PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Effects of Functional Activity in Striated Muscle and the Submaxillary Gland

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IV. *Effects of Functional Activity in Striated Muscle and the Submaxillary Gland.*

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(From the Physiological Laboratory, Cambridge.)

The present paper records an attempt to investigate certain metabolic phases of muscular and glandular activity, and their relationship to the secondary changes which result in the muscle and the gland. By the terms "muscle" and "gland" we mean, not the muscle fibre and the gland cell, but the complex of tissues, contractile, vascular, lymphatic, etc., which form a muscle in the gross sense of the word, and the corresponding structures which form a gland. Either muscular contraction or salivary secretion is accompanied by changes, not only in the contracting or secreting cell, but in the various accessory structures. Our endeavour was to make some analysis of these changes, and of their relation to one another, further than has hitherto been done.

Our method differs from most of those which have been used by previous workers in that we desired to subject our tissues to much longer periods of exercise than others have done. In some crude way we wished to simulate the conditions which obtain when actual exercise is taken by the body. Thus, in our experiments on muscle, we exercised the muscle rhythmically for 15 minutes, in those on gland we gave sufficient pilocarpine to produce a prolonged secretion. In this way we hoped to obtain effects in the metabolism of the muscle, as well as secondary effects in the blood vessels, lymph flow, etc., which we might fairly compare with those which take place in the body.

Skeletal Muscle.

In the case of muscle we have made determinations of

(1) The oxygen used by the muscle.
(2) The rate of flow of blood through the muscle.
(3) The exudation which takes place from the vessels.
(4) In some cases the change in weight.
(5) Specific gravity of the muscle.

Experimental Methods.

Anæsthetics.—Dogs have been used in all cases. The anæsthetic used has been urethane, dose 4 c.c. of the saturated solution per kgrm. The dogs have been put "under" in the first instance by the use of ether.

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Dissections.—Two muscles have been used, the gastrocnemius and the anterior belly of the digastric. The dissection differs slightly in each case since the blood vessels are never quite the same in any two instances. The general principles of the dissection are constant throughout. They consist in tying all the confluents into some large adjacent vein except those from the muscle; all anastomoses must, of course, be tied also. In this case of the gastrocnemius the dissection is essentially the same as that figured by VERZÁR (1) for the cat. The only new feature is that we have dispensed with cannulæ for the collection of the blood. In the case of the gastrocnemius VERZÁR inserted a cannula into the saphenous vein close to its junction with the femoral, breaking down any adjacent valves, then when the femoral was clipped the blood from the popliteal vein ran backwards along the saphenous and could there be collected.

Our procedure has been to open the saphenous vein about half-an-inch from its junction with the femoral, leaving the valves at the junction intact. If the saphenous is tied distally to the opening no hæmorrhage ensues. When a sample of blood is required a special burette (fig. 1) is inserted into the opening.

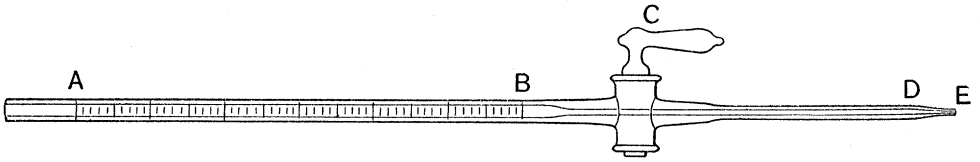

FIG. 1.—Burette.

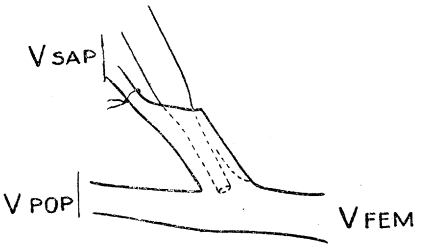

FIG. 2.—V FEM, femoral vein; V POP, popliteal vein; V SAP, great saphenous vein. The dotted lines show a valve and the nose of the burette.

