LVIII. STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE.

III. COMPARISON OF THE REDUCING PROPERTIES OF PLAIN AND STRIATED MUSCLE¹.

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THE purpose of the present experiments was to study the reducing properties of plain and striated muscle in order to ascertain whether there were any pronounced differences in this respect which would be of value in the consideration of their comparative physiology. In making the comparison, the samples of plain and striated muscle have in each case been taken from the same animals, sometimes the frog, more often a mammal, and have been subjected to identical treatment. The actual striated muscle chosen for comparison has varied; in cats the muscle of the thigh, in frogs the sartorius or gastrocnemius, in the bullock, portions of perineal muscle, etc. In view of the greater variability of plain muscle, as regards its physiology, various sources have been used, but no conspicuous differences between them were seen. The tissues used were small intestine, uterus, stomach, and the retractor penis. The reductions studied were those of methylene blue, and of *m*-dinitrobenzene, the former by the well-known technique introduced by Thunberg [1920], the latter as carried out by Lipschitz [1920]. When possible the experiments were carried out on fresh tissues; when material had to be kept it was immersed in 2 % boric acid and kept in the refrigerator; previous to use such preserved muscle was well washed with several changes of distilled water in a shaking machine; such material served very well for the study of the reductions of washed tissue.

It may be said at the outset that the qualitative differences which were found between the two varieties of muscle were negligible, the only important differences being quantitative.

THE REDUCTION OF METHYLENE BLUE.

The reduction of methylene blue *in vacuo* at 35° is usually effected by plain muscle in the fresh state with a velocity only about one-third to one-fifth

¹ Protocols of experiments have been abbreviated or omitted in order to save space, as the technique employed is fully described in the papers quoted.

of that brought about by striated muscle under identical conditions. The following table shows the result of a typical experiment:

Table I. *Reduction of Methylene Blue by Fresh Muscle. (Cat. Intestine and thigh muscle.)*

Mg. m. bl.	0.02	0.02	0.04	0.04	0.06	0.06	0.08	0.08
Plain mus. (g.)	0.2	—	0.2	—	0.2	—	0.2	—
Str. mus. (g.)	—	0.2	—	0.2	—	0.2	—	0.2
Time for reduction (mins.)	15	4	11	12	40	—	40	17
Mg. m. bl.	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Plain m. (g.)	0.1	—	0.2	—	0.3	—	0.4	—
Str. mus. (g.)	—	0.1	—	0.2	—	0.3	—	0.4
Time for reduction (mins.)	>43	21	28	5	27	5	12	1

It will be seen that the plain muscle is much inferior in its reducing power to striated muscle, both when compared with varying amounts of the dye, and also when the amount of tissue is changed, the concentration of dye being kept constant. This result is not surprising in view of the relatively small oxygen usages of plain muscle reported by Evans [1923]. Similar results were obtained with frog tissues; thus in one experiment, using 0.2 g. tissue and 0.4 mg. methylene blue, the times for reduction were 40 minutes for plain muscle, 7.5 minutes for heart muscle, and 5.5–8.0 minutes for striated muscle.

When the finely minced plain muscle is well washed with several changes of water, its reducing power is almost completely lost; this happens also with the striated muscle. The reduction can, however, be restored again to a considerable extent by addition of potassium succinate as also holds good for striated muscle. One sample of plain muscle (0.2 g.) for instance in the fresh state reduced 0.04 mg. methylene blue in 28 minutes; after washing well with water the same tissue (0.2 g.) showed no sign of reduction of 0.02 mg. methylene blue after 1 hour; when 0.2 cc. of 0.1 molar potassium succinate was added, the reduction under otherwise similar conditions was almost complete in 50 minutes.

Optimal Reaction.

