dative browning occurs to a limited extent in wattle (*Acacia mollissima*) and quebracho (*Schinopsis* spp.) extracts, which contain mainly tannins differing from the autoxidation polymers of catechins (Hathway & Seakins, 1957*b*).

SUMMARY

1. The autoxidation of (+)-catechin in aqueous solution is relatively slow and is accompanied by an increase in the average molecular weight of the mixture formed.

2. The percentage of (+)-catechin remaining in the oxidation product is estimated and the average molecular weight of the tannins calculated.

3. The autoxidation reaction appears to be complex. Degradation accompanies the condensation reaction and the percentage of phloroglucinol formed is low.

4. The oxidation is accompanied by a 'browning' reaction; the product is soluble in methanol and acetone–water, and apparently similar to that which accompanies (+)-catechin in commercial gambier extract.

5. Tannins present in cube gambier from the leaves of *Uncaria gambir* are shown to be polymeric.

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The Oxidation of Fatty Acids by a Particulate Fraction from Desert-Locust (*Schistocerca gregaria*) Thorax Tissues

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The oxidation of fatty acids in the insect body has not been the subject of the same amount of research work as has been done with preparations from mammalian tissues. Krogh & Weis-Fogh (1951) and Weis-Fogh (1952) investigated changes in the respiratory quotients of *Schistocerca* at rest and in flight and found that the R.Q. was 0·75 in the flying insect, which pointed to an extensive mobilization of fat reserves, in comparison with the R.Q. 0·82 observed in the resting insect; they suggested that this value resulted from the predominant oxidation of carbohydrates by the insect at rest. Preparations from the flight muscles of the cockroach (*Periplaneta americana*) (Barron & Tahmisian, 1948) and locust-thorax mitochondria (Rees, 1954) were able to oxidize acetate, but butyrate and octanoate were not attacked. McShan, Kramer & Schlegel (1954) reported slight oxidation of octanoate by whole homogenates obtained from wood-roach (*Leucophaea maderae*) thorax muscles. No

* Deceased.

other experimental evidence for the existence of a fatty acid-oxidizing system in insects has been reported, and therefore an attempt has been made to find such a system. The experiments reported in this study describe the isolation of a particulate fraction derived from locust tissues which was found to be capable of oxidizing even-numbered straight-chain fatty acids from C_4 to C_{18}.

EXPERIMENTAL

Materials

Adenosine triphosphate (ATP), flavinadenine dinucleotide (FAD), diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), coenzyme A (CoA) and the hexokinase preparation used were all obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). All fatty acids were high-purity preparations obtained from Mann Research Laboratories (New York, U.S.A.). All other chemicals were of analytical grade.

Insects

Locusts were gathered in various localities in southern Israel during the last quarter of 1958. They were kept until a considerable quantity of eggs had been laid. The insects hatched from this first batch were subsequently used for the production of eggs and insects in various stages of growth. They were reared in cages constructed according to Hunter-Jones's (1956) description. The cages were illuminated day and night with ordinary 15 w bulbs. The diet of the insects consisted of fresh green leaves and was supplemented with coarsely ground wheat.

All insects were grown at 35° until they reached the final moult, at which stage they were divided into two groups, respectively maintained for 2–3 weeks at 35° and 45° until required for dissection. The temperature within the cages was kept constant within $\pm 1°$ by means of a protected electric-heating spiral actuated by a simple bimetallic thermoregulator.

