CCLXXX. FUMARATE AND TISSUE RESPIRATION

II. THE RESPIRATION OF PIGEON BREAST MUSCLE DISPERSIONS

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BANGA has found [Annau *et al.* 1935] that if minced pigeon breast muscle be treated with ice-cold water and then squeezed through muslin, a very fine dispersion of the tissue can be obtained. This, with suitable additions, will show a respiration which resembles that of the original minced muscle, in that it is of the same order of magnitude and can be accelerated by fumarate and inhibited by malonate. It is necessary to add an extract of muscle made with hot trichloroacetic acid, which is said to supply donators and co-dehydrogenases. The dispersion can be separated by centrifuging into a precipitate containing the ferments, and a solution containing an activator ("Zwischensubstanz"), both being essential to the activity. Laki has shown [Annau *et al.* 1936] that the dispersion behaves similarly to minced muscle in that it causes oxaloacetate to react with the formation of malate and pyruvate.

Stare & Baumann [1936] have observed that the oxygen consumption of pigeon breast muscle which has been ground with sand can be greatly increased by the addition of fumarate together with an extract made from the same tissue by digesting with water for 1 hr. at room temperature before deproteinizing by heat. Cozymase would not produce quantitatively the same effect as this extract.

The present paper describes a study of dispersions made by Banga's method, with a view to identifying the coenzymes and substrates concerned in their respiration.

EXPERIMENTAL

Analyses

Lactic acid was determined according to Friedemann & Graeser [1933] after treatment with tungstic acid and copper-lime. Glycogen was determined by a modification of Pflüger's method [Good *et al.* 1933], except that the material was heated for a full 3 hr. with 30% KOH, since Young [1937] has found that this is usually necessary for muscle glycogen.

All measurements of O_2 consumption were made at 38° in the Haldane-Barcroft-Warburg apparatus, in solutions buffered by 0·028 *M* sodium phosphate of *p*H 7·45, and with air in the gas space.

Preparation of the dispersion

Immediately after the death of the pigeon, the breast muscle was dissected, cooled on frozen distilled water and minced in an ice-cold Latapie mill. It was then ground for 10 min. with 1·5 vol. previously cooled distilled water in a mortar surrounded by a freezing mixture, being kept throughout in a thick pasty condition. It was squeezed through muslin, using a rubber glove, and the

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resulting thick dispersion of the muscle was carefully brought to *p*H *ca.* 7·3 with 0·2 *N* NaOH, of which 0·09 vol. was almost invariably needed. Throughout all operations the dispersion was kept in ice. Before use the dispersion was usually diluted with an equal volume of water to increase the accuracy of pipetting into the vessels. In nearly all experiments an amount of the neutralized and diluted dispersion was taken which corresponded to 0·1 ml. of the original dispersion, and this was diluted to a final volume of 2 ml. in the manometer vessel.

Properties of the dispersion

The particles were small enough to pass through a Jena G. 3 glass filter and the dispersions could be accurately pipetted, as shown by numerous multiple determinations. The particles were difficult to centrifuge, except in an angle centrifuge after dilution of the dispersion with water. The solid content of the neutralized and diluted extracts lay between 30 and 50 mg. per ml., the glycogen content between 0·7 and 6·1 mg. per ml., and the lactic acid content between 0·9 and 1·4 mg. per ml.

Coenzymes. The respiration of the dispersion was greatly increased on the addition of an extract made from pigeon breast muscle by stirring it with 1·5 vol. water at 80° for 5 min. and filtering. An extract made from baker's yeast in the same way had a similar effect. With both the respiration was strongly inhibited by 0·01 *M* malonate, just as is the respiration of the minced muscle.

Fig. 1 shows that increasing the amount of muscle extract increases both the initial rate and the total O_2 consumption, whilst the presence of 0·005 *M*

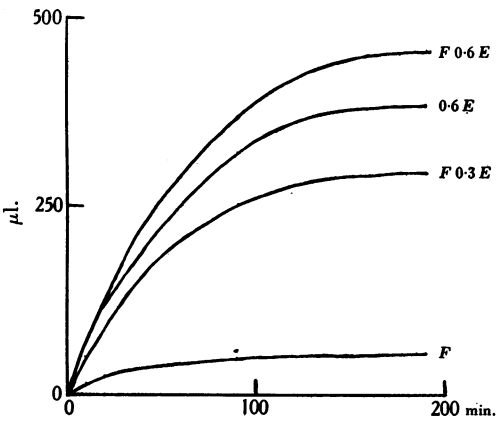

Fig. 1.