When buffered with phosphate solutions, the optimal rate of reduction was obtained with striated muscle at a p_H above 10.5, whilst with plain muscle the optimum was about p_H 9.0–10.0. With borate buffers the optimal p_H was about 8.0 for plain, and about 8.8 for striated muscle. In both instances washed tissue in presence of succinate was used for these tests. The following is the result of the experiments: in each case 0.2 g. of the washed tissue, 0.1 cc. 1 : 5000 methylene blue, 0.1 cc. of $M/10$ potassium succinate, and 0.9 cc. of $M/10$ potassium phosphate buffers were taken. The reduction times were:

p_H	For striated muscle	For plain muscle
3.0–6.7	>60'	>72'
7.2	27'	61'
7.7	28'	37'
9.0	24'	26'
10.5	15'	36'

Alteration in the amount of buffer solution made no appreciable difference to the result, *e.g.* the reduction times were the same with 1 cc. of buffer solution as with 0.2 cc. buffer solution and 0.8 cc. water.

Effect of Temperature.

The optimal temperature, at p_H 9.0 is, according to my experiments, about 50° for both varieties of muscle. Thus in a mixture containing 0.2 g. washed muscle, 0.1 cc. of 1 : 5000 methylene blue, 0.8 cc. of *M*/10 phosphate buffer, and 0.1 cc. of *M*/10 potassium succinate, the reduction times (brought to the same scale for purposes of comparison where different samples were used) were as follows: at 0°, the reduction was scarcely apparent, even after 18 hours; at 20° it was partially complete in 4 hours, at 30° complete in 4 hours, at 40° in 50 minutes, at 50° in 23 minutes, and at 60° in 32 minutes. Experiments were, however, usually carried out at 38°.

Effect of Salts and of KCN.

The reduction of methylene blue by washed plain muscle, in presence of succinate, is accelerated by both sodium and potassium chlorides, precisely as with striated muscle. The effect of cyanide is also identical, that is to say, the reduction by the washed tissue (0.2 g.) of methylene blue (0.2 cc. of 1 : 5000) in presence of succinate (0.2 cc. of *M*/10 potassium succinate) is not inhibited, but slightly, though definitely, accelerated by the presence of potassium cyanide in concentrations up to 0.2 cc. of *M*/100 KCN in 1 cc. of reaction mixture. The reduction was carried out in presence of 0.4 cc. of *M*/10 K_2HPO_4 , and the times for complete reduction were, in one experiment, without cyanide 2 hours; with 0.1 cc. *M*/1000 cyanide, 1 hour 24 minutes; with 0.2 cc. *M*/1000, 1 hour 13 minutes, with 0.05 cc. *M*/100, 1 hour 19 minutes; with 0.1 cc. 1 hour 11 minutes, and with 0.2 cc., 1 hour 15 minutes. Other experiments gave similar results, there being in all cases definite acceleration of the reduction after addition of KCN. The acceleration was possibly due to slight reduction of the H-ion concentration of the mixture, but was not further investigated, the chief point being that there was no inhibition of the reduction. In this respect also there is a close resemblance to the behaviour of striated muscle, as studied by Thunberg [1917].

Restoration of Reducing Power by Addition of Organic Acids.

When plain muscle is washed in the manner described by Thunberg [1920], its reduction power is almost completely lost. On addition of potassium succinate the reducing power is partially restored; but no matter how much succinate is added to it, the reducing power does not approximate to that of the fresh tissue, and still less to that of fresh striated muscle.

It appeared of interest, in connection with the results obtained by Thunberg, to find whether the substances which were capable of restoring the power

of reduction to plain muscle inactivated by washing were the same as those which acted in that way with washed striated muscle. In addition to succinate, I tried lactate, citrate, acetate and formate. The experiments were carried out in every respect according to the Thunberg technique, and need not be described in any detail; in each instance a parallel experiment was carried out with washed striated muscle to serve as a control. The amounts of materials placed in the evacuated tubes were as follows: muscle 0.2 g. $M/10$ potassium lactate (or other organic salt) solution and water together to make up 0.4 cc.; $M/10$ K_2HPO_4 solution 0.5 cc.; methylene blue (1 : 5000) 0.1 cc. Temp. 37° .