Preparation of particle suspensions

Thorax tissues composed mainly of muscle, serving as starting material for the preparation of homogenates, were obtained by the following dissection method, which was found to be time-saving and yielded muscle preparations comparatively uncontaminated with fat: ten insects, five males and five females, each weighing approx. 3 g., were cooled to 2–6° and kept at this temperature before dissection, which, like all the following stages in the preparation of particle suspensions, was carried out at 0–2°. First the legs and wings were removed, then the last two abdominal segments were cut off and the head was nearly separated from the body by cutting the tendons. It was then severed from the body by gentle pulling, which also removed the gut. The remainder of the abdomen was then cut off and the thorax was halved by dorsal and ventral cuts along the body axis. The contents of each half thorax so obtained were spread on filter paper with slight pressure, thus separating the muscle tissue from most of the fat, which was retained by the filter paper. The pooled thorax muscles were suspended in 80 ml. of 0·25 M-sucrose solution (previously brought to pH 6·4 by the addition of 0·1 M-KOH) and blended for 15 sec. in a MSE homogenizer at 12 000 rev./min. The suspension so obtained was then homogenized in a Potter–Elvehjem glass homogenizer with a Teflon plunger by ten slow up-and-down movements. The blending procedure before homogenization was found necessary for the complete separation of the muscles from the hard integument, for this cannot be achieved by the Potter–Elvehjem technique. The homogenate was gently squeezed through four layers of surgical gauze. The 'filtrate' (about 72 ml.) was then poured on to a thin layer of dry cotton wool spread on a 5·5 cm. Büchner funnel, and then filtered again through the same layer of cotton wool, at first by gravity and towards the end of the filtration by gentle suction. This procedure yielded about 58 ml. of filtrate, almost entirely free of integument debris and fat. The filtrate was centrifuged at 18 000 *g* for 10 min. The pellet obtained consisted of a lower reddish layer which was uniform in colour and texture and of a very small 'fluffy' upper layer. After decantation of the supernatant, fat adhering to the walls of the centrifuge vessel was removed by wiping with filter paper and the pellet was suspended in 2·6 ml. of 0·25 M-sucrose solution (pH 6·4) and briefly homogenized in a smaller Potter–Elvehjem apparatus. The total volume of particle suspension thus obtained was 3·2–3·3 ml., so that each millilitre of the suspension contained material derived from about two thoraces. The amount of material present in different batches was more or less constant, as judged by the nitrogen content, which was found to range from 4·2 to 4·6 mg. of nitrogen/ml. of suspension. The particulate matter found in the suspension consisted almost entirely of slightly elongated particles, 2–3 μ long. A portion (0·25 ml.) of the particle suspension, when added to the standard reaction mixture described below, consumed about 1·2 μmole of oxygen during 30 min., at which point respiration ceased altogether.

Analytical methods

The reaction medium, to be referred to in the following as 'standard reaction mixture', had the composition: 0·1 M-potassium phosphate buffer (pH 6·4), 0·2 ml.; $MgCl_2$, 7·5 μmoles; ATP (disodium salt), 1 μmole; KCl, 50 μmoles; particle suspension, 0·25 ml.

Substrates, cofactors, etc., were added as concentrated stock solutions, the volume being made up to 1 ml. All solutions used were previously adjusted to pH 6·4. (In the experiment in which the pH optimum of the oxidizing system was determined, both the phosphate buffer and the substrate and cofactor solutions added were adjusted to the various pH values tested.)

All stages in the preparation of the reaction mixtures were carried out at 4°. The Warburg vessels containing all components of the reaction mixtures except the particle suspension were kept in ice. The freshly prepared particle suspension, also kept in ice, was added and the vessels were immediately connected to the manometers and transferred to the Warburg bath. An equilibration period of 10 min. was required. Oxygen-uptake measurements were carried out by standard Warburg technique at 37° in air.

The same standard reaction mixture was used for the determination of the P/O ratios. It was made to contain 4 μmoles of butyrate and the quantities of glucose and hexokinase described by Copenhaver & Lardy (1952). The vessels were equilibrated in the Warburg bath at 37° for 5 min., glucose and hexokinase were added from the side

arm and the equilibration period was continued for 5 min., of which the last 2 min. served for closing the stopcocks. The initial readings were taken and the zero-time vessels were quickly removed and deproteinized. Phosphate determinations were carried out by Gomori's (1942) method.

Nitrogen was determined by a Kjeldahl–Nessler procedure. Stable suspensions of fatty acids (except for butyric acid) were prepared by the method described by Lehninger (1955) for the preparation of octanoate suspensions. The method was found satisfactory for all the fatty acids investigated in this work.

RESULTS

Two systems bringing about the oxidation of fatty acids are described: (1) The particles isolated from thorax tissues derived from insects finally held at 35° (35° particles); this system can completely oxidize butyric acid but is unable to oxidize any higher fatty acid. (2) The particles isolated from thorax tissues derived from insects finally held at 45° (45° particles); this system completely oxidized all fatty acids from C_4 to C_{18} and differed from the system contained in the 35° particles in its cofactor requirements.

Butyrate oxidation by the 35° particles

pH *dependence of oxidative activity.* The pH for optimum butyrate-oxidizing activity was found to be 6·4 (Fig. 1). All subsequent experiments were therefore carried out at this pH. The optimum pH found was the same both for butyrate and succinate oxidation.

Cofactor requirements. Butyrate oxidation required the presence of ATP and Mg^{2+} ions. It was found that it proceeded at the maximal rate when 0·5 μmole of ATP and 2·5 μmoles of Mg^{2+} ions were present in a reaction mixture containing 4 μmoles of butyrate (Figs. 2, 3). The following cofactors were

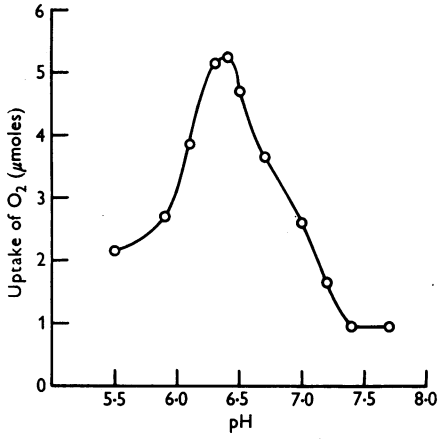

Fig. 1. pH dependence of butyrate oxidation by 35° particles. The vessels contained 4 μmoles of butyrate in the standard reaction mixture. Reaction time, 20 min.

