

## THE OXYGEN USE OF DENERVATED MUSCLE.

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THE rapid loss of weight which occurs in denervated muscle shows that the balance of repair and breakdown is upset to the detriment of the former; but it does not show whether the disturbance of the balance is due solely to a lessened power of repair, or solely to an increased breakdown, or to both of these. That a lessened power of repair is in part responsible for the change is indicated by an experiment published a short time ago by one of us<sup>(1)</sup>. In this, the muscles of both hind legs of a rabbit were denervated, those on one side were made to contract daily for three weeks, those of the other side remaining at rest. The contraction had no certain effect in delaying the loss of weight of the muscles. In normal muscles, contraction carried on for a like period causes either an increase in weight or delays the wasting that would go on without it, so that the absence of increase in the denervated muscle may reasonably be attributed to a lessened power of repair. But this line of argument though suggestive is not wholly conclusive. The question can be approached from another side. Observations which have been made in various ways especially those of Fletcher and Hopkins on the formation of carbonic and lactic acids, of A. V. Hill on heat production, and of Verzar on oxygen use, all tend to show that the oxygen taken up by muscle is used partly for the repair of muscle, and partly for the oxidation of products to be eliminated. In such case if the loss of weight of denervated muscle were due solely to lack of power of repair, the oxygen use<sup>1</sup> would be decreased, if it were due solely to greater breakdown, the oxygen use would be increased. Thus a knowledge of whether the oxygen use in denervated muscle lessens, or remains the same, or increases, would enable a conclusion to be reached as to one or more of the possible

<sup>1</sup> 'Oxygen use' in this paper means the oxygen used by unit weight of tissue in a unit time.

ways in which the loss of muscle substance may conceivably occur and serve as a guide for further investigation on the cause of the atrophy.

*Method.* The animals used were cats. The nerves were cut on the left side. In two experiments the internal popliteal nerve was cut, in two others both internal and external popliteal nerves. For the operation the animals were anæsthetised with chloroform; for the final experiment they were anæsthetised first with chloroform, then with A.C.E. mixture given by a tracheal tube, and with urethane (7 to 8 c.c. injected subcutaneously), except that in Exp. 4 no urethane was given, and about an hour before taking samples of blood the brain was destroyed by injection of starch grains into the carotid artery, then artificial respiration was kept up and the A.C.E. discontinued.

In Verzàr's experiments(2) on the oxygen use of normal muscle, the blood was taken from the gastrocnemius only. The tendo Achillis was cut and the veins entering the deep surface of the muscle were tied. This procedure necessitates considerable exposure of the muscle. In order to avoid this, we have taken either all the leg muscles innervated by the internal popliteal nerve, or the whole of the leg muscles, *i.e.* the muscles exclusive of those of the thigh and foot. In either case the exposure of the muscles to be examined need only be slight. In the former case the anterior tibial artery and vein were tied and the extensor muscles excised. It must be noticed that by our method, the blood obtained though predominantly muscular is not wholly so, since some comes from the tibia, or tibia and fibula, as well as from the deep fascia. For our purpose we regarded this as of no importance, since similar blood, though perhaps not quite in the same quantity, mixed with the blood from the muscles on the undenervated side and was taken for comparison with that of the denervated side. All the veins from the foot, from the skin, the veins running into popliteal vein above the gastrocnemius and the veins running into the vena saphena and femoral near the junction with the popliteal were tied. The internal saphenous vein was isolated just below the popliteal, and the femoral for about two inches upwards.

The blood was collected from the internal saphenous vein in the manner introduced by Barcroft and employed by Verzàr. A cut was made in it a little below the junction with the popliteal: the valve at the junction prevented any blood issuing from the cut. A graduated pipette was passed into the cut, and the nozzle pushed past the valve into the main vein, blood at once began to flow into the pipette, after

$\frac{1}{10}$  to  $\frac{2}{10}$ th c.c. had passed into it—during which the pipette was carefully adjusted so that the nozzle was in the centre of the vein—the main vein was compressed about  $\frac{3}{4}$  to 1 inch higher up, and the pipette rapidly filled. The first portion of blood  $\frac{2}{10}$ – $\frac{3}{10}$  c.c. collected was not taken for oxygen estimation. In preliminary experiments we did not find that clamping the main vein with a bull dog was a convenient or advantageous way of diverting the blood stream. Instead of this we passed a woollen thread under the vein, and an assistant, as soon as he was told to do so, pulled this up, thus blocking the blood flow upwards. The pipette was held in a nearly horizontal position. The time of flow of each  $\frac{1}{10}$  c.c. was marked on a revolving drum by means of a key and time marker. A few minutes after taking a sample of the venous blood, a sample of arterial blood was taken. The oxygen lost by the venous blood was determined by Barcroft and Roberts' (3) differential manometer, the arterial blood being put in one side of the manometer and the venous blood in the other side. The method followed was that given by Barcroft (4) and with apparatus which he kindly lent us.

