CXLII. AN EFFECT OF ASCORBIC ACID ON FATTY ACID OXIDATIONS IN THE LIVER.

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IN the course of experiments on the oxidation of fatty acids by the liver of guinea-pigs it was found that the yield of acetoacetic acid appeared to depend on the state of nutrition of the animal. Preliminary experiments indicated that the livers of guinea-pigs fed for a lengthy period on a scorbutic diet behaved differently from the normal, a lower yield of acetoacetic acid being produced, and for this reason work was commenced on the effects of ascorbic acid on fatty acid oxidations of liver. Although further experiment has not shown a very strict line of demarcation between the activities of the livers of scorbutic and normal guinea-pigs, it has become abundantly clear that ascorbic acid exercises very definite effects on the respiration of, and on the power to oxidise fatty acids by, the livers of both guinea-pigs and rats.

It appears that the most significant effect of the addition of ascorbic acid to liver slices is the maintenance of respiration at a relatively high level for a much longer period than occurs in liver slices to which ascorbic acid has not been added. This is particularly noticeable with the rat where the respiration of a liver slice falls off quickly after an initial period of a fairly constant rate of oxygen uptake.

It is much less noticeable in the guinea-pig where the Q_{O_2} tends to remain constant over a long period; hence results with guinea-pigs are irregular and apparently much more dependent on nutritional conditions than is the case with the rat. Increases of acetoacetic acid production ranging from 30 to 200 % due to the addition of ascorbic acid have been noted with the rat and from 0 to 60 % with the guinea-pig; these increases in the yield of acetoacetic acid are probably closely connected with the maintenance of the relatively high levels in the rate of oxygen uptake, the oxidation of fatty acids being greatly dependent on the general respiratory metabolism of the liver cell.

Technique.

The technique described by the writers [1933] in their work on the oxidation of fatty acids in the liver has been followed. Measurements of oxygen uptake and acetoacetic acid yield (at 37°) have been obtained with the Barcroft differential manometer. Acetoacetic acid has been estimated manometrically by the method described by the writers [1933]. A small change in the technique of this estimation has been adopted: 1 ml. $M/5$ acetate buffer p_H 3.8 has been replaced by 1 ml. $M/2$ acetic acid. The results are equally accurate and consistent.

The acetoacetic yield is expressed by the symbol $Q_{\text{acetoacetic acid}}$ or Q_{ac} which represents the $\mu\text{l. CO}_2$ equivalent to acetoacetic acid produced by 1 mg. dry weight liver tissue per hour.

The only animals used in this work have been guinea-pigs (from various sources) and rats (also from various sources). We have recently confined ourselves, in work with rats, to the Wistar strain and find that this procedure is more satisfactory for obtaining comparable results.

The substrates under investigation have been butyric, crotonic and *dl*- β -hydroxybutyric acids. These have been chosen because they are very good sources of acetoacetic acid and also because the mechanism of their oxidation to acetoacetic acid is at present under investigation in this laboratory. A few experiments with higher fatty acids such as *n*-hexanoic acid have also been carried out and found to give results essentially similar to those found with butyric and crotonic acids.

Buffer solution. It was shown by the writers [1933] that concentrations of phosphate buffer (p_{H} 7.4) of the order $M/15$ appreciably diminish both Q_{O_2} and Q_{ac} of guinea-pig liver, and this was traced to the removal of calcium ions from the medium bathing the liver slices. Low concentrations of phosphate buffer solution do not buffer well in experiments lasting 3 hours or more. The difficulty of adequate buffering has been overcome (or partially so) without affecting the concentration of calcium ions by the use of sodium glycerophosphate buffer. Sodium glycerophosphate (B.D.H.) solution was brought to p_{H} 7.4 by addition of hydrochloric acid, and this was diluted by Locke solution and the substrate under investigation to make its final concentration $M/15$. Experience has shown that with both guinea-pig and rat liver slices of the order of 20 mg. dry weight there is a drop in p_{H} , in an experiment of 3 hours' duration, of about 0.2.

The final medium was made up as follows:

1.0 ml. $M/5$ sodium glycerophosphate solution (p_{H} 7.4).

1.5 ml. Locke solution¹.

0.5 ml. 0.1 M substrate solution.

Such a glycerophosphate-Locke medium has been used in the experiments described below.

Liver slices were added to this medium, and then 0.5 ml. of a neutral solution of ascorbic acid was added. All experiments with liver slices were carried out in an atmosphere of oxygen.

The manometric apparatus was allowed to shake at the rate of 90 times a minute, and readings were taken every 15 minutes.

After 3 hours, the liver slices were carefully removed, washed in distilled water and dried at 105° . Usually very little disintegration of the liver slice occurred. 2.5 or 3 ml. of the medium were then transferred to another Barcroft apparatus for estimation of acetoacetic acid. Determinations of final p_{H} were always made, and the results of any experiment where there had been a considerable change in p_{H} were discarded.

