THE NUMBER AND DISTRIBUTION OF CAPILLARIES IN MUSCLES WITH CALCULATIONS OF THE OXYGEN PRESSURE HEAD NECESSARY FOR SUPPLYING THE TISSUE. By AUGUST KROGH.

(*From the Laboratory of Zoophysiology, University of Copenhagen.*)

In a preceding paper I have recorded measurements of the diffusion constants for certain gases and especially for oxygen in animal tissues. In order to utilise these determinations it is necessary to know further the rate at which oxygen is used up by the tissue in question and the average distance which an oxygen molecule has to travel from a capillary into the tissue before entering into chemical combination. In most tissues the arrangement of capillaries is too irregular to admit of detailed measurements and calculations but in the muscles we have a fairly regular distribution of capillary vessels, which are in the main parallel to the muscle fibres between which they are intercalated. I have therefore made the muscles the basis of my study.

Fig. 1. Longitudinal section of injected M. gastrocnemius of the horse. × 70.

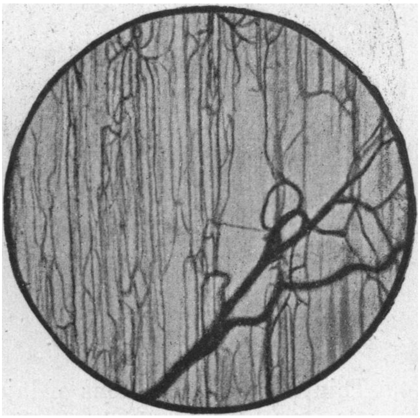

Fig. 2. Injected abdominal muscle wall of the guinea-pig. × 70.

The general arrangement of the vessels in striated muscles of mammals is as follows (Figs. 1 and 2). From small arteries, running in the main parallel to the fibres, arterioles are given off at more or less right angles and at rather constant intervals. These arterioles penetrate among the fibres, dividing freely, and each arteriole gives rise to two bundles of capillaries running along the fibres in opposite directions. In a muscle stretched to its normal length during rest the capillaries are more or less straight, but in a contracted muscle they become very sinuous; they anastomose to a certain extent forming long meshes about the fibres. From two bundles of capillaries meeting each other from opposite directions the blood is collected into small veins running like the arterioles mainly at right angles to the fibres and opening into larger veins following the corresponding arteries along the muscle fibres. The arrangement I have given above is similar to that figured by Ranvier[1] and by Kölliker[2].

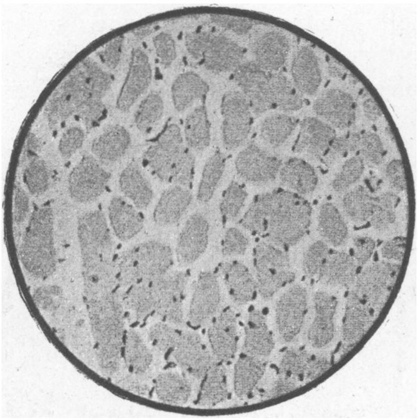

Fig. 3. Transverse section of the M. gastrocnemius of the horse. × 120.

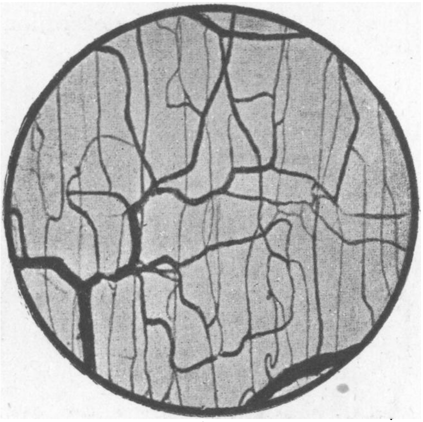

Fig. 4. Injected smooth muscle of the stomach of the frog. × 38. (Referred to later.)

Thin transverse sections of injected muscles show the capillaries as small coloured dots which are distributed with conspicuous regularity among the muscle fibres (Figs. 3 and 5), and it becomes at once apparent that no serious error can be committed by supposing each capillary to supply oxygen independently of all the others to a cylinder of tissue surrounding it. In a transverse section such a cylinder is represented by an area which can be taken as circular and the average area belonging to each capillary can be calculated from a counting of the number of capillaries in a transverse section by division of its total area with the number found.

[1] **Ranvier.** *Traité tech. d'Histologie,* fas. 4, p. 511. 1876.

[2] **Kölliker.** *Hdb. d. Gewebelehre,* 1. p. 380. 1889.