It is desirable to have a number of these burettes so that there may be no difficulty in finding one suitable to the requirements of the particular vessel which is being used. A description of one will suffice, with an indication of the points which may be varied.

The graduated portion of the burette, AB, has a capacity of 2 c.c. and is graduated along its length, each graduation corresponding to 1/50 c.c. The portion of the burette between C and E must be long enough to pass the blood into the bottle of the blood-gas apparatus (2). The nose DE must be suitable to the vessel, that is to say, it must be conical in shape and of such a size that when put into the vessel it makes a tight joint, completely filling the lumen; at the same time the nose must be long enough for E to pass beyond any valves there may be between the opening in

the saphenous vein and its confluence with the popliteal. The opening at E must not be so small as to check the blood flow. It is important that the dead space between E and B should be of suitable size. In the case of a very rapid flow the dead space must be large enough to allow of the burette being properly inserted into the vessel and the flow becoming steady along its course before the surface of the blood reaches B, since the measurement of the time which the blood takes to fill the tube from B to A is fundamental to the experiment. On the other hand if the blood flow is very slow, the dead space may be so large as to cause a commencing tendency to clot. This again is fatal, since the hæmoglobin capacity of the blood is one of the measurements which is made subsequently.

Consequential to the subsequent accurate measurement of the hæmoglobin value of the blood are certain other points in the technique. (1) The amount of blood used must be delivered with more accuracy than can be done rapidly from a pipette. Hence the introduction of the tap C and the conversion of the pipette into a burette. (2) It is not possible to put any fluid into the burette to prevent the clotting. It is found, however, that if the blood be withdrawn direct from the vessel (no cannula being used) and if the burette be quite clean and dry the operation of collecting the blood may be carried out successfully without clotting.

Anti-coagulant.—The only anti-coagulant used has been potassium oxalate. This is finely ground up and a few milligrammes are placed in the blood-gas bottle (which contains 2 c.c. of ammonia) after the ammonia is put into it. The blood then runs from the burette on to the crystalline powder and is rendered uncoagulable.

Rate of Flow.—The measurement of the rate of flow of the blood is made with the help of an assistant. When the meniscus of the blood passes the marks B and A on the burette the observer gives the word and the assistant presses a "knock down" key which is in the same circuit as a signal on the kymograph fitted with a Brodie's clock. Thus a record is obtained of the time taken to collect 1 or 2 c.c. of blood, as the case may be. It is sometimes desirable to get a record on the drum as the meniscus passes each 1/10 c.c. mark on the burette. Thus an estimate can be formed as to whether the blood flows at an even pace.

The Estimation of the Oxygen is carried out by the differential method; the form of apparatus most suitable is that described by Barcroft (2), though we have used the older forms of apparatus (3). One cubic centimetre of arterial blood and one of venous is used for each determination.

The Exudation from the Vessels.—The hæmoglobin value of the blood has, in one form or another, been used as an index of the fluid entering or leaving the blood by numerous observers—Leathes (4), Barcroft (5), Moussu, and Tissot (6). The adaptation of the principle which we have used is as follows:—

One cubic centimetre of blood from the burette is placed in each blood-gas bottle of the differential manometer. This is mixed in the process of gas analysis with 2 c.c. of ammonia solution (4 c.c. of strong ammonia per litre), the only substance

present being the few milligrammes of oxalate crystals which become dissolved. The water of crystallisation in these is so small as not to affect the estimation. The same quantity of oxalate is added to each bottle.

At the end of the blood-gas analysis there are 3 c.c. of fluid in each bottle, the fluid being blood diluted to exactly one-third of its original concentration ; 20 c.c. of the dilute ammonia are now added, making 23 c.c. of fluid in each bottle.