The results, at the reaction given by this mixture (about p_H 9) may be summarised as follows:

Plain muscle		Striated muscle	
Reduction time		Reduction time	
No lactate	Lactate (0.4 cc.)	No lactate	Lactate (0.2 cc.)
+ + in 4 h. 19 m.	Complete in 4 h. 9 m.	+ + in 4 h. 7 m.	Complete in 4 h. 2 m.
No formate	Formate (0.1-0.4 cc.)	No formate	Formate (0.1-0.4 cc.)
0 in 2 h.	0 in 2 h.	0 in 2 h.	0 in 2 h.
No acetate	Acetate (0.1-0.4 cc.)	No acetate	Acetate (0.1-0.4 cc.)
+ in 3 h.	+ in 3 h.	+ in 3 h.	+ in 3 h.
No citrate	Citrate (0.4 cc.)	No citrate	Citrate (0.5 cc.)
+ + in 3 h. 27 m.	+ in 3 h. 23 m.	+ + + in 3 h. 22 m.	+ + + in 3 h. 16 m.

These results show that there was a slight acceleration of reduction by lactate, but, at this hydrogen ion concentration no effect produced by acetate, formate or citrate. The effect of altering the H-ion concentration was, however, quite considerable with citrate, which, as shown by Fleisch [1924], is oxidised by washed muscle and methylene blue much more rapidly at a p_H between 5.0 and 6.5 than at 7.6. I have similarly found that whilst at p_H 9.0 and 7.5 citrate was not oxidised, there was pronounced decolorisation of the methylene blue at p_H 6.3 and 5.5, *e.g.* in experiments with quantities as above, and using washed plain muscle:

At p_H 5.5 without citrate; only slight decolorisation in 3 hours 38 minutes.

At p_H 5.5 with 0.1 cc. citrate; decolorisation almost complete in $3\frac{1}{2}$ hours.

At p_H 6.3 without citrate; slight decolorisation in 3 hours 37 minutes.

At p_H 6.3 with 0.3 cc. citrate; almost completely decolorised in $3\frac{1}{2}$ hours.

At p_H 7.5 without citrate, or with 0.6 cc. citrate; decolorisation nearly complete in $3\frac{1}{2}$ hours.

With lactates it was found that the acceleration of decolorisation was most rapid at p_H 8.0-9.2.

There does not seem to be any noteworthy difference between the properties of striated and those of plain muscle in these respects. I could not confirm the oxidation of acetates and formates by either tissue, though a definite action by striated muscle was found by Thunberg [1920].

REDUCTION OF *m*-DINITROBENZENE.

A comparison was also made between the reducing power of plain and striated muscles and of other tissues, on *m*-dinitrobenzene. W. Lipschitz [1920, 1921] and his collaborators have shown that this substance is reduced by many tissues to the soluble, yellow *m*-nitrophenylhydroxylamine, the amount of which may be estimated colorimetrically. This reduction, unlike that of methylene blue, is not reversible by atmospheric oxygen, though it takes place more readily in the absence of oxygen, and in most ways gives results which are similar to those given by methylene blue reduction.

It was found, using a method similar to that of Lipschitz, that plain muscle reduced the *m*-dinitrobenzene with much greater speed than did striated muscle. Comparison of various tissues showed that with cat's tissues the amounts of reduction obtained in equal lengths of time were in descending order: plain muscle of small intestine; heart muscle; uterus muscle; striated muscle. With frog's tissues liver and testis reduced more rapidly than muscular tissue, plain and striated muscle showing little difference in this experiment. In all the experiments with mammalian tissues however it was found that the plain muscle showed upwards of twice as much reduction in a given time as was shown by striated muscle. In all these experiments the amounts of substances used were, muscle 0.5 g., total fluids 3.0 cc. and *m*-dinitrobenzene, in finely powdered form, 0.05 g. It was found that the reduction with striated muscle soon reached a standstill, whereas that given by the plain muscle was able to proceed for a longer time, as well as at a more rapid rate; it seems highly probable that this remarkable difference between the two tissues, which behave in the opposite way towards methylene blue reduction, may be due to the fact that the reduction product is for some reason more toxic to striated muscle than to plain muscle, and so brings the reduction reaction to an end as soon as it has accumulated in sufficient amount. It was found, in agreement with Lipschitz, that the reduction took place in all instances much more rapidly *in vacuo* than when the contents of the tube were exposed freely to the air. On thorough washing with water, the reducing power was completely lost, but was partially restored by addition of succinates, just as with methylene blue reduction. The reduction was not inhibited by reasonably small amounts of cyanide, such as $M/400$ KCN, or less.