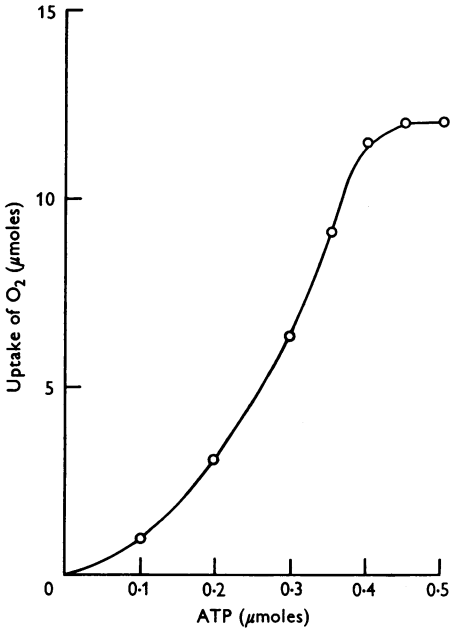

Fig. 2. ATP requirement of the butyrate-oxidizing system in 35° particles. Vessels contained ATP as indicated, and all other ingredients as in the standard reaction mixture together with 4 μmoles of butyrate. Reaction time, 80 min.

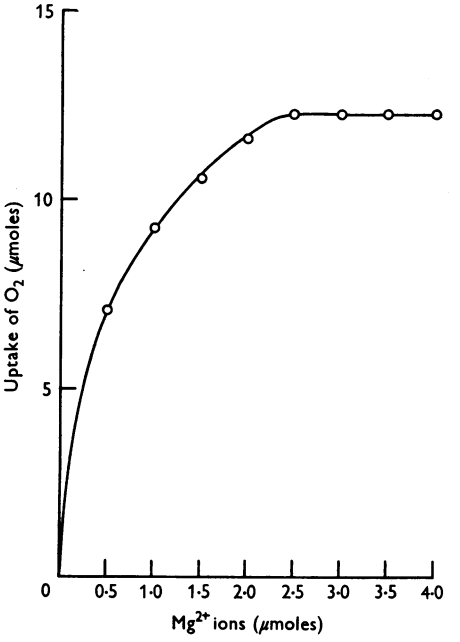

Fig. 3. Requirement for Mg^{2+} ions of the butyrate-oxidizing system in 35° particles. Vessels contained Mg^{2+} ions as indicated, and all other ingredients as in the standard reaction mixture together with 4 μmoles of butyrate. Reaction time, 80 min.

tested and found to be unnecessary (amounts, in μmole, added to the reaction mixture are given in parentheses): cytochrome *c* (0·015); FAD (0·04); DPN (0·15); TPN (0·15) and CoA (0·025).

Substrate concentration and activity. In Fig. 4 the amounts of oxygen taken up are plotted against time, with the amount of butyrate present in the reaction mixture varying from 0·5 to 10 μmoles. It can be seen that the oxidation of up to about 1 μmole of butyrate is virtually complete within a reaction time of 1 hr. The system becomes saturated with substrate, as indicated by the constant initial velocity of the reaction, at amounts of substrate exceeding 3 μmoles. When the oxygen-uptake increments (the amounts of oxygen taken up in excess of the endogenous respiration of the system measured 65 min. after the start of the experiment) are plotted against amounts of substrate (Fig. 5), the first part of the curve so obtained shows that with up to 2 μmoles of butyrate the uptake of oxygen is linearly proportional to the amount of substrate. The slope of the line (uptake of oxygen/μmole of butyrate) is 4·9 and therefore closely approaches the theoretical value of 5 μmoles of oxygen required for the total oxidation of 1 μmole of butyrate.

