhibition of the oxidation of the added substrates, glucose and glutamic acid, to a greater extent than the inhibition of respiration by fatty acids suggested that either the entry of the added substrates into the tissue was inhibited or that their oxidation was specifically inhibited. Since the uptake of both substrates against a concentration gradient requires oxidative energy any inhibition of respiration would be expected to decrease the rate of uptake. However, glucose uptake was only slightly depressed and its conversion into lactic acid was increased. The oxidation of lactic acid must therefore have been inhibited.

There is some information on the effects of free fatty acids on mitochondrial metabolism. Munoz & Leloir (1943) observed that fatty acids inhibited the formation of organic phosphates by liver mitochondria, and Ahmed & Scholefield (1961*a*) demonstrated that the synthesis of ATP in rat-liver slices was inhibited by fatty acids at concentrations that did not inhibit respiration. Pressman & Lardy (1956) demonstrated the stimulation of adenosinetriphosphatase activity of rat-liver mitochondria by a range of fatty acids, the most effective of which were myristic acid and linoleic acid, and Wojtczak & Lehninger (1961) demonstrated that oleic acid was capable of causing the swelling of isolated rat-liver mitochondria in a manner similar to the endogenous 'U' factor, which had the characteristics of a mixture of fatty acids.

The accumulation of lactic acid in the presence of concentrations of fatty acids that inhibit respiration indicates that either the electron-transport chain or the citric acid cycle is inhibited. Dalgarno & Birt (1963) found that carrot mitochondrial succinate dehydrogenase was inhibited by 1·0 mM-oleic acid or -stearic acid, but malate dehydrogenase was not affected. From present knowledge it is not possible to decide whether inhibition of the citric acid cycle such as this could alone explain the inhibition of respiration or whether inhibition of electron transport is the main factor. The results of Ahmed & Scholefield (1961*a*) indicate that fatty acids may act in a similar manner to 2,4-dinitrophenol, inhibiting ATP formation at low concentrations, possibly by stimulating adenosine-triphosphatase activity, and inhibiting electron transport at higher concentrations.

The disappearance of glucose from the serosal fluid during the incubation of everted intestinal sacs with inhibitory concentrations of fatty acids is similar to the effect of phlorrhizin at 0·5 mM observed by Parsons, Smyth & Taylor (1958). However, they subsequently demonstrated that phlorrhizin at this concentration did not affect the complete oxidation of glucose but only inhibited the transfer of glucose through the mucosal surface of the tissue (Newey, Parsons & Smyth, 1959). The uptake of glucose from the serosal side was, however, not inhibited. Thus the action of fatty acids resembles more closely the effect of 2,4-dinitrophenol and anaerobiosis, both of which prevent the accumulation of transportable sugars in the tissue (Crane & Mandelstam, 1960). The failure of the glucose concentration in the tissue in the presence of 2·5 mM-lauric acid to reach the concentration in the medium is evidence that aerobic energy sources were not available although oxygen uptake was not completely abolished. The increased permeability of the tissue as indicated by the rapid loss of lactic acid would be expected to facilitate the entry of glucose.

## SUMMARY

1. The effect of different concentrations of saturated straight-chain $C_6$–$C_{20}$ fatty acids on rings of rat small intestine has been studied *in vitro*.

2. At 0·6 mM the $C_7$–$C_{15}$ fatty acids inhibited respiration by more than 5 % and the $C_{12}$–$C_{15}$ acids inhibited by more than 15 %. At 6·0 mM the $C_6$–$C_{19}$ acids inhibited respiration by 14 % or more and the $C_9$–$C_{13}$ acids inhibited by more than 60 %.

3. The oxidation of glucose (5 mM) by intestinal rings was inhibited by 0·6 mM- and 6·0 mM-fatty acids. The pattern of inhibition resembled the inhibition of respiration. The $C_9$–$C_{13}$ acids were the most potent, causing over 70 % inhibition at 6·0 mM. The incorporation of glucose carbon into lipids was inhibited in a similar manner. Glucose concentrations up to 40 mM did not reverse the inhibition of respiration caused by 1·0 mM-lauric acid.

4. The uptake of glucose by intestinal rings in 10 min. was slightly stimulated (6 %) by 0·4 mM-lauric acid, but was inhibited 13 % by 2·5 mM-lauric acid. Both 10 mM-lauric acid and 7·78 mM-palmitic acid prevented the accumulation of glucose against a concentration gradient in the fluid bathing the serosal surface of intestinal sacs.

5. The formation of lactic acid was stimulated by 0·4 mM- and 2·5 mM-lauric acid by 27 and 41 % respectively after 10 min. The loss of lactic acid from the tissues was stimulated by 2·5 mM-lauric acid.

6. The inhibition of respiration by lauric acid appeared at a concentration of 0·01 mM and increased as the concentration was raised to 10 mM. At 10 mM the inhibition was 36 % of that in the absence of fatty acids.

7. In the presence of succinic acid or glutamic acid, 1·0 mM-lauric acid inhibited the respiration of intestinal rings by 29 %. The oxidation of the glutamic acid to carbon dioxide was inhibited by 63 %.

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# Turnover of Nucleic Acids in a Multiplying Animal Cell

## 1. INCORPORATION STUDIES

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It is widely accepted that RNA is involved in the transfer of information from the DNA to the rest of the cell, but opinions differ about the precise way in which it is involved (Sirlin, 1963). A specific model based on experimental evidence has been proposed for those cells which have well-defined nuclei. The cytoplasmic RNA is thought to be made in the nucleus on the DNA and then transferred to the cytoplasm (Goldstein & Plaut, 1955). This 'translocation' model is based mainly on the evidence obtained from radioautographic studies. These showed, in general, that, when animal or plant cells were grown in a medium containing radioactive precursors of RNA, the RNA of the nucleus became labelled much more rapidly than that of the cytoplasm. In some cases there was virtually no incorporation of radioactivity into cytoplasmic RNA during the first 30 min. of labelling, although incorporation into nuclear RNA was extensive. This observation has been widely assumed to show that most of the RNA in the cytoplasm originates in the nucleus (Zalokar, 1959; Perry, Hell & Errera, 1960).

Perry, Errera, Hell & Durwald (1961) found with radioautographic techniques that the incorporation of label into the nuclear RNA reached a maximum steady value after 2–3 hr., whereas incorporation into the cytoplasmic RNA, after an initial lag, continued to rise steadily throughout the experiment. This result was also considered to support the view that the cytoplasmic RNA was synthesized in the nucleus.

It is known, however, that animal and plant cells normally synthesize the constituents of RNA from relatively simple precursors (Brown & Roll, 1955), and the introduction of preformed bases, nucleosides and nucleotides does not necessarily suppress endogenous synthesis immediately or entirely (Henderson, 1962). It is also known that cells often contain relatively large 'pools' of metabolites which may have to be diluted by added exogenous precursors before the specific radioactivities of the immediate precursors of RNA become the same as those of the added compounds (Harris & Watts, 1962). It has been pointed out (Harris, 1959; Harris & Watts, 1962) that these factors make it