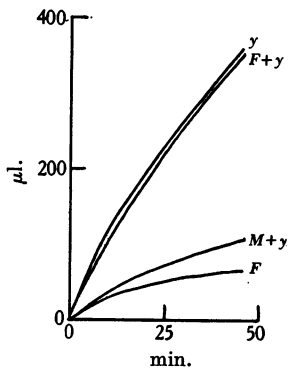

Fig. 2.

Fig. 1. O_2 consumption of dispersion in presence of muscle extract. *F*, 0·005 *M* fumarate; *F* 0·3 *E*, 0·005 *M* fumarate, 0·3 ml. muscle extract; *F* 0·6 *E*, 0·005 *M* fumarate, 0·6 ml. muscle extract; 0·6 *E*, 0·6 ml. muscle extract.

Fig. 2. O_2 consumption of dispersion in presence of yeast extract. *F*, 0·01 *M* fumarate; $y = 0·3$ ml. yeast extract; $M = 0·01$ *M* malonate. (Yeast extract with fumarate, but without dispersion, took up no O_2.)

fumarate has no great influence on them. This is the case also with yeast extract, as is seen from Fig. 2. The effect of muscle extract is seen also from Table I.

Table I

	1	2	3
Dispersion (ml.)	0·17	0·17	0·17
Phosphate (ml.)	0·5	0·5	0·5
Muscle extract (ml.)	—	0·5	—
Cozymase (1 mg. per ml.) (ml.)	—	—	0·3
Magnesium chloride (0·1 *M*) (ml.)	—	—	0·1
H_2O (ml.)	1·3	0·8	0·9
O_2 uptake (60 min.) (μl.)	**50**	**500**	**414**

Further experiments showed that the chief function of the muscle extract in increasing the respiration was to supply coenzymes, an adequate amount of substrate being present in the dispersion. For when the coenzymes adenine-nucleotide and magnesium are added instead of muscle or yeast extract the respiration still undergoes a large increase (Table I) and remains sensitive to the inhibitory action of malonate (Table II). Further, the addition of glycogen, a

Table II. *Dispersion in presence of magnesium and cozymase*

Concentrations as in Table I

Additions ...	—	0·2% NaCl	0·4% NaCl	0·01 *M* malonate
60 min. O_2 consumption (μl.)	238	224	163	21

substrate present in the muscle extract, usually had little effect on the respiration of the dispersion in presence of the coenzymes; although in a few dispersions of low activity, presumably poor in substrate, glycogen considerably increased the respiration (Table III).

Table III. *Dispersion in presence of magnesium and cozymase*

Addition ...	—	1·6 mg. glycogen
60 min. O_2 consumption (μl.)	76	193

The respiration was low in the absence of either adeninenucleotide or magnesium. The effect of varying the concentration of magnesium when the cozymase concentration was constant is seen from Fig. 3. The respiration reached a maximum when the magnesium concentration lay between 0·125 and 0·19 mg. per ml., i.e. between 0·005 and 0·0075 *M*.

The effect of adeninenucleotide concentration may be seen from Figs. 4 and 5. In the experiment of Fig. 4 an almost pure cozymase preparation (ACo > 600,000) was used, whereas in that of Fig. 5 a 50% preparation (ACo 350,000) was used. With the latter, which must contain considerable amounts of adenylic acid, there is an optimum concentration of the order of 200 Co units. A muscle extract prepared as above was found to contain 190 Co units per ml.

Enzyme stability of the dispersions. Increasing the concentrations of the coenzymes towards their optimum values increases not only the initial rate but also the total O_2 uptake. It must be assumed that the system is becoming inactivated during the experiment, so that the total O_2 uptake under these conditions is dependent on the rate of respiration rather than the amount of available substrate. Experiments in which more cozymase was added after the respiration began to decrease did not support the idea that the coenzyme was being inactivated. An inactivation of the enzymes must be assumed.

The dispersions retain their activity well when kept in ice. In one experiment in the presence of muscle extract, the dispersion showed no loss of activity after storing 7 hr. in ice: other dispersions however have proved less stable.

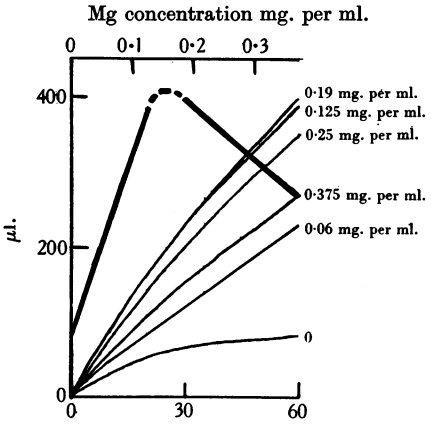

Fig. 3.