As we have said above our aim was to see whether denervated muscle uses more or less oxygen than the undenervated, and we compared the rate of flow and oxygen loss of the venous blood from the muscles of the two sides. The actual numbers obtained for the oxygen use per minute per gram of muscle only hold for the conditions of the experiments and we do not think they show, except in the most approximate way, the oxygen use in un-anæsthetised animals. We may emphasize a little these conditions, since they help to explain the not inconsiderable differences we found in the oxygen use even in the course of the same experiment. The various operations from the time of beginning the administration of anæsthetics to the time of taking the first sample of blood took  $2\frac{1}{2}$  to 3 hours. The prolonged administration of anæsthetics causes progressive fall of blood-pressure, and in general it will be seen from the protocols that the blood flow was less than would be expected from the weight of muscle taken. We did not take a blood-pressure tracing for we thought we should lose more than we gained by further complicating the experiment. Within the possible limits of blood-pressure, variations would affect both sides approximately equally. Prolonged anæsthesia tends to cause shallow respiration, and incomplete oxidation of the blood in passing through the lungs. According to Verzàr (5) muscle uses less oxygen if there is less in the blood. In the first three of our experiments the amount of volatile anæsthetic in the

body was not kept absolutely constant, the difference was always small, but it may have been enough to vary a little the muscle tone on the undenervated side, and the slight reflexes which occasionally occur when the limb is moved in order to place it in the most favourable position for putting the pipette into the vein.

Most of the factors mentioned above tend to cause a variation in muscle metabolism but to a not definitely determinable extent. There are also certain sources of experimental error. The method cannot we think be trusted to give accurately the rate of flow through the muscle. The bore of the nozzle of the pipette is necessarily much smaller than that of the vein above, and in consequence by increased resistance tends to bank up the blood in the muscle veins and to make the estimation of the rate of flow smaller than it really is. In our experiments each pair of pipettes used—one on the right and the other on the left—were as nearly as possible of the same bore, but the bore of the several pairs varied somewhat, so that the resistance of the flow into them was not exactly the same. The nozzle of the pipette cannot be seen in the vein, so that whether it is in the centre of the vein or not can only be judged by the position of the pipette and the length inserted. Whilst with a little practice there is no difficulty in obtaining a perfectly regular flow in the pipette, a slight shifting of it will sometimes cause a distinct variation in rate. When, as in our experiments, hirudin is not used, the blood must be run into the manometer quickly or it will clot; but the viscosity increases some time before clotting takes place, and this will affect the amount of blood which clings to the side of the tube.

We have compared the oxygen use in denervated and undenervated muscle in four experiments. In each experiment the comparative estimation was made twice so that there were eight observations in all. We give the experiments in the order in which they were made since the details were varied as experience suggested. The details of method already given above we need not mention again.

The intervals between collecting the successive samples of blood varied from 7 to 17 minutes. Since we do not find any definite relation between the length of the interval and the variations in oxygen use we do not think it worth while to give the intervals in the tables.

*Exps. 1 and 2. Flexor muscles denervated on one side.*

*Exp. 1.* Cat. Left internal popliteal nerve cut 56 days. Body weight 3.22 kilos. Extensor muscles on both sides excised. After the last sample of blood had been taken, the popliteal nerve was stimulated above the point of section and found to cause slight contraction, so that recovery of nervous connection had taken place in some of the fibres; it will be seen however that the difference of weight of the muscles on the two sides was great. Taking the weight on the undenervated side as the original weight of the denervated muscles, the loss of weight of the latter was 63.1 p.c.

TABLE I.

