The Meyerhof Quotient and the Synthesis of Glycogen from Lactate in Frog and Rabbit Muscle

A REINVESTIGATION

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1. The conversion of lactate into glycogen was demonstrated in frog sartorius muscle in oxygen. The rates and amounts are highest when lactate is added to the bathing medium and are dependent on lactate and CO_2 concentration, as well as pH. The glycogen content of a resting muscle can be doubled in 4 h at 24°C . 2. Sartorius muscle, recovering aerobically in liquid paraffin from a period of anoxia, converts preformed lactate into glycogen at a lower rate and in smaller amounts than when lactate is added in an aqueous medium. The lower rates are similar to those Meyerhof found under the same conditions, after correction for temperature; they can be attributed partly to low muscle pH and partly to the limited amounts of lactate present. 3. Rabbit psoas muscle also shows the ability to convert added lactate into glycogen under aerobic conditions. The rates are low and similar to those in frog sartorius muscle recovering from anoxia. 4. The present experiments yield a Meyerhof quotient of 6.2, compared with Meyerhof's value of 4–5. However, these values are not significantly different from one another. 5. It is suggested that the glycogen coefficient, i.e. mol of glycogen formed/mol of lactate disappearing, is a more reliable way of assessing the resynthetic mechanism than the original quotient, i.e. mol of lactate disappearing/mol of lactate oxidized. The found coefficient is 0.419 ± 0.024 .

Meyerhof (1920a, 1922) was the first to discover a feature of oxidative recovery in frog muscle in which some of the lactate formed during fatigue disappears when the muscle is allowed to recover in oxygen, but only about 20–25% of this lactate can be accounted for by oxidation to CO_2 and water. Meyerhof (1920a, 1922) expressed his results in the form of the quotient (total lactate disappearing)/(lactate oxidized), the numerator of which was based on direct measurement of lactate and the denominator on the O_2 uptake divided by 3. Altogether Meyerhof (1920a, 1922) recorded 21 separate experiments on muscles fatigued in various ways; a re-plot of these data in their raw form yields a quotient of 4.1 ± 0.383 (S.E.M. for 19 degrees of freedom), i.e. only about one-quarter of the missing lactate could have been oxidized. Two later experiments of Meyerhof & Lohmann (1926) yielded quotients of 4 and 6. Thus there can be no doubt about the general validity of the quotient.

Meyerhof (1920a, 1922) concluded from his earlier experiments that the lactate unaccounted for by oxidation had been converted into carbohydrate, mostly in the form of glycogen. In that case the mean value of the quotient given above shows that 0.38 ± 0.13 mol of glucose units of glycogen or of

other carbohydrate should have been resynthesized/mol of lactate disappearing. To confirm this hypothetical formation of carbohydrate, Meyerhof (1920a, 1922) seems to have carried out only two direct experiments, in both of which the gains in glycogen did, in fact, account for most of the missing lactate. However, the experiments were of very long duration, and it can be questioned whether this was a sufficient basis on which to build the far-reaching and oft-quoted hypothesis that finally emerged. The only other confirmatory evidence seems to be that of Foster & Moyle (1921) and Meyerhof, Lohmann & Meier (1925). The latter authors perfused the hind limbs of frogs with 0.12% lactate and reported significant gains in the glycogen content of the muscles. Eggleton & Evans (1930) repeated these latter experiments but could find only small and erratic gains in glycogen which were not statistically significantly different from zero.