Inhibitors. Fluoride, cyanide, azide and arsenite (10 μmoles of each), tested separately by addition to the standard reaction mixture containing 4 μmoles of butyrate, caused a 70% inhibition of the butyrate-oxidizing system. 2:4-Dinitrophenol (DNP) (0·1 μmole) also brought about a 70% inhibition of respiration, whereas the addition of 1 μmole of ethylenediaminetetra-acetic acid (EDTA), added as its magnesium complex, produced a 50% inhibition. The inhibition of butyrate oxidation by malonate is shown in Fig. 6. In reaction mixtures containing amounts of butyrate ranging from 3 to

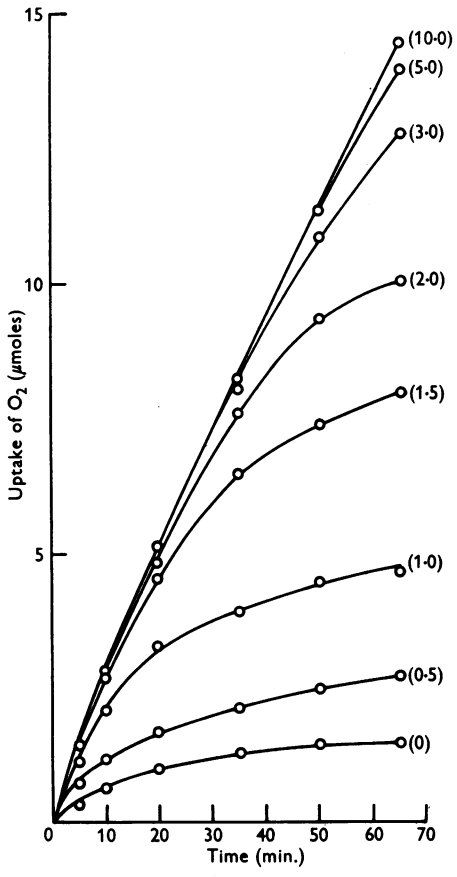

Fig. 4. Effect of increasing amounts of butyrate on O_2 uptake by 35° particles. Numbers attached to the curves denote the amounts of butyrate (μmoles) added to the standard reaction mixture.

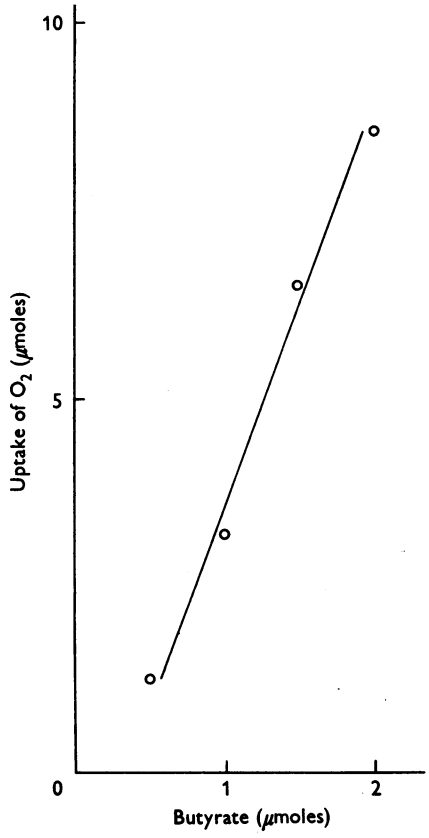

Fig. 5. Uptake of O_2 by 35° particles as a function of the amount of butyrate added. O_2 consumed during a reaction time of 65 min. against the amounts of butyrate initially present in the standard reaction mixture. Uptake of O_2/μmole of butyrate = 4·9 (theoretically expected value for total oxidation of butyrate = 5·0).

10 μmoles, 85% inhibition was produced by the addition of 10 μmoles of malonate; this figure did not alter when the amount of butyrate was varied.

Oxidative phosphorylation. Determinations of P/O ratio were carried out in reaction mixtures containing 4 μmoles of butyrate. The results are shown in Table 1.

Oxidation of butyric acid and higher fatty acids by particles prepared from locusts grown at 45°

Butyrate oxidation. The 45° particles differed from the 35° particles mainly in requiring CoA, in addition to ATP and Mg^{2+} ions, for butyrate oxidation. When CoA was omitted, the reaction proceeded initially at about half the rate observed with the 35° system and slowed down to about 20% of the rate observed in the 35° particle system after about 1 hr. reaction time. The addition of 0·025 μmole of CoA restored the activity of the 45° system to that of the 35° system (Fig. 7). The system present in the 45° particles had its activity optimum at pH 6·4. The substances found to inhibit butyrate oxidation in the 35° system also produced inhibitory effects of the same order in the 45° system. In all the experiments in which inhibitors were tested on the 45° system the reaction mixtures employed contained, in addition to all the ingredients of the standard reaction mixture, 0·025 μmole of CoA and 4 μmoles of butyrate.

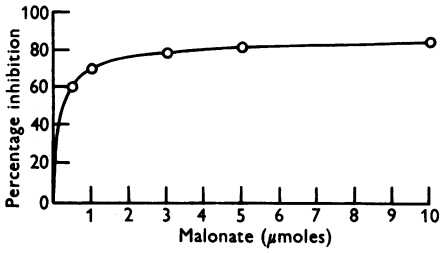

Fig. 6. Inhibition of butyrate oxidation by malonate in the 35° particle system. Vessels contained the standard reaction mixture with 4 μmoles of butyrate.