Choice of liver slices. It is important for the investigation of fatty acid oxidation with liver slices to select slices of appropriate sizes and thicknesses. It has been shown by the writers [1933] that mincing liver results in a cessation of fatty acid oxidation and it would be expected that very thin slices of liver

¹ Occasionally we found it desirable to use only 1 ml. Locke solution. Care was always taken, however, to use the same quantity of Locke solution in all vessels in any one experiment. The Locke solution was composed of 0.003 M CaCl_2 , 0.0065 M KCl and 0.1445 M NaCl .

would approximate to the condition of minced tissue. Experience shows that liver slices less than 0.2 mm. thick give irregular results; the Q_{O_2} usually falls off rapidly and there is a diminished Q_{ac} . There is also an upper limit to the thickness of the slices used, determined probably by the diffusion of oxygen. Slices thicker than 0.4 mm. give a diminished Q_{O_2} and Q_{ac} . A few descriptive results are shown in Table I.

Table I. *Effects of varying thicknesses of liver slices (guinea-pig) on $Q_{acetoacetic}$ acid in presence of crotonate, 0.017 M. (Glycerophosphate-Locke medium.)*

Exp.	Thickness mm.	Q_{ac}
1	0.28	2.3
	0.43	2.4
	0.56	1.6
2	0.29	2.0
	0.27	2.0
	0.67	1.1

We have therefore tried in all experiments to cut slices varying between 0.2 and 0.4 mm. Moreover in any one experiment where comparisons are made on one tissue it has been our endeavour to use slices of approximately the same thickness. Rat liver is more difficult to cut than guinea-pig liver and it is usually not an easy matter to obtain a large slice of uniform thickness. Hence we have used two or three small slices of approximately uniform thickness (as determined superficially by opacity or colour) rather than one large slice. Slices are always trimmed—a slice with frayed edges tends to disintegrate in the manometer vessel and to give, therefore, too low a weight after removal at the termination of the experiment.

The thickness of the slices is determined by floating them in a Petri dish containing saline, on the bottom of which is a sheet of squared paper. The area of the slice is outlined on the paper as carefully as possible and the area calculated in mm.² With trimmed, rectangular slices the area can be obtained in this way with considerable accuracy. The thickness of a slice can now be calculated from the volume ($= 5 \times$ dry wt. in mg.) and the area (mm.²). With experience, slices of thicknesses varying from 0.2 to 0.4 mm. can be chosen with ease.

The livers are cut immediately after removal from an animal which has been killed by cutting the throat and then well bled.

Source of ascorbic acid. Two samples of ascorbic acid have been used in the work; one was kindly presented to us by Prof. A. Szent-Györgyi. The other was kindly given to us by the Hoffmann-La Roche Co. according to whom the analytical figures were: $[\alpha]_D = +24^\circ$; m.p. 188–189°; equiv. wt. (iodine titration) = 88. The two samples behaved in the same way.

Differences between guinea-pig liver and rat liver.

Representative results indicating the respiration of, and acetoacetic acid production by, guinea-pig and rat livers are shown in Table II. Detailed results concerning guinea-pig liver oxidations are given in our earlier paper [1933] and will not be further referred to here. The most striking difference between rat and guinea-pig livers is the high Q_{O_2} of the former compared with that of the latter. Rat liver usually gives a Q_{O_2} of 10–12; guinea-pig liver gives a Q_{O_2} of 4–6.

Another difference, which will be commented upon again later, is that whilst the oxygen uptake of guinea-pig liver (under the experimental conditions

Table II. *Respiration of, and acetoacetic acid production by, liver slices from guinea-pig and rat.*

Substrates present as Na salts.

Exp.	Substrate (0.017 <i>M</i>)	Q_{O_2}	Q_{ac}
1. Guinea-pig liver	Nil	4.0	0.2
„	Crotonate	9.0	3.6
„	Butyrate	10.5	4.4
„	<i>dl</i> - β -Hydroxybutyrate	6.1	1.8
2. Rat liver	Nil	11.6	0.8
„	Crotonate	12.8	4.9
3. Rat liver	Nil	10.7	0.9
„	Butyrate	12.6	3.0
4. Rat liver	Nil	9.5	0.7
„	<i>dl</i> - β -Hydroxybutyrate	11.9	6.4

already described) is usually constant for 2 or 3 hours, that of rat liver begins to fall off rapidly after a preliminary period of fairly constant oxygen uptake which varies from 45 to 90 mins. As a general rule the thinner the slice the quicker is the tendency for the oxygen uptake to fall from a linear rate.

When sodium butyrate or sodium crotonate at a concentration of 0.014 *M* is added to guinea-pig liver there is a decided increase in Q_{O_2} , the figures in Table II being quite typical. The rise in Q_{O_2} with rat liver due to butyrate or crotonate is always very much less and we have had cases when the Q_{O_2} of rat liver has not been changed by the addition of butyrate or crotonate, although it was clear from the acetoacetic acid production that oxidation of the fatty acid had been proceeding.