There seem to have been very few further attempts to repeat Meyerhof's experiments until quite recently, e.g. Bar & Blanchaer (1965) and Gourley & Suh (1969). The latter authors showed some glycogen formation from added lactate in isolated sartorius muscles, but the lactate concentrations they employed (3 mM) were far below that to be expected

in a fatigued muscle. There is thus considerable justification *prima facie* for the severe criticism of the entire hypothesis by Krebs & Woodford (1965), who objected on the grounds of the thermodynamic 'barrier' to glycogen formation which would exist unless the following enzymes were present: L-malate-NADP oxidoreductase (decarboxylating) (EC 1.1.1.40), or alternatively pyruvate carboxylase (EC 6.4.1.1), phosphoenolpyruvate carboxykinase (EC 4.1.1.32) and fructose diphosphatase (EC 3.1.3.11). They were able to demonstrate only the presence of the last of these in 'white' muscle. However, Opie & Newsholme (1967) later discovered significant quantities of the first and third of these enzymes, so the distinct possibility now exists that they are involved in the first steps of the conversion of lactate into glycogen. In view of this it seemed timely to repeat Meyerhof's experiments, with the more refined techniques of assay now available. The results demonstrate that glycogen can be formed in large amounts from lactate, added to isolated frog sartorius muscles.

MATERIALS AND METHODS

Preparation of muscle samples. The sartorius muscles of English frogs (*Rana temporaria*) were carefully dissected out, cut free from their origin and tendon of insertion and placed in Warburg vessels with 1 ml of medium (see below) until required.

In some experiments the muscles were dissected out immediately after the death of the animals by decapitation and pithing; in others the animals were pinned out after death on boards, placed in large bottles with moist N_2 , sealed and stored in a room at 10°C for 16–20 h to allow anaerobic accumulation of lactate. The muscles were then dissected out as above.

Ringer solution and other bathing fluids. When Ringer solution was used, its composition was as follows (Hill, 1965, p. 244): NaCl, 115.5 mm; KCl, 2.5 mm; $CaCl_2$, 1.8 mm; phosphate buffer, 3 mm, at pH 7.0 (sodium salt). In several experiments lactate was added to it in various concentrations as noted in the text. It was prepared from pure lactic acid (Sigma Chemical Co., St Louis, Mo., U.S.A.), neutralized with NaOH, and the lactate content was assayed enzymically (see below).

In some experiments, designed to reproduce the conditions of Meyerhof's (1920b) experiments, the muscles were suspended in neutral paraffin, density 0.83–0.87 (BDH Chemicals Ltd., Poole, Dorset, U.K.), previously saturated with water (Meyerhof, 1920b).

Measurement of oxygen uptake. The O_2 uptake was measured in a standard Warburg apparatus at 24°C, usually with 25% KOH in the central well of the vessels to absorb the CO_2 formed.

The sartorius muscles of ten frogs were used in each experiment and after being weighed were placed in pairs in Warburg vessels containing 1 ml of medium. They were then equilibrated with pure O_2 for 15 min at the bath temperature of 24°C. The arrangement of the muscle pairs, in order of killing the frogs, was as follows (R, right

sartorius; L, left sartorius): vessel no. 1, muscle no. 1 L, 6R; vessel no. 2, muscle no. 2R, 7L; vessel no. 3, muscle no. 3L, 8R; vessel no. 4, muscle no. 4R, 9L; vessel no. 5, muscle no. 5L, 10R; vessel no. 6, muscle no. 1R, 6L; vessel no. 7, muscle no. 2L, 7R; vessel no. 8, muscle no. 3R, 8L; vessel no. 9, muscle no. 4L, 9R; vessel no. 10, muscle no. 5R, 10L. Immediately after equilibration with O_2 , the contents of vessels 1–5 were combined and added to 6 ml of 50% KOH solution for assay of initial glycogen and lactate content. Vessels 6–10 were allowed to take up O_2 for various times and then their contents combined and placed in KOH as above.

In some experiments, where it was desired to allow CO_2 to accumulate during the experiment, KOH solution was omitted from the central wells of the vessels. The approximate O_2 uptake was then measured from the observed pressure changes by Warburg's direct method, assuming a respiratory quotient of 1.0 (Umbreit, Burris & Stauffer, 1947) and correcting for the pH of the bathing solutions. The initial pH varied between 6.9 and 7, and the final pH between 7.3 and 7.54. The latter values are probably too high because of loss of CO_2 during measurement.