Oxidation of higher fatty acids. The higher fatty acid-oxidizing system in 45° particles had the following properties: (1) Oxidation of all the higher fatty acids investigated went to completion and the presence of butyrate was obligatory. (2) In addition to the cofactors needed for the oxidation of butyrate the presence of additional factor(s) contained in a sheep-liver extract was found to be essential. (3) Higher fatty acids were oxidized only when their amount in the reaction mixture did not exceed certain limiting values.

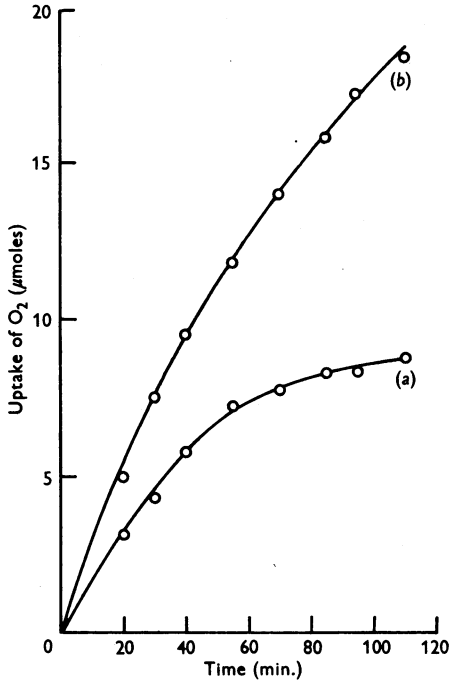

Fig. 7. Coenzyme A requirement of the 45° particle system. (*a*), Standard reaction mixture containing 4 μmoles of butyrate; (*b*), the same system with the addition of 0·025 μmole of CoA.

Table 1. *Oxidative phosphorylation with butyrate as substrate*

Values are the means of results from four experiments. The vessels contained, in addition to the standard reaction mixture, 4 μmoles of butyrate and the inhibitors listed; glucose and hexokinase were added from the side arm. Reaction time, 30 min. Temp., 37°. Further experimental details are given in the Experimental section.

Inhibitor	Uptake of oxygen* (μg.atoms)	Phosphate esterified (μmoles)	Ratio P/O
None	21	9	0·43 (±0·05)
DNP (0·1 μmole)	6·6	0	0
EDTA (magnesium complex 1 μmole)	10·6	0	0

* In control vessels containing all ingredients except glucose and hexokinase the oxygen uptake was 13·7 μg.atoms (the presence of hexokinase and glucose produced a 54% increase in oxygen uptake).

(1) Since the presence of tricarboxylic acid-cycle intermediates in reaction mixtures containing particles of mammalian origin has been found to be necessary for effecting the oxidation of higher fatty acids (Knox, Noyce & Auerbach, 1948), we first attempted to find an analogous effect in the locust system. Preliminary experiments, in which oxidation of succinate, fumarate, citrate and α-oxoglutarate was compared, showed that, in accordance with the results reported by Rees (1954), succinate was the substrate most efficiently oxidized. Accordingly, succinate was added to the standard system containing higher fatty acids. Only in a reaction mixture containing 0·5 μmole of hexanoate and 2 μmoles of succinate could any effect be observed, and even then it was comparatively small, the oxygen uptake reaching 25% of the value theoretically expected for total oxidation of the amount of hexanoate present. An attempt was therefore made to find more efficient priming substances and it was found that butyrate (2 μmoles) was able to induce the total oxidation of all the higher fatty acids added.

(2) The reaction mixture containing all the cofactors (including CoA) found to be necessary for the oxidation of butyrate by the 45° particles was unable to bring about oxidation of the higher fatty acids. A number of additional cofactors were therefore investigated, of which DPN and TPN proved to be ineffective. Varying results were obtained when FAD was tested and it was found that the nature of the FAD preparation used had a profound influence on the results obtained. At first a low-purity FAD preparation was used and was found to be effective. Later on, when a commercial FAD preparation of high purity was used instead, no oxidation of higher fatty acids could be observed.

The low-purity FAD preparation initially used and found to be effective was obtained according to the method described by Dimant, Sanadi & Huennekens (1952), by extraction of sheep liver with aqueous trichloroacetic acid, adsorption on a Florisil column, consecutive elution with 2% acetic acid, water, aqueous 0·5% pyridine and finally with 5% pyridine and freeze-drying of the 5% pyridine eluate. On re-investigation of the various eluates it was found that the catalytic activity for the oxidation of higher fatty acids resided in the residues obtained after freeze-drying of the 0·5% and the 5% pyridine eluates, the 0·5% pyridine eluate being slightly more effective than the 5% eluate. No attempts were made to identify the active substance.