	Blood sample from muscles	Blood flow per minute	Weight of muscles	Oxygen used per 1 c.c. of blood	Oxygen used per min. per gram.
I	1. Denervated (Left)	1.39	13.912	.0726	.0073
	2. Denervated (Left)	1.545	13.912	.103	.0114
II	3. Normal (Right)	1.63	37.745	.02922	.0013
	4. Normal (Right)	2.822	37.745	.0475	.0036

*Exp. 2.* Cat. Left internal popliteal nerve cut 21 days. Body weight 3.35 kilos. Preparation as in the preceding, but the blood samples were taken alternately from the left and right sides.

TABLE II.

	Blood sample from muscles	Blood flow per minute	Weight of muscles	Oxygen used per 1 c.c. of blood	Oxygen used per min. per gram.
III	1. Denervated (Left)	3.24	23.755	.0507	.0069
	2. Normal (Right)	2.84	38.764	.036	.0026
IV	3. Denervated (Left)	3.6	23.755	.0465	.0071
	4. Normal (Right)	3.37	38.764	.0384	.0033

Both of these experiments show a much greater oxygen use in the denervated than in the normal muscles but *Exp. 2* is clearly much more satisfactory than *Exp. 1*. In *Exp. 1* there is a considerable difference in the oxygen use as determined in two different samples of blood taken from the same muscles, and this suggests either that the metabolism of the muscles was varying greatly or experimental error. Taken by itself *Exp. 1* would not have much weight, but since its main result is in harmony with that of the other experiments, it may we think be regarded as confirmatory evidence.

*Exps. 3 and 4. Flexor and extensor muscles denervated on one side.*

*Exp. 3.* Cat. Both left popliteal nerves cut 29 days. Body weight 3.5 kilos. Stimulation of the peripheral end of the left sciatic was without effect. The blood was taken from the whole of the muscles of the leg. The right sciatic was cut about an hour before taking the blood samples.

TABLE III.

	Blood sample from muscles	Blood flow per minute	Weight of muscles	Oxygen used per 1 c.c. of blood	Oxygen used per min. per grm.
V	1. Denervated (Left)	6.25	26.921	.03122	.0073
	2. Normal (Right)	13.95	47.684	.00323	.001*
VI	3. Denervated (Left)	10.17	26.921	.03702	.0141
	4. Normal (Right)	12.00	47.684	.0298	.0075

\* This number is probably too low and due to some experimental error.

Percentage saturation of arterial blood with oxygen 73.7%, 72.5%.

*Exp. 4.* Cat. Both popliteal nerves cut on left side 17 days. Body weight 3.8 kilos. Blood taken from the whole of the muscles of the leg. In this experiment anaesthetics were given for the first part only of the dissection. The brain was destroyed about an hour before taking the blood samples by injecting starch grains into the peripheral end of the carotid artery; respiration ceased, and artificial respiration was kept up for the remainder of the experiment.

TABLE IV.

	Blood sample from muscles	Blood flow per minute	Weight of muscles	Oxygen used per 1 c.c. of blood	Oxygen used per min. per grm.
VII	1. Denervated (Left)	3.93	36.18	.147	.016
	2. Normal (Right)	3.71	58.69	.04115	.0026
VIII	3. Denervated (Left)	3.72	36.18	.12725	.0131
	4. Normal (Right)	4.7	58.69	.045	.0036

Percentage saturation of arterial blood with oxygen 82%, 81.3%.

*Exp. 3* shows the same characters as *Exp. 1*. Whilst it shows a much greater oxygen use in the denervated than in the undenervated muscle, the oxygen use in the successive estimations varies greatly. In this case the chief cause was we think a variation in the oxidation of the arterial blood. The blood in the carotid was more or less dark; and a sample taken at the end of the experiment had a percentage saturation of only 73.1. It was for this reason that in *Exp. 4*, we destroyed the brain and kept up artificial respiration. Although this causes a great fall of blood-pressure—it will be noticed that the rate of blood flow was much less than in *Exp. 3*—the conditions throughout are kept much more constant. In this experiment the difference in the oxygen use found in the two determinations of the same side although taken at an interval of 20 to 30 minutes are relatively small. Variation in constancy of internal conditions is, however, clearly only one factor in the variation of oxygen use as determined by this method, for in *Exp. 6* (cp. below) made in the same way, the oxygen use was found to be markedly greater in Nos. 3 and 4, than in those taken either before or after.