The acetoacetic acid production from guinea-pig liver in presence of butyrate or crotonate is variable and seems to be dependent on the size of the liver. As a general rule the larger the liver in a well fed animal the smaller is the yield of acetoacetic acid. This observation will be commented upon at greater length in another communication. The acetoacetic acid production from *dl*- β -hydroxybutyric acid (0.014 *M*) with guinea-pig liver is much less variable than that from butyric or crotonic acid. With this animal the Q_{ac} is usually of the order 1.8 whilst that from butyrate and crotonate may vary from 1.5 to 4.5. With rat liver the yield of acetoacetic acid from *dl*- β -hydroxybutyric acid is considerably higher than with guinea-pig and the Q_{ac} is of the order 4–6. The Q_{ac} with rat liver from butyrate and crotonate is lower and has about the same range of variation as with guinea-pig.

We have observed that the rate of oxygen uptake tends to fall off to a greater extent in the presence of crotonate than in the presence of butyrate, and this seems to apply to both guinea-pig and rat.

Oxidation of ascorbic acid in the liver.

Harrison [1933] in his experiments on the effect of ascorbic acid on the respiration of scorbutic and normal guinea-pig tissue “balances” the ascorbic acid in the manometric vessels by placing the acid in phosphate buffer without tissue in the left-hand vessel of the Barcroft apparatus. This we have not done in our experiments because there is no proof that the rate of oxidation of ascorbic acid in the presence of tissue is equal to or is of the same order as that in the absence of tissue. This constitutes only a minor criticism of Harrison’s results, for he used smaller quantities of ascorbic acid than we have used.

The following experiments indicate, with intact liver slices, that after 3 hours at 37° ascorbic acid is by no means fully oxidised, although it is so in the absence of tissue.

Exp. 1. 0.5 ml. of a neutral solution of ascorbic acid containing approximately 3 mg. per ml. was added to 3 ml. glycerophosphate-Locke medium and allowed to autoxidise in the presence of oxygen under the same conditions as were used for work with liver slices. After 3 hours 1 ml. of the medium was added to 4 ml. 3 % trichloroacetic acid and titrated with *N*/200 iodine solution. 0.04 ml. was required. The ascorbic acid was therefore practically completely oxidised under the given experimental conditions.

Exp. 2. 0.5 ml. of a neutral solution of ascorbic acid containing approximately 3 mg. per ml. was added to a glycerophosphate-Locke medium containing rat liver slices and to a glycerophosphate-Locke-crotonate (0.014 *M*) medium containing rat liver slices. After 3 hours' shaking in oxygen at 37°, the slices were removed and 1 ml. of each of the media was added to 4 ml. 3 % trichloroacetic acid and the solution titrated with *N*/200 iodine solution. The same procedure was carried out in control experiments where no ascorbic acid had been added. The following iodine titres were obtained:

			<i>N</i> /200 iodine titre ml.
(a) Glycerophosphate-Locke medium	Liver slices	No ascorbic acid	0.16
(b) Glycerophosphate-Locke-crotonate medium	"	"	0.12
(c) Glycerophosphate-Locke medium	"	Ascorbic acid present	0.48
(d) Glycerophosphate-Locke-crotonate medium	"	"	0.49

An iodine titration on the ascorbic acid itself showed that 1 ml. of the medium would have required 0.95 ml. *N*/200 iodine had there been no oxidation of ascorbic acid. Hence from (a) and (c) 32/95, i.e. 33 % of the original quantity of ascorbic acid still remains and from (b) and (d) 38 % remains. Another experiment carried out under similar conditions showed that 49 % of the original quantity of ascorbic acid still remained at the termination of the experiment.

These experiments prove either (1) that the oxidation of ascorbic acid is retarded by liver slices, or (2) that oxidised ascorbic acid is reduced by liver slices, an equilibrium being established.

The second conclusion is the more likely one in view of Szent-Györgyi's experiments on the reduction of oxidised hexuronic acid by animal tissues [1928].

The action of ascorbic acid on the respiration of, and acetoacetic acid production by, normal guinea-pig liver.

According to Harrison [1933] ascorbic acid has but little effect on the respiration of normal guinea-pig liver. The probability is that there is a small but variable increase if the criticism mentioned above is taken into consideration. Our own experiments bear out this conclusion. Table III shows the effects of the addition of ascorbic acid¹ on the Q_{O_2} and Q_{ac} of normal guinea-pig liver slices in the presence of sodium crotonate or butyrate. It will be observed that the Q_{O_2} is in all cases raised, but the amount of rise is only about that to be expected by the oxidation of part of the ascorbic acid added. The expected change in Q_{O_2} (taken over 3 hours) for liver slices of 20 mg. dry weight is a rise of about 1.6 with an initial concentration of ascorbic acid of 0.5 mg./ml. but it is difficult to be certain of this as estimations of the amounts of ascorbic acid remaining at the end of the experiments were not made. It is clear that no significant increase attributable to the action of the added ascorbic acid on the oxidative metabolism of liver was obtained. Exp. 258 showed definitely a higher value of Q_{O_2} in presence of ascorbic acid but it is to be noted that the Q_{O_2} of the liver in this experiment in

¹ The ascorbic acid was neutralised with NaOH just before being added to the Barcroft vessels.