The reaction mixtures used for the experiments described in the following part of this study were all made to contain 1 mg. of the freeze-dried 0·5% pyridine eluate fraction.

(3) The general course of oxidation of higher fatty acids in the presence of butyrate can be seen from the results presented in Figs. 8 and 9. Fig. 8

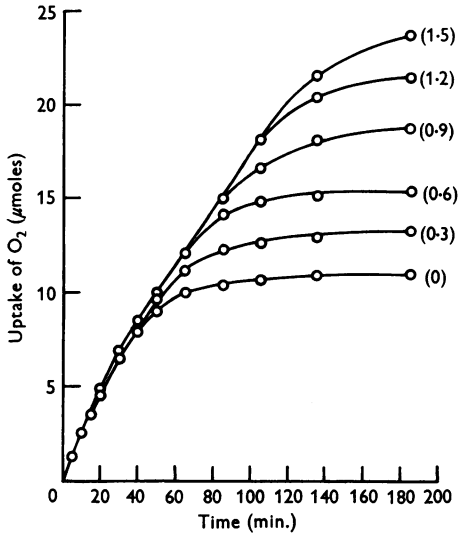

Fig. 8. Oxidation of hexanoate by the 45° particles. Numbers attached to the curves indicate the amounts of hexanoate (μmoles) added to the standard reaction mixtures in addition to 2 μmoles of butyrate.

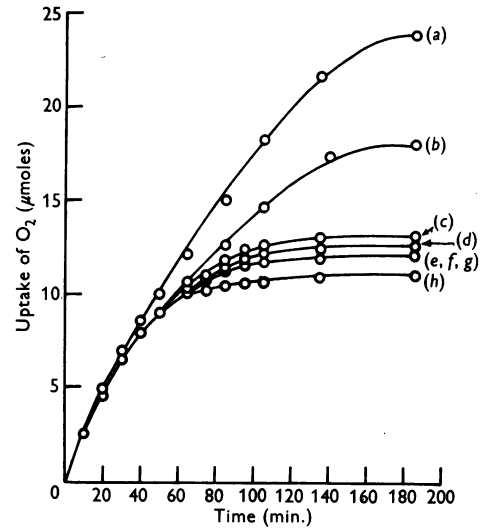

Fig. 9. Oxidation of higher fatty acids by the 45° particle system. All vessels contained the standard reaction mixture with 2 μmoles of butyrate. (*a*) 1·5 μmole of hexanoate; (*b*) 0·6 μmole of octanoate; (*c*) 0·17 μmole of decanoate; (*d*) 0·1 μmole of dodecanoate; (*e*) 0·06 μmole of tetradecanoate; (*f*) 0·05 μmole of hexadecanoate; (*g*) 0·04 μmole of octadecanoate; (*h*) 2 μmoles of butyrate only, no higher fatty acid present.

shows the oxidation of 0·3–1·5 μmole of hexanoate in the presence of 2 μmoles of butyrate. The amounts of oxygen taken up increase in linear proportion to the amount of hexanoate present and reach the values required for the total oxidation of hexanoate (8 μmoles of oxygen/μmole of hexanoate). The experiment was repeated with all saturated even-numbered straight-chain fatty acids from C_8 to C_{18}. The oxidation of all the fatty acids investigated was concluded within 180 min. (Fig. 9). The total oxidation of fatty acids as appearing from an oxygen uptake corresponding to the theoretically expected one could be demonstrated for the C_6–C_{12} acids (Table 2). The oxidation of fatty acids containing more than 12 carbon atoms might probably also come to completion but this could not be quantitatively demonstrated, since the increments of oxygen uptake observed were too small to be significant. The initial velocities observed were practically the same both with butyrate alone and with butyrate together with any higher fatty acids. The amounts of higher fatty acids employed are seen to decrease with increasing chain length. This was due to the fact that the oxidation of all fatty acids containing more than six carbon atoms was found to be inhibited when their amount present in the reaction mixtures exceeded a limiting value, which was found to be characteristic of each fatty acid. When these limiting values were further exceeded not only was the oxidation of the respective higher fatty acid completely abolished but butyrate oxidation, too, was inhibited to a significant degree. In Fig. 10 the maximal amounts of higher fatty acids for which complete oxidation still occurred are plotted against chain length. (The same figure shows the solubilities of the respective fatty acids in water