Assay of glycogen. The muscle samples, after digestion in hot 25% KOH, were prepared for determination of glycogen by the method of Good, Kramer & Somogyi (1933). Glycogen was hydrolysed to glucose in 1.2 M-HCl for 1 h at 100°C. The glucose was measured, after oxidation by alkaline ferricyanide, by back-titration with ceric sulphate; Xylene Cyanol FF (BDH Chemicals Ltd.) was used as an internal redox indicator (modified method of Cole, 1944). The method is capable of measuring as little as 0.01 μ mol of glucose. For this reason it was preferred to the more usual anthrone method. Experiments in which glycogen was added to the samples showed that the recovery was 99–101% over the range of glycogen concentrations actually encountered in practice (15–17 μ mol/g of muscle).

Assay of lactate. Lactate was assayed enzymically by using lactate dehydrogenase and NAD^+ at alkaline pH and measuring the NADH formed spectrophotometrically at 340 nm (Hohorst, 1963). By this method lactate can be accurately assayed in the KOH-ethanol supernatants left over from glycogen assays, after neutralization with $HClO_4$, decantation from the $KClO_4$ precipitate and appropriate dilution (R. K. Scopes, personal communication). The recovery of lactate from test samples was 100 $\pm$ 1%. This simple procedure obviates the need to double the number of muscle samples for the two separate assays, and thereby decreases very considerably the variability in the results.

Assay of hexose 6-phosphate. This was assayed enzymically by the method of Hohorst (1963). Because the assay had to be made on neutralized $HClO_4$ extracts of sartorius muscles the preparations used for the O_2 uptake and glycogen and lactate assays were not suitable. Separate groups of frogs were therefore used for the assay of hexose 6-phosphate.

RESULTS

Experiments with sartorius muscles from freshly killed frogs. The experiments of Gourley & Suh (1969) showed that some glycogen formation from

Table 1. *Experiments with sartorius muscles of freshly killed frogs at 24°C, with and without addition of lactate to the Ringer solution*

The parameters are given in terms of $\mu\text{mol/g}$ of muscle, except where noted. The initial hexose 6-phosphate content was approx. $3.5 \mu\text{mol/g}$. The mean weight of a muscle pair was 0.15 g (in 1 ml of Ringer solution). Five pairs were combined for initial assays and five for final assays. The initial pH varied between 6.95 and 7.05.

Expt. no.	1	2	3	4	5	6	7
Duration (h)	3.50	24.0	4.72	4.00	4.25	4.25	4.25
[Lactate] added in Ringer (mm)	0	0	5.0	38.0	18.0	38.0	41.5
Initial [lactate] in Ringer + muscle (mm)	3.0	3.2	7.4	39.0	18.2	39.0	42.5
Lactate disappearing (A) ($\mu\text{mol/g}$)	7.0	17.8	23.2	26.0	42.5	60.0	50.0
Initial glycogen (glucose units)	16.8	26.0	24.8	29.8	24.6	30.2	31.1
Glycogen synthesized (B)	-2.9	-23.7	+1.3	+8.1	+13.6	+19.6	+15.0
O ₂ uptake (C)	33.7	—	43.1	56.2	49.6	—	—
Calc. lactate disappearing (D = C/3 + 2B)	5.5	—	17.0	34.9	43.7	—	—
CO ₂ absorbed into KOH or not	Yes	Yes	Yes	Yes	No	No	No

lactate occurred in frog muscle, even at the very low concentration of lactate they employed (3mm). Our own preliminary experiments confirmed this and suggested that much larger amounts of glycogen would be formed at higher lactate concentrations. In the following experiments therefore concentrations of lactate from 3 to 50mm were added to the Ringer solution bathing the muscles, respiratory CO₂ being allowed to accumulate in some cases, whereas in others it was absorbed into potassium hydroxide.