This fact is not in agreement with the observations of Lipschitz and Gottschalk [1921] who found considerable inhibition at low concentrations of cyanide with frog's striated muscle.

THE REDUCING POWER OF AQUEOUS EXTRACTS.

Aqueous extracts of fresh plain and striated muscle were made by treating the minced tissue with twice its weight of water, to which some boric acid was added when it was to be kept at room temperature for many hours, or on ice for many days. The extraction was carried out at 0° as a rule, but in some instances at room temperature or at 60–70°. The filtered extracts ob-

tained differed in appearance, that from plain muscle being colourless but opalescent, while that from striated muscle was red, owing to the presence of haemoglobin, and clear. In some of the experiments filtration was carried out through a Berkfeld filter, but the results were the same as when the filtration was through paper alone. The filtered extracts showed definite reducing power towards methylene blue *in vacuo*, but the plain muscle extract had much the more powerful effect in this respect. Thus in one experiment 0.2 g. of fresh plain muscle reduced 0.02 mg. of methylene blue in 1 cc. water in 35 minutes; the same amount of striated muscle brought about the reduction in 8 minutes. An extract of samples of each tissue was made at 60° with twice its weight of water, and filtered after 1 hour. To 0.9 cc. of each filtrate 0.1 cc. of methylene blue (0.02 mg.) was added; reduction occurred *in vacuo* in 21 minutes with the plain muscle extract, whilst the striated muscle extract showed no sign of reduction after 36 minutes. On adding to 0.9 cc. of each extract 0.2 g. of washed muscle from which the extracts had been made, and 0.1 cc. of the methylene blue solution, there was reduction in 15 minutes with the plain muscle extract plus washed plain muscle, and in 20 minutes incomplete reduction with the striated muscle extract to which the washed striated tissue had been added. It was found that plain muscle extract re-activated washed striated muscle much more than striated muscle extract. Parallel with this greater reducing and re-activating power of the plain muscle extract is a much more intense nitroprusside reaction for glutathione [Hopkins, 1921], and the fresh tissues themselves also show the same difference in this respect when tested directly. On boiling, or on being allowed to stand in air for 2 or 3 days the extract loses its reducing power, and no longer shows the nitroprusside reaction; it is however still able to some extent to restore the reducing power to washed tissue. When the boiled extract is treated with a little fresh tissue ($\frac{1}{10}$ th its weight), and again filtered off after half an hour, it is found that the reducing power and the nitroprusside reaction have been restored to the extract. It is evident from a consideration of these results that whilst the fresh extract of plain muscle contains a relatively large amount of glutathione, this is lost by oxidation on standing, but can be converted to the reduced form again by treatment with either fresh or washed tissue [Hopkins, 1921]. It was also found that the reduction of washed tissue was accelerated to a greater extent when plain muscle extract and potassium succinate were added than when either of these was added alone; in fact in one experiment the reduction of methylene blue by washed muscle and fresh extract was no quicker than that by the extract alone.

Apparently some other factor is concerned in addition to glutathione, because it was found that when the extract was heated to 65° for 30 minutes in slightly acid solution, and then neutralised, much of its reducing power had been lost, although the nitroprusside reaction remained as intense as before. This was possibly due to destruction of some soluble catalyser or hydrogen acceptor.

CONCLUSIONS.

1. Plain muscle reduces methylene blue more slowly, but *m*-dinitrobenzene more quickly than striated muscle. Neither reduction is inhibited by cyanide.
2. Both types of reduction are almost abolished by washing, but are partly restored by addition of succinate.
3. The optimal p_H for plain muscle reduction of methylene blue is about 9.0.
4. The differences between the reducing action of plain and striated muscle appear to be quantitative rather than qualitative. It seems unlikely that there is any essential difference between them as regards the nature of their intermediary metabolic products.
5. Plain muscle and its extracts are richer in glutathione than striated muscle or its extracts, and show properties in accordance with this.

I hope to continue these experiments in the near future on my return to Japan.

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