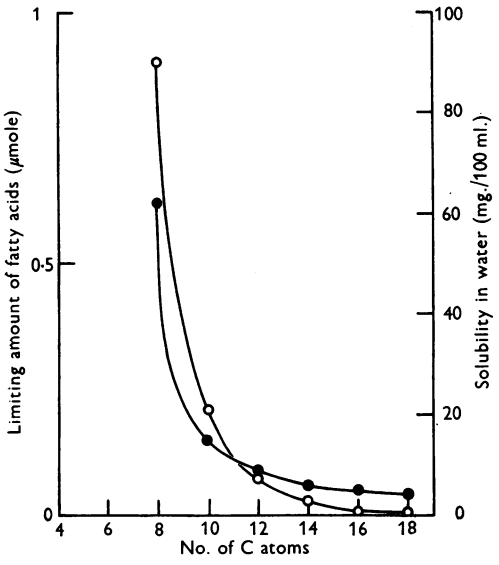

Fig. 10. Maximal concentrations of higher fatty acids which could be oxidized by the 45° particles (○) and solubilities of the fatty acids in water (●). Solubilities were obtained by extrapolating to 37° the solubility data for 30° and 40° given by Deuel (1951).

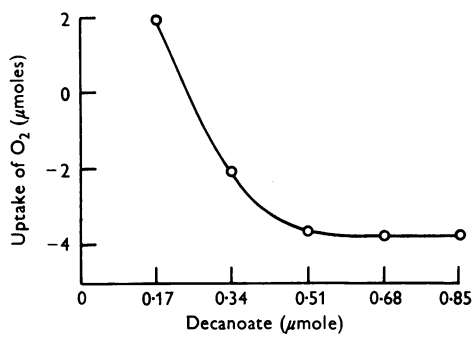

Fig. 11. Inhibition of decanoate and butyrate oxidation by increasing decanoate concentrations in standard reaction mixtures containing 2 μmoles of butyrate and the indicated amounts of decanoate. Reaction time, 95 min. The smallest amount of decanoate employed is that which still allows complete oxidation of decanoate. The zero point on the ordinate corresponds to the uptake of O_2 of the control, a reaction mixture containing only 2 μmoles of butyrate.

Table 2. *Oxidation of higher fatty acids by the 45° particle system in the presence of butyrate*

Vessels contained the standard reaction mixture, to which 0·025 μmole of CoA and 2 μmoles of butyrate were added. Duration of experiment, 180 min.

Fatty acid	Amount of fatty acid in reaction mixture (μmoles)	Total uptake of O_2 (μmoles)	Uptake of O_2 for fatty acid* (μmoles)	Expected uptake assuming complete oxidation
Hexanoic	1·5	23·80	12·60	12·0
Octanoic	0·6	18·80	7·60	6·6
Decanoic	0·17	13·30	2·10	2·3
Dodecanoic	0·1	12·75	1·55	1·7

* The total O_2 uptake, from which the amount of 11·2 μmoles of O_2 measured for the oxidation of butyrate alone has been subtracted.

plotted against chain length.) Fig. 11 presents a characteristic example (decanoate) of the inhibition of oxidation of a higher fatty acid and of butyrate by increasing amounts of the same higher fatty acid.

DISCUSSION

In discussing the experimental evidence for the existence of a system oxidizing fatty acids in particles isolated from locust-thorax tissue one must take into account both the enzymic aspects of the problem and also factors which might be regarded as resulting from membrane-permeability effects. The fatty acid-oxidizing system in the locust tissues investigated, though similar to that found by Grafflin & Green (1948) in mammalian-kidney particles in that it can bring about the complete oxidation of fatty acids and requires ATP and Mg^{2+} ions, differs from it in some important respects. The locust system does not require cytochrome *c*, a fact which may be due to a sufficiently high cytochrome concentration within the particles. The occurrence of high cytochrome concentrations in insect sarcosomes was reported by Watanabe & Williams (1951, 1953) and by Levenbook (1953). It differs from the fatty acid-oxidizing mitochondrial preparations from liver and kidney (Kennedy & Lehninger, 1952; Judah & Rees, 1953) in its requirement for CoA, which, though demonstrated only in the 45° particles, is assumed for reasons to be discussed below to exist also in the 35° particles. Attention may also be drawn to the fact that the pH optimum of fatty acid-oxidizing activity in the locust particles lies at 6·4, a much lower value than that of 7 and above at which similar systems are usually examined. In this connexion the earlier experiments of Bodine (1925) should be recalled, in which he found a pH of 6·6 in the haemolymph of the locust *Schistocerca americana*. A specific property of the locust system is the role played by butyric acid, which is completely oxidized in the absence of any tricarboxylic acid-cycle intermediate and can induce the oxidation of higher fatty acids more effectively than succinate. It might be suggested that butyrate acts as a 'primer' for both the fatty acid cycle and the tricarboxylic acid cycle. As a substrate, butyrate occupies a somewhat special position. It is oxidized at a faster rate than all the other fatty acids, as can be seen from the fact that the rate of oxygen uptake when butyrate alone is oxidized does not differ from that observed when any higher fatty acid is present as additional substrate (Fig. 9). Butyrate alone can be oxidized in the absence of the sheep-liver factor. The inhibition by cyanide, azide and arsenite follows the pattern usually found in mammalian oxidative systems functioning via the tricarboxylic acid cycle and the cytochromes. As expected, inhibition by malonate was found to depend upon the malonate concentration, but was not affected by increasing the amount of butyrate. It can therefore be concluded that the factor limiting the capacity of the system operates at some point on the pathway leading to the production of succinate. Oxidative phosphorylation, here demonstrated in an insect system with butyric acid as substrate for the first time, was found to be comparatively inefficient (P/O ratio 0·43). However, the reaction conditions under which the degree of oxidative phosphorylation was determined were not specially chosen to produce optimum P/O ratios. These might be found by suitably varying the composition of the reaction mixture and other experimental conditions. The significant increase (54%) in the rate of oxygen uptake of the system, when hexokinase and glucose were added, as compared with that of the same reaction system not containing hexokinase, is in agreement with the results obtained by Lardy & Wellman (1952) with mammalian particles. They suggested that the effect may be due to the regulation of respiration by the availability of suitable high-energy phosphate acceptors. The same conclusion might therefore be drawn with respect to the system here described.