Table III. *Effects of ascorbic acid on the respiration of, and acetoacetic acid production by, liver slices of normal guinea-pig in presence of butyrate and crotonate.*

Substrate concentration 0.014 M. Glycerophosphate-Locke medium. Initial $p_H = 7.4$.
 Final p_H = usually 7.1–7.2. Each experiment refers to a single animal.

Exp.	Weight of liver slices taken (mg. dry wt.)	Substrate	mg. per ml. ascorbic acid added	Q_{O_2} (av. over 3 hours)	Q_{ac}
128	24.3	Crotonate	Nil	9.5	3.1
	21.2	"	0.43	10.4	3.5
139	24.2	Butyrate	Nil	8.5	3.0
	27.0	"	0.50	9.6	2.9
140	38.8	"	Nil	10.6	3.5
	34.5	"	0.50	12.1	4.3
141	22.5	Crotonate	Nil	9.0	3.2
	18.5	"	0.50	9.6	3.4
256	17.4	"	Nil	7.6	2.4
	17.6	"	0.86	9.8	3.2
258	14.6	"	Nil	7.0	2.3
	12.1	"	0.86	10.6	3.6

the absence of ascorbic acid was lower than usual; this was due to the fact that the rate of oxygen uptake fell off more rapidly than commonly occurred. In the presence of ascorbic acid the rate of oxygen uptake was constant throughout most of the experiment (see Table VI).

It will be noted that in most cases the presence of ascorbic acid produced an increase in Q_{ac} . The increase is usually small, but occasionally, as in Exp. 258, of the order of 50 to 60 %.

The results given in Table III are typical of a large number of experiments with normal guinea-pigs and show that whilst the effect of added ascorbic acid on the whole is small, irregularities (*i.e.* significant increases in Q_{O_2} and Q_{ac}) may occur.

Ascorbic acid and scorbutic guinea-pig liver¹.

The livers of guinea-pigs fed on a scorbutic diet for 15 days or longer usually show rises of Q_{O_2} and Q_{ac} in the presence of ascorbic acid, which are significantly greater than the average rises found with normal guinea-pigs. The animals were killed when they showed definite signs of scurvy and after their weights had reached a maximum and fallen 10–15 % of the maximum value. There is less irregularity about the results with scorbutic guinea-pig livers and an increase

 Table IV. *Effects of addition of ascorbic acid on respiration of, and acetoacetic acid production by, liver slices from guinea-pigs fed on a scorbutic diet for varying periods.*

Substrate, crotonate 0.014 M. Conditions as in Table III.

Exp.	Period of scorbutic diet days	Weight of liver slices taken mg. dry wt.	mg. per ml. ascorbic acid added	Q_{O_2} (av. over 3 hours)	Q_{ac}
260	12	21.5	Nil	9.4	2.7
		21.0	0.86	11.2	3.3
264	15	17.6	Nil	8.6	2.9
		16.6	0.86	12.3	4.1
131	17	23.8	Nil	8.5	2.9
		24.4	0.50	10.1	4.0

¹ We are much indebted to the authorities of the Veterinary Laboratory, Weybridge, for supplying us with guinea-pigs in various stages of scurvy.

in Q_{ac} has always been found. The results of Table IV are typical. The percentage increase in Q_{ac} due to the addition of ascorbic acid is found to be always greater than the percentage increase in Q_{O_2} . It is worthy of note, however, that increases of the same order have been found occasionally with normal and apparently healthy guinea-pigs.

Ascorbic acid and normal rat liver.

Ascorbic acid has very definite effects on the Q_{O_2} and Q_{ac} of rat liver in the presence of sodium butyrate or sodium crotonate. The results given in Table V

Table V. *Effects of addition of ascorbic acid on the respiration of, and the production of acetoacetic acid by, normal rat liver in the presence of butyrate or crotonate.*

Substrate concentration 0.014 M. Conditions as in Table III.