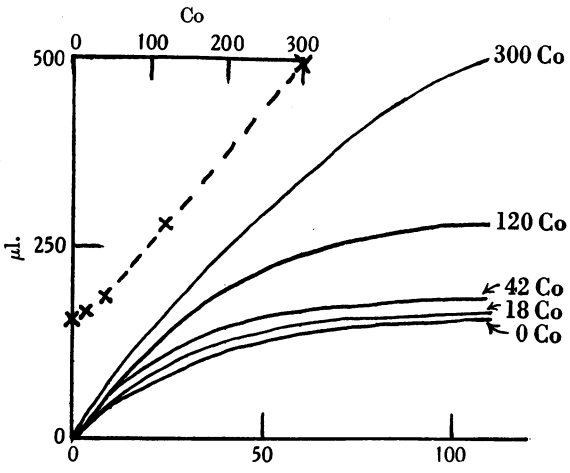

Fig. 4.

Fig. 3. O_2 uptake of dispersion with varying magnesium concentrations. The thick curve shows respiration (60 min.) plotted against magnesium concentration.

Fig. 4. Oxygen consumption of dispersion with varying amounts of cozymase per 2 ml. (1 mg. = 600 Co). 0·125 mg. Mg per ml. present. Broken curve gives oxygen uptake in 105 min. plotted against Co units.

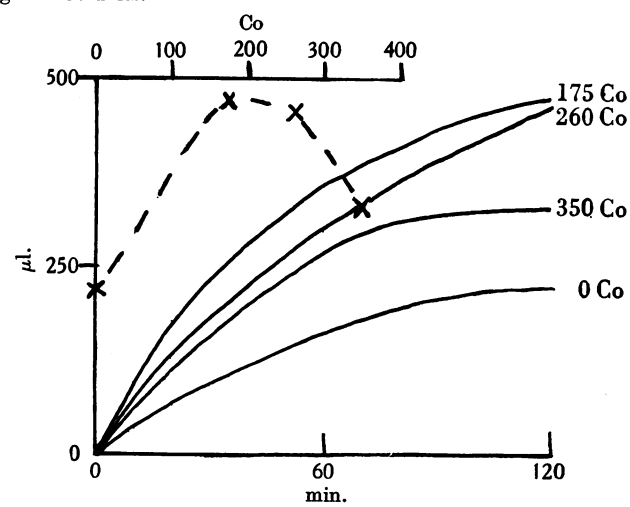

Fig. 5. Oxygen consumption of dispersion with varying amounts of cozymase per 2 ml. (1 mg. = 350 Co). 0·125 mg. Mg per ml. present. Broken curve gives oxygen uptake in 120 min. plotted against Co units.

Effect of fumarate. When cozymase and magnesium are used instead of muscle or yeast extract, the dispersions become more sensitive to the accelerating

action of low concentrations of fumarate. In the experiment of Fig. 6 10^{-3} *M* fumarate has caused an increased O_2 consumption of 294 μl., whereas complete combustion of the added fumarate would need only 134 μl. The corresponding values for the 3×10^{-4} *M* fumarate are 136 and 40 μl. respectively. This shows that the action of fumarate is a catalytic one.

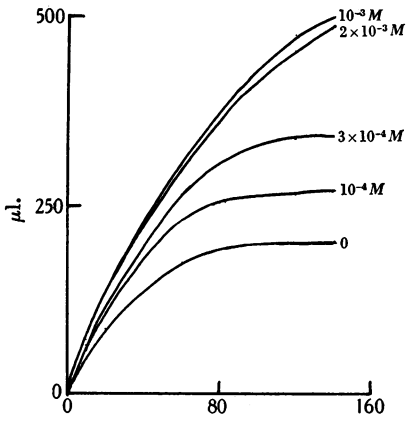

Fig. 6.