Supposing Fig. 6 to represent the cross-section of a capillary (r) with the cylinder of tissue (R) supplied by it we shall have oxygen molecules constantly leaving the capillary through the wall and entering the surrounding tissue where they will be used up at a rate determined by the gas exchange. The oxygen pressure difference between the capillary wall and a point at the distance x from the centre of the capillary must be proportional to the gas exchange p and inversely proportional to the diffusion rate d, and when these are known, together with the dimensions of the capillary and cylinder (their radii r and R) and the distance x, it becomes possible to establish a mathematical formula from which the pressure difference can be calculated. The mathematician Mr K. Erlang has shown me the kindness to work out such a formula which runs

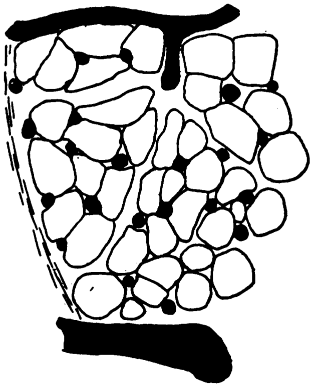

Fig. 5. Transverse section of injected muscle of the tongue of the cat. × 268.

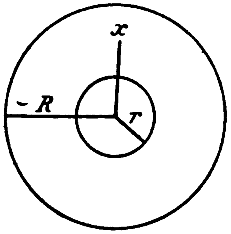

Fig. 6. See text.

$$T_0 - T_x = \frac{p}{d}\left(\tfrac{1}{2}R^2 \log_{\text{nat}}\frac{x}{r} - \frac{x^2-r^2}{4}\right),$$

T_0 and T_x being the oxygen tensions in the capillary and the point x respectively. Putting $x = R$ and substituting ordinary logarithms for the natural we get the formula transformed to

$$T_0 - T_R = \frac{p}{d}\left(1{\cdot}15R^2 \log\frac{R}{r} - \frac{R^2-r^2}{4}\right),$$

which will give us the maximum tension difference necessary to supply the muscle with oxygen. If this difference is found to be larger than the O_2 tension of the venous blood it follows that some portions at least of the muscle (near the venous ends of the capillaries) must suffer from want of oxygen, while if it is smaller the oxygen tension must be positive everywhere within the muscle.

In the above formula all distances are calculated in cm. d is the rate of diffusion per minute through 1 cm.^{2} and the distance of 1 cm. From the determinations given in the preceding paper we have for warm-blooded animals $d_{37} = 0{\cdot}164{\cdot}10^{-4}$ and for cold-blooded $d_{15} = 0{\cdot}133{\cdot}10^{-4}$. p is the absorption of oxygen per cm.^{3} per minute, and in the calculations given below I have taken the gas exchange of muscular tissue to be equal to that of the animal as a whole. R is calculated from countings of capillaries on transverse sections of injected preparations, while r, which cannot safely be measured directly, can be taken provisionally to be determined by the size of the red corpuscles which have to pass through the capillaries. In most mammals the red corpuscles are not very different in size while those of frogs are much larger. It can be shown from the formula that $T_0 - T_R$ decreases but slowly with increasing r.

Between cold-blooded and warm-blooded animals and between warm-blooded of different size there are very large differences in the gas exchange (p) which must involve corresponding differences in the oxygen pressure heads necessary for supplying the muscles ($T_0 - T_R$), unless they are compensated in some way. The only compensation which is possible according to the formula is a variation of R, and one is led therefore to expect that the average distances between capillaries in muscles should vary inversely with the gas exchange or, what amounts to the same thing, the number of capillaries per square mm. of the cross-section should vary directly with the gas exchange.

I intended to test this deduction on a series of injection preparations of muscles from a number of different animals, but I have had to reduce the programme very considerably on account of the unexpected difficulties met with in obtaining complete injections of muscular tissue. Injections from the aorta or any large arteries, which produce excellent results in most organs, have invariably failed in the case of muscles though the technique was varied in several ways. The vessels were washed out carefully with saline at body temperature and nitrites were added to produce a vaso-dilatation. In some experiments on the cod the muscles were tetanised at intervals before and during the injections, but though more capillaries became filled than without tetanisation the injections were far from complete. The best results have been secured by injecting directly through a muscle artery, tying as far as possible all the arterial anastomoses and employing a high pressure. When practicable I have finally tied the vein and set up a very considerable pressure before tying the artery. I believe that the material to be injected is best left for a day or two after killing the animal before the injection is made.

As injection fluid I have used gelatine stained with freshly prepared Prussian blue or with Indian ink.

I have obtained good injections of the following muscles: M. gastrocnemius of a horse[1], M. semimembranosus of a dog[1], M. masseter of a guinea-pig, Mm. sartorius and submaxillaris of a frog. Certain portions of a prætracheal muscle of the guinea-pig and of an eye muscle of a cod are also probably completely injected.