Exp.	Weight of liver slices taken mg. dry wt.	Thickness of slices mm.	Substrate	mg. per ml. ascorbic acid added	Q_{O_2} (av. over 3 hours)	Q_{ac}
134	21.7	—	Butyrate	Nil	13.2	3.6
	25.5	—	"	0.50	15.6	3.9
	17.2	—	Crotonate	Nil	10.0	2.5
	25.5	—	"	0.50	14.3	4.4
160	22.8	—	"	Nil	11.1	3.6
	20.5	—	"	0.5	13.9	4.5
	18.3	—	"	1.0	15.3	4.9
	19.7	—	"	2.0	17.7	5.6
263	13.6	0.26	"	Nil	12.2	3.1
	14.5	0.26	"	0.86	13.0	4.3
266	14.0	0.27	"	Nil	12.0	3.6
	12.8	0.26	"	0.86	16.6	6.6
267	22.3	0.33	"	Nil	12.4	3.2
	20.4	0.37	"	0.86	13.2	4.5
268	16.6	0.30	Butyrate	Nil	14.1	3.2
	17.6	0.27	"	0.86	16.5	4.5
In the following experiments only Wistar rats were used.						
274	20.9	0.38	Nil	Nil	11.6	0.8
	19.6	0.37	Crotonate	Nil	12.9	4.9
	24.2	0.43	Nil	0.86	13.0	1.6
	20.2	0.39	Crotonate	0.86	16.7	8.4
275	13.6	0.24	Nil	Nil	8.0	0.5
	13.3	0.23	Butyrate	Nil	10.0	1.6
	16.5	0.25	Nil	0.86	11.2	1.0
	17.5	0.26	Butyrate	0.86	13.7	2.5
276	23.8	0.37	Nil	Nil	10.7	0.9
	22.4	0.33	Butyrate	Nil	12.6	2.9
	20.0	0.32	Nil	0.86	12.7	1.4
	18.3	0.30	Butyrate	0.86	17.8	5.3
283	15.9	0.28	Crotonate	Nil	9.4	1.8
	16.2	0.30	"	0.43	15.6	4.1
	14.5	0.28	"	0.86	15.1	4.5
	15.8	0.30	"	1.3	14.7	5.1
285	20.3	0.22	Nil	Nil	8.4	0.3
	29.2	0.28	Crotonate	Nil	8.1	1.5
	20.7	0.23	Nil	0.43	12.2	0.4
	25.2	0.29	Crotonate	0.43	13.9	3.8
287	23.7	0.33	"	Nil	11.3	2.2
	18.6	0.28	"	0.43	15.1	2.6
288	26.8	0.33	"	Nil	7.0	0.9
	20.0	0.30	"	0.43	11.9	1.4

are typical of those found in about 50 experiments. The increases in Q_{O_2} and Q_{ac} are not regular and vary from rat to rat, but increases in Q_{ac} of the order of 80 % or more have often been found (see Exps. 134, 160, 266, 274, 276, 283, 285). Large increases in Q_{O_2} have also been obtained but as stated earlier these are more difficult to interpret owing to the fact that analyses of the ascorbic acid contents of the vessels at the termination of the experiments were not carried out. Rat liver usually gives a significant Q_{ac} in the absence of added substrates, and this value is invariably increased by the addition of ascorbic acid (see Exps. 274, 275, 276).

The problem now remains as to how ascorbic acid brings about the increased oxidation of butyric and crotonic acids—a very definite phenomenon in the case of rat liver and of scorbutic guinea-pig liver but doubtful, and at all events irregular, with normal guinea-pig liver. Control experiments have shown that the autoxidation of ascorbic acid does not induce oxidation of butyrate or crotonate (*i.e.* in the absence of tissue) and the negative results found with some guinea-pig livers confirm this.

It seems at first sight unlikely that ascorbic acid is essential for the oxidation of fatty acids, for scorbutic guinea-pig liver gives a Q_{ac} not far removed from normal. On the other hand no tests for ascorbic acid were carried out on the livers of the scorbutic animals and it is impossible for us to say what were the actual concentrations of ascorbic acid in the livers in question.

An explanation of this phenomenon, more satisfactory than one involving the assumption that ascorbic acid specifically affects fatty acid oxidation, comes from a consideration of the effects of ascorbic acid on the rates of oxygen uptake.

The effect of ascorbic acid on the rates of oxygen uptake by liver slices.

The rate of oxygen uptake by guinea-pig liver either alone or in the presence of crotonate and butyrate (0.014 *M*) is usually constant, the Q_{O_2} at the end of a 3 hours' experiment not being appreciably different from that half way through the experiment.

The presence of ascorbic acid does not alter the rate apart from a rise in Q_{O_2} at the commencement of an experiment owing probably to autoxidation of a portion of the ascorbic acid itself.

Rat livers, however, when slices are used of the thickness already quoted almost invariably show a fairly rapid fall in Q_{O_2} after an initial fairly steady rate of oxygen uptake, and at the end of 3 hours the Q_{O_2} may be less than 50 % of that half way through the experiment. The presence of ascorbic acid always diminishes this rate of fall, so much so that the rate of oxygen uptake may be the same at the end of 3 hours as it was at the end of 1½ hours. In Table VI an analysis of most of the results already quoted in earlier tables is given.

Turning to the first part of the table dealing with guinea-pigs it will be seen that the percentage decrease in Q_{O_2} at the end of the experiment from that half way through is small and not appreciably affected by ascorbic acid. The percentage increase in Q_{ac} due to the ascorbic acid is also small, though appreciable in Exp. 256. In Exp. 258, there is a 17 % drop in Q_{O_2} which is removed by the presence of ascorbic acid; in this experiment the percentage increase in Q_{ac} is highest (50 %). There is little doubt from our experience that had these experiments been carried on for a longer period the percentage decrease in Q_{O_2} in the absence of ascorbic acid would have been greater and the percentage increase in Q_{ac} due to ascorbic acid considerably larger.