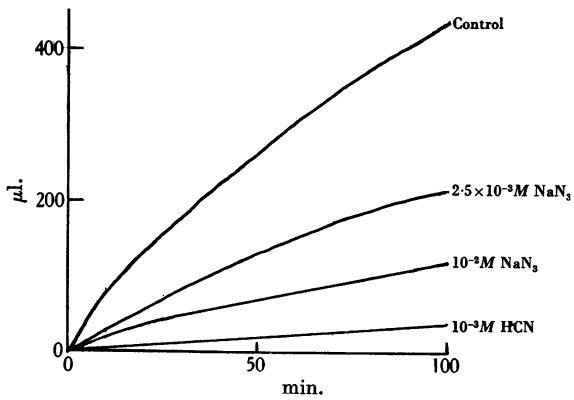

Fig. 7.

Fig. 6. O_2 consumption of dispersion with varying fumarate concentrations. 0·125 mg. Mg per ml., and 260 Co per 2 ml., present.

Fig. 7. Effect of cyanide and azide on respiration of dispersion.

Effect of inhibitors. That the respiration of the dispersions was strongly inhibited by sodium cyanide and by sodium azide [Keilin, 1936], as seen from Fig. 7, is consistent with the idea that it is catalysed by Warburg's *Atmungsferment* or some other heavy metal catalyst. The CO_2 absorbent in the vessels containing cyanide was the KOH–KCN mixture suggested by Krebs [1935].

Inhibition by salts. The respiration of minced pigeon breast muscle is strongly inhibited by Ca ions in concentrations of the order in which they are put in Ringer's solution (for references see Greville [1936]). They have an equally strong action on the respiration of the dispersions. Thus the respiration was reduced to less than a quarter by the addition of 0·00275 *M* Ca. The inhibiting action of NaCl is shown in Table II.

Respiration. The Q_{O_2} (μl. per hr. per mg. dry weight) of the most active dispersions, in presence of magnesium, cozymase and fumarate, is about 50 for the first 60 min. The initial rate is somewhat higher.

Respiratory quotient. The R.Q. of the dispersions in presence and absence of fumarate was measured by the method of Dickens & Šimer [1930; 1933] for phosphate media. It was found that the respiration was reduced if the dispersions stood in the vessels for the time necessary to fill the latter with O_2; so the measurements were made with air in the gas space. This could be done without unduly increasing the preformed CO_2, which in any case is relatively very high, since the activity of the dispersions decreases with time. The vessels were put in the bath in pairs. Results are given in Table IV.

It will be seen that the increased O_2 uptake due to fumarate is accompanied by increased CO_2 production. The R.Q. is in the neighbourhood of unity. Banga found [Annau *et al.* 1936] that fumarate raised the R.Q. of minced muscle from

Table IV

Exp.	Time min.	Fumarate *M*	Preformed CO_2 (μl.) Solutions	Preformed CO_2 (μl.) Total	O_2 μl.	CO_2 μl.	R.Q.
1	85	—	—	76	−226	240	**1·06**
		10^{-3}	18	105	−425	419	**0·98**
		10^{-3}	18	99	−432	430	**0·99**
2	60	—	—	87	−299	283	**0·95**
		10^{-3}	25	103	−378	359	**0·95**
		10^{-3}	25	86	−384	377	**0·98**
3	90	—	—	88	−271	260	**0·96**
		10^{-3}	23	115	−566	516	**0·91**
		10^{-3}	23	112	−575	532	**0·92**

about 0·86 to about 1·05; whilst Stare & Baumann [1936] found that the addition of fumarate increased the R.Q. from 0·90 to from 1·00 to 1·20, depending on the amount of fumarate added. The smaller amounts of fumarate had comparatively little effect on the R.Q., even though the O_2 uptake had been markedly increased.

Centrifuging of dispersions. In the experiment of Table V and Fig. 8, 1 vol. of dispersion was diluted with 3 vol. water, brought to *p*H 5–6 with HCl and

Table V

	1	2	3
Deposit suspension (ml.)	0·16	—	0·16
Supernatant fluid (ml.)	—	0·6	0·6
Phosphate (ml.)	0·5	0·5	0·5
Cozymase (1 mg. per ml.) (ml.)	0·3	0·3	0·3
Magnesium chloride (0·1 *M*) (ml.)	0·1	0·1	0·1
Water (ml.)	0·9	0·5	0·3
O_2 consumption (85 min.) (μl.)	**19**	**5·5**	**495**