The marked effect of lactate concentration on the formation of glycogen and on lactate utilization is seen clearly from the results in Table 1 for sartorius muscles removed immediately after death. When no lactate was added to the Ringer solution, as in Expts. 1 and 2, and the initial lactate concentration was about 3mm, glycogen was actually lost from the muscle despite oxidation of some of the preformed lactate. In such a case the loss of glycogen and disappearance of lactate in relation to the O₂ taken up indicated that both these sources of C₃ compounds were being oxidized via the tricarboxylic acid cycle. However, when the lactate concentration was increased above 7mm, larger amounts of glycogen were synthesized; at 39mm-lactate $8.1 \mu\text{mol}$ (1.45mg) of glycogen (glucose units) was synthesized/g of fresh muscle in 4h in the absence of respiratory CO₂, whereas in its presence the amount increased to 15–19.6 μmol (2.7–3.5mg)/g. The latter value represents a doubling of the initial glycogen content at the expense of added lactate.

CO₂ had a marked effect on the rate of glycogen resynthesis. A possible explanation is that it is needed for the operation of the 'malic' enzyme [malate-NADP oxidoreductase (decarboxylating)], which might be the first step in the resynthetic chain from pyruvate to glycogen. The amount of

CO₂ actually available will depend on the pH of the solution bathing the muscle. In Expt. 6 in Table 1 the initial pH was 6.97 and the final pH 7.54. Assuming both the rise in pH and the liberation of CO₂ were linear with time, the total CO₂ liberated half-way through the experiment was about $35 \mu\text{mol/g}$ of muscle and the pH was 7.25. Under the actual conditions of 0.17g of muscle in 1ml of Ringer solution with a gas head-space of 19ml and a temperature of 24°C, the calculated maximal concentration of CO₂ + HCO₃⁻ in the Ringer solution was 0.2mm. It would in fact be lower than this, because the measured final pH is probably too high (see the Materials and Methods section).

To decide to what degree the spontaneous accumulation of CO₂ was optimum, two identical experiments were carried out, in which sodium hydrogen carbonate was added to the Ringer solution to a final concentration of 11.9mm, and an atmosphere of O₂ + CO₂ (95:5) was used instead of pure O₂. Lactate was also added to a concentration of 42mm; the initial pH was 7.10 and the final pH about 7.5. The total concentration of CO₂ + HCO₃⁻ was initially 13.6mm (about 68 times that in Expt. 6 in Table 1). The amounts of glycogen formed/g in $4\frac{1}{2}$ h were 28.0 and 24.6 μmol of glucose units. When these values are compared with that of 19.6 $\mu\text{mol/g}$ in Expt. 6 in Table 1 it is clear that even the low concentration of CO₂ present during the latter experiment (0.2mm) was at least 75% optimum.

The combined results of these lactate addition experiments cover a wide range of glycogen synthesis, and therefore serve to establish the general relation between glycogen synthesized and total lactate disappearing. It was found that glycogen synthesis was linear with time, providing the lactate concentration in the bathing fluid did not fall

Fig. 1. Plot of the rate of lactate disappearance ($\Delta\text{Lactate}/\Delta t$) against the rate of glycogen formation ($\Delta\text{Glycogen}/\Delta t$) in freshly excised frog sartorius muscles exposed to O_2 . Bathing solution was Hill's frog Ringer to which lactate had been added in various amounts (see Table 1 and text). The temperature was 24°C . $\circ$, Direct measurement of glycogen formed and lactate disappearing. Δ , Lactate disappearing estimated from $2\Delta\text{Glycogen}/\Delta t - (\Delta\text{O}_2/\Delta t)/3$. $\bullet$, Rabbit experiments from Table 3.

appreciably during the experiment. For this reason the observed parameters are plotted in Fig. 1 as rates, together with the calculated rates of glycogen synthesis, based on the assumptions: (a) that the measured O_2 uptake was exclusively utilized to oxidize lactate; (b) that subtraction of this amount of lactate from the total disappearing gives a measure of the lactate converted into glycogen. It is seen that the observed and calculated values agree reasonably well. Values for the observed parameters in rabbit psoas muscle are shown in Fig. 1 and are seen to fall close to the regression line for frog sartorius muscle.