In the foregoing experiments, the controls consisted of the blood samples taken from the undenervated side. We tried a different form of control, viz. by comparing the blood samples taken from the two sides in a normal animal. Two experiments were made, one early in the series, anæsthetics being given and the extensors excised as in Exps. 1 and 2; the other at the end of the series in the manner of Exp. 4, *i.e.* preliminary anæsthesia, destruction of the brain, artificial respiration, blood samples from the whole of the leg muscles. It will be seen that the difference in oxygen use on the two sides is of quite a different order from that in the experiments in which the muscles were denervated on one side.

*Exp. 5.* Cat. Weight 2.76 kilos. Anæsthetics. Blood from flexor muscles.

TABLE V.

	Blood flow per min. in c.c.	Weight of muscles	Oxygen used per min. per grm.
{ 1. Right side	8.58	28.525	.0044
{ 2. Left side	3.00	28.373	.0034
			Mean .0039

*Exp. 6.* Cat. Weight 3.5 kilos. Brain destroyed. Artificial respiration. Blood from flexors and extensors. Weight of muscles: left = 53.05 grm., right = 54.83 grm.

TABLE VI.

	Blood flow per min. in c.c.	Oxygen used per 1 c.c. blood	Oxygen used per min. per grm.
{ 1. Left	5.215	.0543	.0053
{ 2. Right	4.000	.07325	.0053
{ 3. Left	8.787	.0461	.0076
{ 4. Right	6.573	.0578	.0070
{ 5. Left	4.615	.0606	.0053
{ 6. Right	3.859	.0759	.0052
			Mean .0059

Percentage saturation of arterial blood taken at end of the experiment, 90.5, 89.

Taking the experiments as a whole, they bring out a clear difference between the oxygen use per gram per minute in denervated and in undenervated muscle. In each of the eight cases in which comparison was made with one side denervated and the other not, the oxygen use was greater in the former than in the latter. The mean of the eight cases of the former was .0104 c.c., the mean of seven cases of the latter (one being omitted as probably too low) was .0035 c.c. If we take the oxygen use for 1 c.c. of blood, the mean was .077 c.c. for that coming from the denervated and .038 c.c. for that coming from

the undenervated muscles. The eight observations made in a similar manner on animals in which the nerves were not cut on either side also show an oxygen use less than that in the observations on denervated muscles. The unsatisfactory feature of the experiments is the variation in oxygen use found in some cases in successive samples of blood coming from the same muscles. Verzàr(2) only gives one experiment in which two successive blood samples were taken from the gastrocnemius, but here also there was a considerable variation. A large factor in the variation we believe to be due to the difficulty by the method used of determining the real rate of blood flow through the muscles. However this may be, the variation prevents any conclusion being drawn from these experiments as to the degree of oxygen use in different stages of denervation. We had hoped, as we made the observations at different intervals after nerve section, that some information might be obtained with regard to this point.

An increase in the oxygen use by the denervated muscle shows that the atrophy of muscle cannot be due simply to a decrease in the rate of building up of the muscle substance. It must be due to the breakdown being quicker than in the normal muscle. This breakdown might conceivably be accompanied by quicker building up if the new formed product were unstable, but this seems very unlikely in itself, and the experiment on the effect of contraction on denervated muscle mentioned in the introduction tends to show that the power of repair is decreased. Consequently the probability is that the atrophy is caused partly by a decreased power of repair, but mainly by increased breakdown. It has already been suggested by one of us(1) that the atrophy may be the result of the fibrillation which begins soon after denervation. The greater oxygen use is consistent with this suggestion, but does not afford definite evidence in proof of it.



## SUMMARY.

Eight determinations were made on the oxygen use of denervated muscles on one side of the body and of the undenervated muscles on the opposite side. In all cases a much greater oxygen use per gram per minute was found to be present in the denervated muscles. We conclude from this that the atrophy of denervated muscle is not solely due to a decreased power of repair, the breakdown remaining constant, but that it must be due chiefly to an increase in the rate of breakdown of the muscle substance.

## REFERENCES.

- (1) Langley. *This Journal*, 50. p. 339. 1916.
- (2) Verzàr. *Ibid.* 44. p. 243. 1912.
- (3) Barcroft and Roberts. *Ibid.* 39. p. 429. 1910.
- (4) Barcroft. *The Respiratory Function of the Blood* (Cambridge), p. 298. 1914.
- (5) Verzàr. *This Journal*, 45. p. 39. 1912.