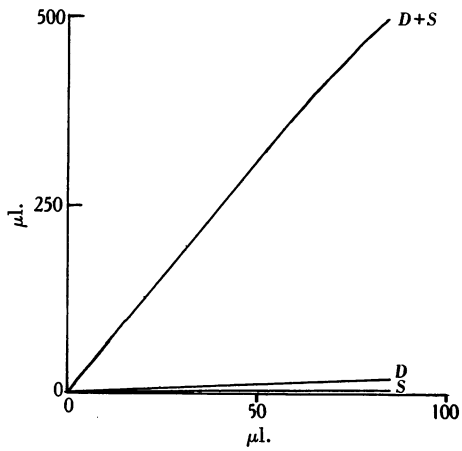

Fig. 8. Respiration of dispersion after centrifuging. *D*, deposit suspension; *S*, supernatant fluid.

centrifuged. The deposit was resuspended in 1 vol. water, and both it and the clear supernatant fluid were neutralized. 4 vol. of the latter were, of course, equivalent to 1 vol. of the former.

It is clear from this experiment that both deposit and supernatant fluid contain different factors essential to the respiration. It was found that the respiration when deposit and supernatant fluid were both present was still strongly inhibited by malonate, and that the activity of the supernatant fluid was reduced by about 50% when it was heated to 85° for 5 min.

In a further analysis of the system, one portion of the supernatant fluid was heated to 100° and another portion was dialysed in a collodion bag for 90 min. against 0·5% NaCl at room temperature (Table VI). Since the respiration in

Table VI

Phosphate, cozymase and magnesium present in usual concentrations

Vessel no. ...	1	2	3	4
Deposit suspension (ml.)	0·125	0·125	0·125	0·125
Supernatant fluid (S.F.) (ml.)	0·3	—	—	—
S.F. heated (ml.)	—	0·15	—	0·15
S.F. dialysed (ml.)	—	—	0·35	0·35
O_2 uptake 0–128 min. (μl.)	**293**	**89**	**84**	**300**
O_2 uptake 61–128 min. (μl.)	**117**	**27**	**21**	**120**

vessel 4 greatly exceeds the sum of those in vessels 2 and 3, it is clear that both the dialysed and the heated supernatant fluids contain different factors essential to the respiration. It is highly probable that the former contains Banga's "activator" or "Zwischensubstanz", whilst the latter contains substrate.

Carbohydrate consumption

When a dispersion respired in the presence of fumarate, lactic acid was rapidly formed at first and then partially or wholly disappeared again (Fig. 9);

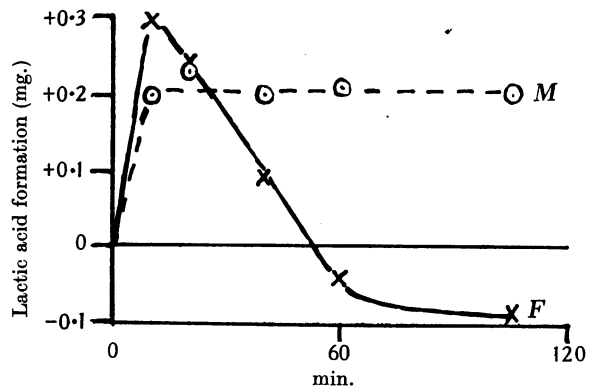

Fig. 9. Lactic acid formation of dispersion. *M*, in the presence of 0·01 *M* malonate. *F*, in presence of 0·001 *M* fumarate.

hence lactate was oxidized by the dispersions. The question arose as to whether the disappearance of carbohydrate or carbohydrate breakdown products is catalysed by fumarate; and one of a number of experiments on the action of added fumarate is given in Table VII. The O_2 uptakes were larger than the figures given because the O_2 taken up during the 10 min. temperature equilibration must be added. The figures giving carbohydrate disappearance in μl. assume complete combustion to CO_2 and H_2O. The R.Q. values given above do

Table VII

In each vessel an amount of dispersion corresponding to 0·1 ml. original dispersion: 8·7 mg. dry weight: 0·21 mg. preformed lactic acid. Time of exp. 133 min.

Vessels	Fumarate	*Glycogen initially present mg.	Lactic acid formed mg.	Disappearance mg.	Disappearance µl.	Oxygen uptake µl.
1+2	—	2·41	1·90	0·51	380	>446
3+4	—	2·41	1·90	0·51	380	>452
5+6	10^{-3} *M*	2·41	1·52	0·89	665	>845
7+8	10^{-3} *M*	2·41	1·51	0·90	672	>866

* Expressed as glucose.

not contradict this assumption. There is, of course, no justification for assuming that the difference between the original amount of glycogen and the lactic acid formed represented carbohydrate consumed; but the experiment does show that not more than 70 % of the respiration in the presence of fumarate could be due to the ultimate oxidation of the glycogen initially present. This maximum proportion was much lower in some other experiments. In the vessels containing fumarate (Table VII), taking means, 0·385 mg. less lactic acid appeared than in those without fumarate. This would require 288 µl. O_2 for complete combustion. The difference actually observed in the O_2 uptakes was 406 µl. Hence not all the extra O_2 uptake caused by fumarate could be due to the oxidation of glycogen.