2:4-Dinitrophenol completely abolished oxidative phosphorylation and also caused a 70% inhibition of respiration. This effect and the above-mentioned inhibition by fluoride are in agreement with known data on inhibition of fatty acid oxidation in mammalian systems (Kennedy & Lehninger, 1952; Judah & Rees, 1953). Ethylenediaminetetraacetic acid, besides producing a 50% inhibition of respiration, completely inhibited the esterification of phosphate. This confirms the results obtained by Sacktor (1954) who found the same effect in fly-muscle sarcosomes.

The 45° particles were shown to differ from the 35° particles in their coenzyme requirements and in their ability to oxidize higher fatty acids. Both these effects may be attributed to an enhanced and persisting permeability of the particle surface caused by the higher temperature at which the insects were kept. This enhanced permeability may allow certain cofactors, in the present case CoA, to diffuse through the particle wall and therefore be absent from the isolated particles or present in greatly diminished quantities. As corroboratory evidence for the possible existence of such effects, mention may be made of Bellamy's (1958) findings. He reported that the content of the pigment insectorubin in particles from locust integument fell from 13% in insects reared at 28° to less than 0·01% in insects reared at 35°. This difference in permeability may also mean that higher fatty acids are able to enter the 45° particle but not the

less-permeable 35° particle. The inhibitory effect of higher fatty acid concentrations may be due to the fatty acids being absorbed on the particle's surface, thereby decreasing its permeability for all substrates. The absorption of the fatty acid molecule on the particle surface probably involves its aliphatic chain, since the inhibiting concentrations decrease with increasing chain length and decreasing solubility in water. The wider scope of oxidative activity observed in the 45° particles may be of physiological importance. It is well known that the respiratory activity of the locust in flight is up to 50 times as great as that of the locust at rest (Krogh & Weis-Fogh, 1951). Assuming that the internal body temperature of the locust in flight can rise sufficiently to affect the particle membrane, additional combustible substrates would be made available (in the present case higher fatty acids), thus accounting for the enhanced respiration and supplying the increased energy demands of the flying insect.

SUMMARY

1. The isolation of a particle system from locust-thorax tissue is described; the tissues were derived from insects kept at 35° and at 45° (35° particles and 45° particles).

2. The 35° particles oxidized butyrate completely, but were unable to oxidize higher fatty acids; the 45° particles oxidized both.

3. The 35° particle system required adenosine triphosphate and Mg^{2+} ions; the 45° particle system showed, for the oxidation of butyrate and higher fatty acids, the additional requirement for coenzyme A, and required for the oxidation of higher fatty acids a factor present in sheep-liver extracts.

4. Both systems were partially inhibited by azide, cyanide, arsenite, malonate, fluoride, 2:4-dinitrophenol and ethylenediaminetetra-acetic acid.

5. The oxidation of higher fatty acids and butyrate in the 45° system was inhibited by higher concentrations of the C_8–C_{18} fatty acids. The minimal inhibiting concentration of each fatty acid decreased with increasing chain length.

6. The 35° system when oxidizing butyrate showed oxidative phosphorylation, which could be abolished by 2:4-dinitrophenol and ethylenediaminetetra-acetic acid.

7. The fatty acid-oxidizing systems found were compared with similar systems of mammalian origin.

Reasons are advanced for believing that the differences between the 35° particles and the 45° particles are due to changes in the properties of the particle surface.

8. The possible physiological significance of the results is discussed.

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