The regression line relating observed rates of glycogen synthesis to rates of lactate disappearance in freshly excised frog muscle is given by:

$$\Delta\text{Glycogen}/\Delta t = -0.419(\Delta\text{Lactate}_{\text{total}}/\Delta t) - 1.3 \quad (1)$$

$r = 0.99$ for $n = 9$; S.E.M. of regression coefficient = ± 0.024 .

The additive constant represents the resting metabolic rate, i.e. the rate of glycogen oxidation via pyruvate and the tricarboxylic acid cycle to supply the energy for resynthesis of the ATP broken down slowly in resting muscle (Bendall & Lawrie, 1962).

Experiments on sartorius muscles recovering in oxygen from 16–20 h anoxia. The experiments described above were carried out to discover whether glycogen synthesis from added lactate was possible in frog muscle under optimum conditions; as we have seen, the answer is clearly in the affirmative.

The actual conditions obtaining in Meyerhof's (1920a, 1922) original experiments were, however, very far removed from this optimum state, because the muscles had usually been tetanized for upwards of 15 min at 14°C so that they could accumulate as much lactate as possible. The result of this rather fierce treatment was to lower the pH to about 6.5 and to decrease the creatine phosphate and ATP reserves of the muscle almost to zero. By suspending the muscles in an atmosphere of moist O_2 Meyerhof (1920a) also limited the amount of lactate available to the intrinsic value of about $30 \mu\text{mol/g}$, and the possible resynthesis of glycogen to somewhat less than $12 \mu\text{mol}$ of glucose units/g. In practice, he never obtained a resynthesis of more than $8 \mu\text{mol}$ of glucose/g [see Meyerhof (1920a), p. 313, for a direct estimation, and Tabelle III, expt. 3, p. 294, for a similar value that can be derived indirectly].

We have preferred to Meyerhof's (1920a) prolonged and vigorous electrical stimulation the gentler procedure of leaving the muscles intact in the decapitated animals for 16–20 h under anaerobic conditions at 10°C ; by this means nearly as much lactate accumulates, due to the resting metabolism, as by Meyerhof's (1920a) method. We also used Meyerhof's (1920b) technique of placing the muscles in pairs in 1 ml of paraffin, instead of suspending them in moist O_2 , during the O_2 uptake experiments. This avoids drying out of the muscles and provides a medium with a very high O_2 solubility. In spite of the long holding time, the bacterial counts of muscles under these conditions are less than $10^6/\text{g}$ at the end of the experiment.

The results are summarized in Table 2, which shows that there is some glycogen resynthesis in every case, although the amounts are much smaller than might have been expected from Table 1. As we implied above, this is probably attributable partly to the limited amount of lactate available, but mainly to the low initial pH values (6.45–6.76, compared with about 7.0 in Table 1) and the lower creatine phosphate and ATP contents.

To confirm the effect of pH, we carried out two experiments of the same type as those in Table 1, but on muscles allowed to accumulate lactate anaerobically for 16 h or so. A 1 ml volume of Ringer solution was used as bathing medium and lactate was added in one case to a final concentration of 21 mM and in the other to 40 mM. CO_2 was allowed to accumulate in both cases. The rates of resynthesis of glycogen were respectively 2.55 and $2.84 \mu\text{mol/g}$ per h and the pH was initially about 6.5; these values compare with 3.2 and $4.6 \mu\text{mol/g}$ per h for the experiments in Table 1 (Expts. 5 and 6 respectively), which had an initial pH of about 7.0 but were otherwise under the same conditions.

In Table 2, it is noteworthy that Expts. 10 and 11 show a considerable discrepancy between the

Table 2. *Experiments with sartorius muscles recovering in oxygen from 16–20 h anoxia*

The temperature was 24°C. The suspension medium was 1 ml of paraffin. The mean weight of a muscle pair was 0.16 g. The initial hexose 6-phosphate content was 3–6 $\mu\text{mol/g}$. All parameters are given in terms of $\mu\text{mol/g}$ of muscle.