Oxidation of glycogen: "autolysed dispersions"

The question as to whether the dispersions could oxidize glycogen by a fumarate catalysis was attacked in another way. The neutralized but not diluted dispersion was put in a flat-bottomed vessel and shaken gently at room temperature. At 1 min. intervals 0·2 *N* NaOH was carefully added to keep the dispersion at *p*H *ca.* 7·3. When no more NaOH was needed (10–40 min.) all the glycogen had been converted into lactic acid, as confirmed by glycogen analyses. The "autolysed dispersion" was then brought to 2 vol. with H_2O and its respiration measured in the presence and absence of added glycogen or lactate (Figs. 10 and 11). It was found that autolysis had greatly reduced the respiration of the dispersion, but that this could be almost restored by the addition of glycogen in the amount originally present together with fumarate, but not by the addition of either alone. That the action of these substances is not merely additive is seen from the following calculation for the experiment of Fig. 10:

For autolysed dispersion, in 110 min.:

$G = O_2$ uptake in presence of glycogen.

$F = O_2$ uptake in presence of fumarate.

$GF = O_2$ uptake in presence of fumarate and glycogen.

$C = O_2$ uptake with no addition, then $(G-C)+(F-C) = 146$ µl., $GF-C = 335$ µl.

O_2 for complete combustion of 0·5 mg. added glycogen = 415 µl.

Extra O_2 uptake observed with autolysed dispersion due to 0·5 mg. added glycogen $(GF-F) = 268$ µl.

O_2 consumption of non-autolysed dispersion in same time = 645 µl.

It will be seen also that the extra O_2 uptake of the autolysed dispersion due to glycogen is equal to slightly less than half the total O_2 uptake of the non-autolysed suspension in the same time, whilst that which can be proved to be due to fumarate-catalysed glycogen oxidation is equal to about a third. Similar results were obtained in several other experiments.

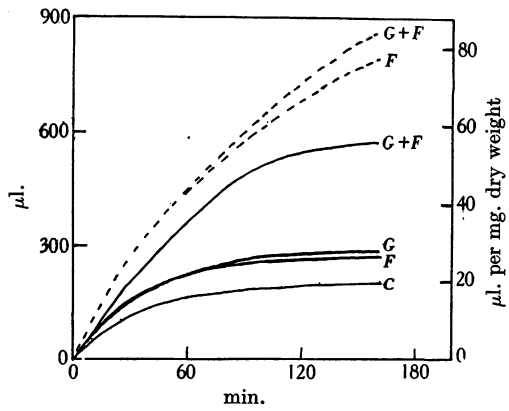

Fig. 10.

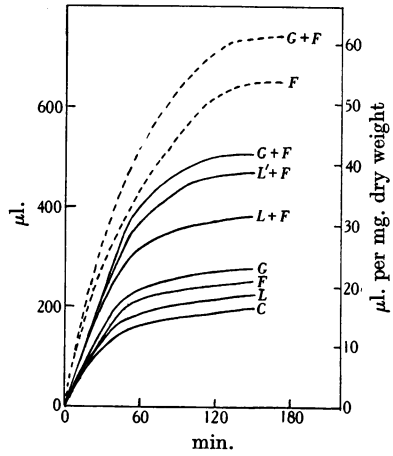

Fig. 11.

Fig. 10. Solid curves, "autolysed dispersion". Broken curves, untreated dispersion. *C*, no addition; *F*, 10^{-3} *M* fumarate; *G*, 0·5 mg. glycogen added to the 2 ml. in vessel. Initial glycogen in untreated dispersion, 0·41 mg. per vessel. Cozymase and magnesium added.

Fig. 11. Symbols as Fig. 10. In addition: $L = 0{\cdot}5$ mg. *d*-lactate, $L' = 0{\cdot}5$ mg. *d*-lactate (half-neutralized).