Table VI. *A comparison between the effects of ascorbic acid on acetoacetic acid production and the rate of fall of Q_{O_2} of liver slices.*

Exp.	Substrate	mg. per ml. ascorbic acid added	% increase in Q_{ac} due to added ascorbic acid	% decrease in Q_{O_2} at end of 3 hours from that at end of $1\frac{1}{2}$ hours
Guinea-pigs (normal).				
128	Crotonate	Nil	—	0
	"	0.43	13	0
129	Butyrate	Nil	—	10
	"	0.5	0	10
139	"	Nil	—	0
	"	0.5	0	10
140	"	Nil	—	10
	"	0.5	22	10
141	Crotonate	Nil	—	0
	"	0.5	6	0
256	"	Nil	—	23
	"	0.86	33	20
258	"	Nil	—	17
	"	0.86	57	0
Rats.				
263	"	Nil	—	70
	"	0.86	39	0
266	"	Nil	—	40
	"	0.86	83	8
267	"	Nil	—	20
	"	0.86	41	8
268	Butyrate	Nil	—	40
	"	0.86	41	11
274	Crotonate	Nil	—	70
	"	0.86	71	15
275	Butyrate	Nil	—	30
	"	0.86	55	9
276	"	Nil	—	37
	"	0.86	81	9
283	Crotonate	Nil	—	66
	"	0.43	126	10
	"	0.86	150	11
	"	1.3	188	9
285	"	Nil	—	67
	"	0.43	153	15

Turning to the second part of the table dealing with the rats we observe percentage decreases in Q_{O_2} varying from 20 to 70, these decreases being practically eliminated by the presence of ascorbic acid. It is in these experiments that the largest effects of ascorbic acid in increasing Q_{ac} are to be found.

Figs. 1–4 illustrate the action of ascorbic acid on the rate of change of Q_{O_2} of rat liver slices throughout an experiment. Fig. 1 shows the action of ascorbic acid on the change of Q_{O_2} with time of rat liver slices in a glycerophosphate-Locke medium containing no added substrate. Figs. 2 and 3 show the effects of differing quantities of ascorbic acid on the respiration of rat liver in presence of sodium crotonate. Fig. 4 refers to the effect of ascorbic acid on the change of Q_{O_2} of rat liver in presence of sodium butyrate. The following points are of note:

(a) There is an initial sharp fall in the rate of change of Q_{O_2} in the presence of ascorbic acid, the Q_{O_2} then arriving at a fairly constant value. The initial sharp fall is probably due to autoxidation of part of the ascorbic acid added.

(b) The constant Q_{O_2} found at the end of an experiment in which ascorbic acid has been added to the liver slices is somewhat less than, but usually not far removed from, the initial rates of oxygen uptake found with those liver slices to which ascorbic acid has not been added.

Fig. 1.

Fig. 1. Effect of addition of ascorbic acid (0.43 mg./ml.) on oxygen uptake by normal rat liver slices.

Fig. 2.

Fig. 2. Effect of addition of ascorbic acid (0.86 mg./ml.) on oxygen uptake of rat liver slices in presence of sodium crotonate (0.014 M).

Fig. 3.

Fig. 3. Effect of addition of ascorbic acid (0.43 mg./ml.) on oxygen uptake of rat liver slices in presence of sodium crotonate (0.014 M).

Fig. 4.

Fig. 4. Effect of addition of ascorbic acid (0.86 mg./ml.) on oxygen uptake by rat liver slices in presence of sodium butyrate (0.014 M).

(c) Essentially the same phenomenon—a maintenance of a relatively high rate of respiration induced by the added ascorbic acid—occurs in the presence of either crotonate or butyrate and also in the absence of any added substrate.

The clearest inference from these results is that ascorbic acid plays a significant part in maintaining an intact respiratory metabolism in the liver. The efficiency of fatty acid oxidation depends upon the integrity of this respiratory metabolism. This hypothesis makes the action of ascorbic acid on fatty acid oxidation an indirect one but it serves to give a rational interpretation of the phenomena described. Further experiment alone can inform us whether ascorbic acid has a direct action on fatty acid oxidation or whether its action takes place *via* a number of intermediate steps. Such a hypothesis allows us to conclude that substances, other than ascorbic acid, and intimately associated with the respiratory metabolism of liver tissue may also affect fatty acid oxidation in a similar way; such a conclusion is obviously open to experimental verification.