The completeness or otherwise of the injection can practically always be judged from longitudinal sections and in certain cases, especially in the cod and frog, the capillaries have been counted on thick longitudinal sections by taking optical transverse sections of them. This has been done as follows: A micrometer in the eyepiece of the microscope is arranged at right angles to the muscle fibres and capillaries, the surface of the section is focussed and the position of the distinctly visible capillaries marked on squared paper. The microscope tube is lowered a certain small amount (usually five scale divisions corresponding to 4·5μ absolutely or, when the section is mounted in balsam of 1·5 refractive index, to 6·75μ). The capillaries which thereby become visible are put into the diagram and the process repeated through the whole thickness of the section or to such a depth as is compatible with obtaining clear images of the capillaries.

In most cases countings have been made on actual transverse sections by means of a micrometer ruled in squares. I give as an example the countings made on five different transverse sections from the gastrocnemius muscle of the horse.

	1	2	3	4	5
	45	34	38	38	31
	40	34	42	43	33
	42	40	43	47	43
	41	46	41	49	39
	44	44	46	33	36
	36	41	—	—	—
	49	38	—	—	—
Average	42	39	42	42	36

The general average is 40·5 and the dispersion (standard deviation) of the single countings ± 5, which shows the regularity of the distribution. As the area of the square counted is 0·0300 mm.^{2} the result of one counting corresponds to 1350 ± 170 (12·4 p.c.) and the general result to 1350 ± 31 capillaries per square millimetre.

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[1] For the material for these and kind help in carrying out the preparation and injection I am indebted to Prof. Paulli of the Veterinary High School, Copenhagen.

In like manner the number of capillaries per mm.^{2} of the dog's semimembranosus has been determined from 30 countings to 2630 ± 51 with a dispersion of ± 280 (10·6 p.c.) and the number in the masseter muscle of the guinea-pig from nine countings to 2840 ± 100 with a dispersion of 300 (10·6 p.c.). From the prætracheal muscle of the guinea-pig only three countings of an area of 0·0150 mm.^{2} have been obtained giving the results 55, 55 and 58 capillaries corresponding to 3700 per mm.^{2} In the muscles of frogs and a cod I have found from 300 to 500 capillaries per mm.^{2}.

It is of course impossible to draw valid conclusions from such a small number of determinations, but as far as they go the countings would seem to indicate that the capillary network is closer in warm-blooded than in cold-blooded animals and closer in small mammals than in larger forms. With a reliable injection technique it would certainly be worth while to count the number of capillaries on transverse sections of various muscles from a larger number of vertebrates.

The results of the counting and other necessary data have been put together in a small table and the resulting maximum oxygen tension difference ($T_0 - T_R$) between the blood and the muscular tissue deduced. By the formula $T_0 - T_R$ is expressed in atmospheres. In the table I have reduced the figures to mm. mercury pressure.

	Weight kg.	Metabolism cal. per kg. and hour	Number of capillaries per mm.^{2} of striped muscle	R μ	Diam. of red corpuscles $2r$ μ	$T_0 - T_R$ mm. Hg.
Cod	1	0·4	400	28	8·5	0·4
Frog	0·04	0·4	400	28	15	0·25
Horse	500	0·5	1400	15	5·5	0·1
Dog	5	3	2500	11·3	7·2	0·2
Guinea-pig	0·5	6	3000	10·3	7·2	0·3

The table shows that the oxygen pressure head necessary for supplying the muscles with oxygen is remarkably small, so small indeed that even during the heaviest muscular work, which may possibly increase the metabolism twenty-fold, the oxygen tension of the muscular tissue will be practically equal to that of the venous blood, the maximum difference in the case of the guinea-pig being only 6 mm. or less than 1 p.c. of an atmosphere.

In other tissues than muscles the arrangement of the capillaries is not sufficiently regular to allow direct calculations to be made but a simple inspection of injection preparations is sufficient to show that in most glands and in the central nervous system the capillary network is even closer than in striated muscles. In smooth muscles (Fig. 4 *supra*) and in connective tissue it is more open, but we have every reason to suppose that here also the oxygen consumption is much lower, and it would seem reasonable to conclude therefore from the measurements and calculations that there is in the tissues of all organs a positive oxygen pressure equal to or even higher than that of the venous blood leaving each organ. For reasons which will be fully discussed in the succeeding paper such a conclusion is not warranted.

SUMMARY.

In striated muscles the capillaries are arranged with such regularity along the muscle fibres that each capillary can be taken to supply a definite cylinder of tissue the average cross-section of which can be determined by counting the capillaries in a known area of the transverse section.

A formula is given which allows the calculation of the oxygen pressure head which is necessary and sufficient to supply the muscle with oxygen from the capillaries.

The number of capillaries per square mm. of the transverse section of striated muscle appears to be a function of the intensity of the metabolism, being higher in small mammals than in larger forms.

The necessary oxygen pressure head deduced from the total number of capillaries is in all cases extremely low.