It might be possible that glycogen increased the respiration merely by making the dispersion more acid. This possibility was eliminated by experiment:

Table VIII

*p*H of buffer	6·61	6·81	6·98	7·20	7·43	7·43
Added glycogen (mg.)	—	—	—	—	—	1·0
O_2 uptake in 100 min. (μl.)	102	113	139	135	131	239

Lactic acid also increases the respiration of autolysed dispersion though to a less extent than the equivalent amount of glycogen (Fig. 11). Since half-neutralized lactic acid is more efficient than sodium lactate, it is presumed that the action of the lactate is reduced by the solution becoming alkaline.

The R.Q. *of "autolysed dispersions"* has been measured in presence and absence of added glycogen (Table IX). It appears that the R.Q. is about unity, but probably somewhat lower in the absence of added glycogen. The added glycogen is burnt at a R.Q. of unity, although in Exp. 1 some substrate of lower R.Q. obviously present in abundance has apparently been "spared" by the added glycogen.

Magnesium and "autolysed dispersions". The residual respiration, i.e. in the absence of glycogen, is still dependent on magnesium. This cannot be due to

Table IX

$F = 10^{-3}$ *M* fumarate. $G = 1$ mg. glycogen per vessel

Exp.	Time min.	Addition	Preformed CO_2	O_2	CO_2	R.Q.
1	108	*F*	96	−411	352	0·86
		G	76	−280	282	1·01
		F + *G*	96	−476	467	0·98
2	110	*F*	100	−195	162	0·83
		G	80	−339	320	0·94
		F + *G*	90	−532	500	0·94
3	110	*F*	89	−137	120	0·87
		G	70	−222	222	1·00
		F + *G*	79	−453	443	0·98
4	90	*F*	166	−240	218	0·91
		G	140	−382	359	0·94
		F + *G*	163	−496	467	0·94

Respiration due to added glycogen $(F + G) - F$:

Exp.	Extra O_2	Extra CO_2	R.Q. of extra respiration
1	− 65	115	1·77
2	−337	338	1·00
3	−316	323	1·02
4	−256	249	0·97

traces of glycogen in the autolysed dispersion, as shown by the following typical experiment:

Time 100 min.	Mg 0·005 *M*
O_2 uptake in presence of magnesium	153 μl.
O_2 uptake in absence of magnesium	38 μl.
Difference	115 μl.
Glycogen in autolysed dispersion	22γ
O_2 needed for combustion of glycogen	18 μl.

If the residual O_2 uptake be due to the oxidation of phosphorylated intermediary products of glycolysis, this observation is at once explained.

Centrifuging of "autolysed dispersions". An experiment in which the autolysed dispersion was centrifuged, and the deposit (P.) and supernatant fluid (S.F.) tested separately and together (Table X) shows that both precipitate and supernatant fluid are essential for the oxidation of glycogen and lactate.

Table X

Fumarate 10^{-3} *M*. Glycogen 0·75 mg. *d*-Lactate (half-neutralized) 0·75 mg. Cozymase. Mg

O_2 uptakes in 163 min. (μl.):

Original extract	810
Original extract + glycogen	785

P.	35	S.F.	85	P. + S.F.	430
P. + glycogen	36	S.F. + glycogen	79	P. + S.F. + glycogen	633
P. + lactate	56	S.F. + lactate	91	P. + S.F. + lactate	523

Discussion

Since the completion of these experiments (end of October), a paper has appeared by Banga [1937] which shows that hexosephosphates are oxidized by the dispersion by means of the C_4 dicarboxylic acid catalysis, and that "Glykogen konnte die Substanzen einigermassen (40 %) ersetzen". The dispersions will reduce oxaloacetate to malate in the presence of hexosediphosphate. She

describes an experiment similar to that given in Table VI of the present paper, in which instead of dialysed supernatant fluid she uses "activator" prepared by precipitating the supernatant fluid with acetone and drying. With deposit and heated supernatant fluid the O_2 uptake is 40 μl., whilst with deposit, heated supernatant fluid and activator it is 420 μl. However, the uptake with deposit and activator is not given.

Substances which behave in the same way as the heated supernatant fluid were obtained from it by barium precipitation. Also hexosediphosphate (10 mg.) and Robison ester (10 mg.) behaved in the same way. It does not, however, seem possible to judge from these experiments what proportion of the fumarate-catalysed respiration is due to the oxidation of hexosephosphates.