*Action of ascorbic acid on acetoacetic acid production by rat liver slices
in presence of dl- β -hydroxybutyric acid.*

In contrast to the effects of ascorbic acid on the oxidation of butyric and crotonic acids in presence of rat liver, there is little or no effect on the oxidation of *dl*- β -hydroxybutyric acid. Typical results are shown in Table VII. It follows

Table VII. *Effect of addition of ascorbic acid on the production of acetoacetic acid by rat liver slices in the presence of dl- β -hydroxybutyric acid.*

Exp.	Weight of liver taken mg. dry wt.	Thickness of slices mm.	Conc. of substrate <i>M</i>	mg. per ml. ascorbic acid added	Q_{O_2} (av. over 3 hours)	Q_{ac}
269	16.1	0.26	0.023	Nil	8.5	3.9
	16.3	0.28	0.023	0.86	10.8	4.3
270	14.4	0.27	0.023	Nil	13.4	6.3
	15.0	0.33	0.023	0.86	15.2	7.1
277	14.7	0.27	0.014	Nil	11.9	6.4
	15.8	0.29	0.014	0.86	13.8	5.8

that the oxidation of this hydroxy-acid does not depend upon the same mechanisms as those governing the oxidation of butyric and crotonic acids. This conclusion is supported by quite independent evidence, which it is hoped to report in due course in a further publication.

Action of iodoacetic acid on fatty acid oxidation by the liver.

It was found early in the course of our work on fatty acid oxidations by liver slices that iodoacetic acid (used as the sodium salt) exercised highly toxic effects at very low concentrations. Concentrations of the order of *N*/5000 will reduce the acetoacetic acid yield from butyrate or crotonate by over 50 %. The toxic action of the halogen-acid is a progressive one, the apparent inhibiting action on acetoacetic acid yield increasing with the length of exposure of the tissue to the iodoacetic acid.

On making a closer study of this phenomenon it was found that the apparent inhibitory effect of iodoacetic acid was greatly dependent on the thickness of slices used. The thicker the slice the smaller was the inhibition of acetoacetic acid production and experience showed that the most suitable thickness of slices to be used in demonstrating the highly toxic action of iodoacetic acid in an experiment of 3 hours' duration was of the order 0.2–0.3 mm. (using iodoacetic acid at a concentration about *N*/5000). Thinner slices than these suffered very rapid poisoning by the halogen acid. A series of typical results is shown in Table VIII.

Table VIII. *Action of iodoacetic acid on the production of acetoacetic acid by liver slices in presence of sodium crotonate (0.014 M).*

Exp.	Animal	Weight of liver slices taken mg. dry wt.	Thickness of slices mm.	Conc. of iodoacetic acid	Q_{O_2} (av. over 3 hours)	Q_{ac}
230	Guinea-pig	15.2	—	Nil	9.4	4.0
		15.9	—	N/6000	6.0	1.3
254	„	22.7	0.33	Nil	7.7	1.8
		11.8	0.15	N/6000	3.4	0.3
		22.1	0.38	N/6000	7.8	1.4
		32.9	0.58	N/6000	7.7	1.8
255	„	27.8	0.41	Nil	6.4	2.1
		31.9	0.38	N/7000	7.2	2.1
256	„	17.4	0.22	Nil	7.6	2.4
		12.7	0.21	N/5250	5.9	1.1
257	Rat	8.6	0.18	Nil	13.9	4.3
		13.1	0.24	N/5250	5.7	1.6
258	Guinea-pig	14.6	0.26	Nil	7.0	2.2
		11.8	0.23	N/5250	3.0	1.3
260	„	21.5	0.30	Nil	9.0	2.7
		21.3	0.27	N/5250	7.7	1.4

Attention may be drawn to Exps. 254 and 255 where it will be seen that with slices about 0.4 mm. thick but little change is shown in Q_{ac} in the presence of iodoacetic acid. Concentrations of iodoacetic acid higher than N/5000 will of course affect even the thicker slices. Exps. 256, 257, 258, 260 show that with slices of 0.2–0.3 mm. thickness, N/5250 iodoacetic acid brings about a drop in Q_{ac} of the order of 50 %.

Q_{O_2} is also decreased by the iodoacetic acid. A study of the rates of oxygen uptake of guinea-pig liver slices exposed to iodoacetic acid shows that the rate of oxygen uptake does not fall off immediately; the rate remains approximately constant for a variable period—45 to 90 mins.—and then falls rapidly. The phenomenon is reminiscent of that obtaining with rat liver slices (*i.e.* in the absence of iodoacetic acid). It could be explained by assuming that iodoacetic acid reacts slowly in the liver slice with some substance essential for the respiration of liver (and possibly for fatty acid oxidation) and that when the concentration of this substance falls below a certain limit there occurs a rapid fall in Q_{O_2} and Q_{ac} .

Action of ascorbic acid on guinea-pig liver poisoned by iodoacetic acid.

In view of the results already obtained with rat liver it was decided to investigate the action of ascorbic acid on normal guinea-pig liver slices exposed to iodoacetic acid.