Expt. no.	8	9	10	11
Duration (h)	4.40	4.17	5.00	2.00
Initial pH	6.45	6.75	6.76	6.58
Initial lactate	26.8	20.0	19.3	23.5
Lactate disappearing (A)	6.2	9.7	14.3	5.7
Initial glycogen	15.2	20.8	28.9	26.7
Glycogen synthesized (B)	1.3	1.7	5.3	3.1
O ₂ uptake (C)	—	—	46.0	13.7
Calc. lactate disappearing (D = C/3 + 2B)	—	—	25.9	10.9
CO ₂ absorbed into KOH	No	No	Yes	Yes

Table 3. *Experiments with strips of psoas major muscle of freshly killed and immobilized rabbits (see the text)*

Lactate was added to Ringer solution, as shown. The mean weight of a strip was 0.2 g. Four strips were combined for initial assays and four for final assays. Parameters are in $\mu\text{mol/g}$, unless otherwise stated.

Expt. no.	12 (normal)	13	14
Duration (h)	4.17	6.00	5.00
Initial pH of Ringer plus muscle	7.02	7.10	7.09
[Lactate] added in Ringer (mm)	43.3	47.0	46.0
Lactate disappearing (A)	18.0	—	15.0
Initial glycogen (glucose units)	62.4	8.9	2.1
Glycogen synthesized (B)	3.0	5.7	4.3
	(= 0.72/h)	(= 0.95/h)	(= 0.86/h)
O ₂ uptake (C)	34.8	37.3	33.0
Calc. lactate disappearing (D = C/3 + 2B)	17.6	23.8	19.6
	(= 4.2/h)	(= 4.0/h)	(= 3.9/h)
CO ₂ absorbed into KOH	Yes	Yes	No

measured disappearance of lactate and that needed to account for the O₂ uptake plus the synthesis of glycogen. It is possible that this discrepancy was made up by utilization of hexose 6-phosphate as an extra source of lactate or pyruvate. Under the conditions of these experiments, the initial hexose 6-phosphate contents lie between 3 and 6 $\mu\text{mol/g}$, which would yield double these contents of lactate equivalents. This would satisfactorily explain the discrepancy, which only plays a major role when the amount of lactate available is limited in this way by the particular experimental design.

Glycogen synthesis from lactate in rabbit psoas muscle. For these experiments the rabbits were paralysed with curare for 10 min before death to ensure a high initial content of ATP and creatine phosphate. Two of them had also been injected with 2 ml of 0.1% adrenaline 1½ h before death to decrease the glycogen content (Bendall & Lawrie, 1962) whereas the third was a normal well-fed animal. Strips were prepared from the psoas major muscle (each about 5 cm long and weighing about 0.2 g) and placed in 1 ml of Krebs–Ringer phosphate solution, with the addition of lactate to a concentration of 43–47 mm. Four strips were combined for

determination of the initial parameters, and four were allowed to take up O₂ for various times and then combined and assayed in the same way (see the Materials and Methods section).

As seen from Table 3, all the muscles synthesized about the same rather low amount of glycogen from lactate, although in Expt. 12 the initial glycogen content was 7 times that in Expt. 13 and 30 times that in Expt. 14. All utilized lactate at a rate of about 4 $\mu\text{mol/g}$ per h (accepting the calculated values as a basis). The amounts of glycogen synthesized are about half those shown by frog sartorius muscles under identical conditions (cf. Expt. 4 in Table 1) and nearly the same as those of the sartorius muscles recovering from a period of anoxia (Expts. 10 and 11 in Table 2). When the results are plotted as in Fig. 1, as glycogen synthesized against total lactate disappearing, the points (as solid circles) fall quite close to the regression line for frog sartorius muscles.

DISCUSSION

The present results appear to vindicate Meyerhof's original hypothesis in its two most important

aspects: first, that the total amount of lactate disappearing from frog muscle during aerobic recovery from a tetanus or a period of anoxia is 5–6 times the amount that is oxidized; secondly, that the missing lactate reappears mostly in the form of glycogen. It is the latter point that has been most in dispute because of its great theoretical interest (Eggleton & Evans, 1930; Krebs & Woodford, 1965).