As regards the mechanism of the oxygen activation, it was first thought by Gözsy & Szent-Györgyi [1934] that the substrates were oxidized by the succin-oxidase system involving the Atmungsferment. On this view the relief of the malonate inhibition by added fumarate could not be explained, but the later fumarate-oxaloacetate theory [Annau *et al.* 1935; 1936] offered an explanation. The latest theory [Straub, 1937; Szent-Györgyi, 1937], however, is that the activated hydrogen of the substrate reduces oxaloacetate to malate. The malate reduces yellow enzyme regenerating oxaloacetate, and the reduced yellow enzyme reduces fumarate to succinate. The succinate is finally oxidized by succinic dehydrogenase and cytochrome. Since malonate must inhibit the last-named stage, it is once again difficult to understand the relief of malonate inhibition by added fumarate, unless the latter is being irreversibly oxidized [cf. Innes, 1936; Stare & Baumann, 1936; Annau & Straub, 1937], and it would seem that an explanation may still be needed.

Krebs [1937] has very recently shown that citrate, like fumarate, is a catalyst of pigeon breast muscle respiration. The effect of citrate on the oxidation by the dispersions has not yet been tested. Krebs's "citric acid cycle" explains the catalytic action of the C_4 dicarboxylic acids and provides a new explanation, different from Szent-Györgyi's "over-reduction" hypothesis, for the production of succinate from fumarate aerobically in the presence of malonate.

Szent-Györgyi's [1937] theory apparently demands that fumarate should give rise to succinate anaerobically, and that this reaction should be inhibited by malonate. This reaction was not observed to occur at any considerable rate by Annau *et al.* [1935]. Some observations by the writer on the formation of succinate in the presence of fumarate are given in the Appendix. They confirm that the aerobic reaction occurs most rapidly in the presence of malonate and show that there is an equally rapid anaerobic reaction which is inhibited by malonate.

Summary

1. The respiration of dispersions made by grinding pigeon breast muscle with water at the freezing-point and squeezing through muslin has been studied.

2. When 0·1 or 0·2 ml. of the dispersion is diluted to 2 ml. in the manometer vessel with suitable buffering, the respiration is small, but becomes large on the addition of extracts made with hot water from muscle or yeast.

3. The chief function of these muscle or yeast extracts is to supply coenzymes, the dispersion usually containing enough substrate.

4. The necessary coenzymes are magnesium and adeninenucleotide.

5. In the presence of these coenzymes the respiration is accelerated by low concentrations of fumarate, which acts catalytically. The Q_{O_2} may then reach 50. The R.Q. is about unity.

6. The respiration is inhibited strongly by cyanide in low concentrations, by azide, by malonate and by calcium.

7. If the dispersion be centrifuged, both deposit and supernatant fluid are essential for the respiration, as previously shown by Banga. If the supernatant fluid be dialysed, an essential factor, probably substrate, is lost, which can be replaced by the addition of heated supernatant fluid.

8. When the dispersions are respiring, lactic acid is first formed from glycogen, and then partially or wholly disappears. The lactate disappearance is usually greater in the presence of fumarate. However, oxidation of products derived from stored carbohydrate during the experiment cannot account for more than 70% of the respiration, and often accounts for considerably less.

9. "Autolysed dispersions" obtained by allowing the dispersions to cause all their glycogen to break down at room temperature, show a low respiration which can be restored almost to the original value by addition of glycogen and fumarate. It can be proved that a large part of the extra respiration due to glycogen is catalysed by fumarate; this part corresponds to about a third of the original respiration. The total extra respiration due to glycogen has R.Q. unity.

10. If the "autolysed dispersions" be centrifuged, both deposit and supernatant fluid are essential for the respiration with added glycogen.

11. The residual respiration of the "autolysed dispersions", in absence of glycogen, still needs magnesium as coenzyme.

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APPENDIX

Succinic acid formation with minced pigeon breast muscle

Estimation. Extraction with ether, autoclaving dry to remove malonate, determination with a succinic oxidase preparation from pigeon breast muscle which did not oxidize α-ketoglutaric acid.

In the anaerobic experiments the vessels were washed out with nitrogen and evacuated. They were shaken continuously during the experiment.

0·75 g. muscle in 8 ml. solution.

M = 0·01 M malonate; F = fumarate (7 mg.).

Preformed succinic acid between 0·04 and 0·17 mg. per g. tissue.

Exp.	Time min.	Gas space	Addition	mg. succinic acid per g. muscle
1	30	Air	—	0·07
			F	0·57
			$M+F$	1·94
2	30	Oxygen	F	0·21
			M	0·24
			$M+F$	3·26
3	30	Anaerobic	—	0·37
			F	1·87
			$M+F$	0·76
4	30	Anaerobic	F	1·56
			M	0·28
			$M+F$	0·52

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