It was found at once that the addition of ascorbic acid had precisely the same action on guinea-pig liver poisoned by iodoacetic acid as on normal rat liver. There was a prolongation of the steady rate of oxygen uptake and a decided increase in Q_{ac} . Representative results are shown in Table IX. In the presence of small concentrations of iodoacetic acid the decrease in Q_{O_2} at the end of 3 hours from that at the end of the first hour is 80 % or over. This decrease is lessened to the extent of about a half by the presence of ascorbic acid—a fact which is reflected in the increased average Q_{O_2} . The Q_{ac} may be increased as much as 200 % by the presence of ascorbic acid. We have never found however that ascorbic acid entirely neutralises the action of iodoacetic acid—the main effect, as already stated, appears to be a prolongation of the

Table IX. *Action of ascorbic acid on the production of acetoacetic acid by normal guinea-pig liver slices poisoned by iodoacetic acid.*

Substrate, sodium crotonate 0.014 *M*. The ascorbic acid was added at the same time as the iodoacetic acid to the liver slices (*i.e.* at the commencement of the experiment).

Exp.	Weight of liver slices mg. dry wt.	Thick-ness of slices mm.	Conc. of iodoacetic acid	mg. per ml. ascorbic acid added	Q_{ac}	Q_{O_2}	% decrease in Q_{O_2} at end of 3 hours from that at end of 1 hour
226	19.3	—	<i>N</i> /3000	Nil	0.9	4.9	80
	23.0	—	"	0.43	2.5	8.2	40
251	13.3	0.21	<i>N</i> /7000	Nil	3.3	8.9	86
	16.7	0.22	"	0.86	4.4	13.7	54
256	12.7	0.21	<i>N</i> /5250	Nil	1.1	6.5	80
	16.7	0.25	"	0.86	2.2	8.5	42

maintenance of a relatively high rate of oxygen uptake. Experiments have failed so far to indicate a reaction between iodoacetic acid and ascorbic acid such as that which occurs between iodoacetic acid and glutathione.

These experiments with iodoacetic acid serve to confirm the conclusions already reached on the mechanism of action of ascorbic acid. If ascorbic acid were directly concerned with fatty acid oxidation and the toxic effect of iodoacetic acid were due to a reaction with ascorbic acid, then it would be possible to neutralise the action of the halogen acid by placing it in contact with the ascorbic acid. Such a neutralisation occurred with glutathione and iodoacetic acid in the case of oxidation of glucose by brain tissue [Quastel and Wheatley, 1932]. Ascorbic acid, however, does not neutralise the action of iodoacetic acid—even when present in considerable excess—it simply diminishes the rapidity of action of the latter.

One inference would be that iodoacetic acid in the liver slice removes a substance from the respiratory system, the production of which is greatly influenced by the presence of ascorbic acid. The substance in question would not only be essential for the maintenance of the normal respiratory level but also for the efficiency of fatty acid oxidation in the liver.

Action of glycogen on fatty acid oxidations.

In our previous paper [1933] we reported an inhibitory action of glycogen on acetoacetic acid production by guinea-pig liver slices. Over 12 experiments were carried out with consistent results. Repetition of this work 9 months later both with the same sample of glycogen and with a new sample of glycogen has failed to confirm this inhibition and at present we are at a loss to understand this irregularity of results. Our former conclusion, however, that added carbohydrate does not increase fatty acid oxidation in liver slices is not affected. Analysis of many of the livers used in the work described in this paper has not shown any distinct correlation between Q_{ac} and the glycogen content of the livers.

SUMMARY.

1. Ascorbic acid, which is fully oxidised at 37° at p_H 7.4 in presence of oxygen, is only partially oxidised under the same conditions in presence of liver slices.

2. The presence of ascorbic acid secures an increase in Q_{O_2} and $Q_{\text{acetoacetic acid}}$ (in the latter case ranging from 30 to 200 %) of rat liver slices, when these are in a glycerophosphate-Locke medium containing sodium butyrate or crotonate.

3. The presence of ascorbic acid secures a significant increase in $Q_{\text{acetoacetic acid}}$ of scorbutic guinea-pig liver slices in media containing butyrate or crotonate; with normal guinea-pig liver slices, the increase in $Q_{\text{acetoacetic acid}}$ is small and irregular.

4. Ascorbic acid brings about a prolongation of the maintenance of the steady rate of oxygen uptake by guinea-pig and rat liver slices.

5. It is suggested that the effects of ascorbic acid on $Q_{\text{acetoacetic acid}}$ are due to the oxidation of fatty acids (*e.g.* butyric and also crotonic acids) being inherently connected with the general respiratory metabolism of the liver cell, and that ascorbic acid is important in maintaining this metabolism at a normal level.

6. Ascorbic acid appears not to affect the oxidation of *dl*- β -hydroxybutyric acid in presence of rat liver slices.

7. Iodoacetic acid at concentrations of the order of $N/5000$ has a large inhibitory action on the production of acetoacetic acid from butyric or crotonic acid by liver slices. This inhibition is reduced by the presence of added ascorbic acid, the reduction not being due to a combination between iodoacetic acid and ascorbic acid.

8. The action of glycogen on acetoacetic acid production by liver slices is discussed.

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