In discussing the synthesis of glycogen from lactate in muscle, we must distinguish clearly between what is possible under optimum conditions of high pH, lactate and CO_2 concentrations and what occurs during recovery from actual anoxia. Optimum or near-optimum conditions were clearly present at the higher lactate and CO_2 concentrations shown in Table 1 (Expts. 5, 6 and 7) and in two experiments mentioned in the text where an atmosphere of $\text{O}_2 + \text{CO}_2$ (95:5) was substituted for pure O_2 . These results, shown as the upper points in Fig. 1, demonstrate that between 13.6 and $28.0 \mu\text{mol}$ of glucose units of glycogen/g can be formed from lactate added to a respiring sartorius muscle during an experimental period of about $4\frac{1}{2}$ h giving in the best cases a final glycogen content of 1.1% of the muscle weight. The latter is certainly a high value for frog muscle (Meyerhof, 1920a, 1922; Eggleton & Evans, 1930) and is about the same as the initial glycogen content of many resting mammalian muscles, e.g. rabbit psoas muscle at 1.0–1.3% and ox longissimus dorsi muscle at 1.5–2.0% (J. R. Bendall, unpublished work). Thus there can be no doubt that glycogen can be synthesized from lactate by a respiring frog sartorius muscle in amounts that are much too large to be accounted for by the thermodynamically unfavourable route of mere reversal of the normal glycolytic pathway (Krebs & Woodford, 1965).

The question how important the aerobic resynthesis of glycogen may be in an isolated muscle, recovering from a tetanus or from anoxia, is not quite as simple to answer as the above remarks might indicate, although it is clear from Table 2 that such resynthesis does occur to a limited extent, but at a rather low rate. For example, the resynthesis observed in such isolated muscles was only $5.3 \mu\text{mol}$ in 5 h at 24°C , compared with $28 \mu\text{mol}$ in 4 h under the optimum conditions discussed above. Such amounts are of about the same magnitude as would be expected from Meyerhof's (1920a, 1922) results under similar conditions, after correcting for the temperature difference. As pointed out in the Results section, the lower rates are probably mainly due to the lower pH (approx. 6.5) obtaining in muscles recovering from a period of anoxia.

Even a white muscle of the rabbit, the psoas major, appears to be capable of resynthesizing glycogen from lactate to some degree, although the amounts are small even under optimum conditions

of high pH and high lactate and CO_2 concentrations (see Table 3).

Having demonstrated that glycogen can be resynthesized from lactate in respiring frog and rabbit white muscle, the next question that arises is the stoichiometric relation between the two parameters. As we have shown, 0.419 mol of glycogen (glucose units) is synthesized/mol of lactate disappearing in frog muscle (see Fig. 1 and eqn. 1); this we have termed the glycogen coefficient. It can be readily converted into the more conventional Meyerhof quotient (Q_M), since it follows from it that, per 1 mol of lactate disappearing, 0.838 mol is formed into glycogen and $1 - 0.838 = 0.162 \text{ mol}$ is oxidized to provide the necessary energy. Therefore $Q_M = 1/0.162 = 6.2$. From the standard error of the glycogen coefficient (± 0.024), however, the confidence limits for the quotient at the 5% level of probability are set at 3.6 and 20.0, and even at the 25% level they are decreased only to 4.5 and 9.8.

Meyerhof's own results show that where the lactate disappearing and the lactate oxidized were directly measured, the mean value of Q_M was 4.1 (Tabellen II, III and V in Meyerhof, 1920a). The standard error for the 21 values available is ± 0.383 , giving confidence limits of 4.9 and 3.3 at the 5% level of probability. The narrower limits set to Q_M in this case are due to the fact that the parameters were directly measured, whereas to convert our values into these terms it is necessary first to subtract a large value (0.838) from 1.0 and then to take the reciprocal, which automatically decreases confidence in the derived quotient. The result is that our Q_M value does not differ significantly from Meyerhof's (1920a, 1922) despite the apparently large difference between them. It is not worthwhile taking this argument further, since the Q_M is of no direct use in determining the nature of the reactions that underlie the process.

All the enzymes necessary for glycogen synthesis from lactate have been demonstrated to be present in frog muscle (Opie & Newsholme, 1967) including the so-called 'malic' enzyme, which actually consists of L-malate-NADP oxidoreductase (decarboxylating) coupled with L-malate-NAD oxidoreductase (EC 1.1.1.37). These steps need CO_2 to operate in the required direction, but not ATP. An alternative pathway might be via pyruvate carboxylase, which requires both CO_2 and ATP, but it is extremely doubtful whether this enzyme is present in muscle in significant quantities (Opie & Newsholme, 1967). The next enzyme required, after these initial steps, is phosphoenolpyruvate carboxykinase, which the latter authors also showed to be present. This requires GTP, which can be resynthesized from ATP via the ubiquitous nucleoside diphosphate kinase (EC 2.7.4.6). Lastly, but much later in the chain, there is a requirement for fructose

diphosphatase which Krebs & Woodford (1965) demonstrated in muscle.

If the 'malic' enzyme operates, the overall reaction from lactate to glycogen can be formulated as follows:

As a result of the production of ADP^{3-} , the mitochondrial electron-transport chain would operate, with either pyruvate or lactate as fuel, to produce 17 mol of ATP from ADP on oxidation of 1 mol to CO_2 and water (assuming that the α -glycerophosphate shunt is operating). Thus 2.5 mol of ATP is used up/mol of lactate converted into glycogen (eqn. 2) and 17 mol is produced/mol of lactate oxidized: or for every 1 mol of lactate disappearing, $2.5/17 = 0.147$ mol is oxidized and 0.853 mol is converted into glycogen. Thus the theoretical glycogen coefficient as defined by eqn. (1) is 0.427 against the found value of 0.419. From the given standard error of the latter (0.024), the confidence limits at the 5% level of probability are 0.362 and 0.476, so that the difference between the predicted and found values of the coefficient is not statistically significant. It is also noteworthy that Meyerhof's (1920a, 1922) mean Q_M value of 4.1 yields a glycogen coefficient of 0.38, but because of the wide confidence limits of ± 0.05 this value does not differ significantly from either ours or the theoretical value.

The alternative route of resynthesis via pyruvate carboxylase would require 1 more mol of ATP/mol of lactate utilized and would thus yield a glycogen coefficient of 0.397. This again does not differ significantly from the found value of 0.419, although a line drawn with this slope fails to pass through the majority of the experimental points in Fig. 1. For these reasons, together with Opie & Newsholme's (1967) failure to find pyruvate carboxylase in 'white' muscle, we consider the 'malic' enzyme pathway to be the more probable route of resynthesis. This would also explain the finding that CO_2 concentration plays an important role in the overall reaction, since L-malate-NADP oxidoreductase (decarboxylating) has a rather low affinity (high K_m) for CO_2 (Mahler & Cordes, 1966).

Conversion of lactate into glycogen may play a part in contraction experiments with isolated muscles, such as those reported so extensively by Hill (1965, pp. 183, 200). In such experiments lactate is certainly produced during short tetani in oxygen (Hill, 1965, pp. 183, 200; von Muralt, 1934), but the amounts are too small to lower the pH drastically, so that resynthesis would not be much inhibited by the latter factor. It might therefore be

expected that some of this lactate would be converted into glycogen during recovery in O_2 . This indeed may explain why the apparent P/O ratio in contracting frog muscle is so low (Wilkie, 1968). When calculated on the basis of the fact that the heat liberated during recovery in oxygen is about equal to the initial contraction heat, and from the known enthalpies of phosphocreatine hydrolysis and glycogen combustion, it is found that 32 mol of ATP is resynthesized/mol of hexose oxidized, and the P/O ratio is therefore 2.67 instead of the generally accepted value of 3.08.

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