A standard hæmoglobin solution is now made by the dilution of 1 c.c. of the animal's defibrinated blood to 100 c.c. To this standard the bloods for estimation are compared as follows :—

Two test-tubes of the same bore are taken. Into one is put about 10 c.c. of the standard. Into the other 2·3 c.c. of the solution for estimation. This quantity contains 0·1 c.c. of the original blood. Water is added until the tint is the same as that of the standard. Suppose 7·7 c.c. of water is added, the volume of fluid in the test-tube would then be 10 c.c., which contains 1/10 c.c. of the blood. Thus the blood would have been diluted 100 times, the result indicating that the blood had neither lost nor gained water as compared with that from which the standard had been made up. The fluid in the bottles amply sufficed for eight determinations of this character on each sample of blood which we withdrew from the animal. This number we usually performed.

The method of titration, etc., was that described by Haldane (7), with the addition of two points :—

(1) Tap-water (Cambridge) is unsuitable for the titration, as the calcium present is precipitated by the oxalate already added to the blood, the fluid therefore becomes turbid.

(2) In carrying out the eight titrations we reversed the test-tubes after four of the titrations had been performed, placing the standard for the last four titrations in the test-tube which we had previously used for the fluid titrated.

A few words must be said concerning the accuracy of this method. In the first place some examples may be given, taken at random from our book, which will show the comparative accuracy of successive titrations of the same blood.

(a) Ammonia Solution added to 2·3 c.c. of Dilute Blood.

	1.	2.	3.	4.	5.	6.	7.	8.	Mean.
Arterial	7·8	7·7	7·6	7·7	7·6	7·8	7·7	7·8	7·71
Venous	8·0	8·0	7·9	8·0	8·0	7·9	7·9	8·0	7·93

There seems to be good ground for supposing that the accuracy of the mean of determinations of this kind is about 0·4 per cent. This point may be tested by adding known but small quantities of Ringer's solution to blood and attempting to estimate the amount added.

The following are some data of this character :—

TABLE I.—Hæmoglobin Value of Blood.

	Per cent.				
Calculated	100	99	98	97	96
Observed	100·3	99·2	97·9	96·6	96·2
Error	0·3	0·2	0·1	0·4	0·2

The question then arises, What difference in concentration exists between the arterial and venous bloods? Is it such that estimations which are correct to 0·4 per cent. can observe it? In the estimations which we have performed on muscle the hæmoglobin value of the venous blood has exceeded that of the arterial by over 4 per cent. in one case, by between 3 and 4 per cent. in five cases, between 2 and 3 per cent. in one case, between 1 and 2 per cent. in 20 cases, between 0 and 1 per cent. in four cases, and in no case has the arterial blood had the greater hæmoglobin value. It seems clear that in a qualitative test the hæmoglobin estimations demonstrate the loss of water from the blood. From the quantitative point of view, one can lay little stress on a single comparison, except in a few cases, but in order to be sure that one has a true picture one must ascertain whether the same features occur in successive experiments.

Stimulation.—The stimulation of the muscle was performed by a weak induction current. In the case of the digastric this was passed through the muscle from end to end; in the case of the gastrocnemius it was led into the nerve by Ludwig electrodes. In both cases the duration of the faradic current was 0·3 second, repeated each second for 15 minutes.

The muscles were fixed at each end so that the contraction was roughly isometric. No tracing was taken of the tension set up in the muscle.

The Oxygen used by Muscle.

Estimations of the oxygen used by resting and active muscle have formed the subject of numerous investigations, which practically started with those which took place in the laboratory of LUDWIG, but till recently those which were best conceived were those of CHAUVEAU and KAUFFMANN (8). These observers investigated the oxygen used by the muscles of the horse in two series of observations, one upon the masseter, the other on the levator labii superioris, always getting increase in the coefficient of oxidation.* Their experiments had the advantage of being extremely

* The term "coefficient of oxidation" was used by CHAUVEAU and KAUFFMANN to indicate the oxygen used, expressed in cubic centimetres per gramme of muscle per minute, and will be used in the same sense in this paper.

physiological in conception, for the activity was produced, not by any artificial method of stimulation, but by the natural one of giving the horse a feed of oats which he proceeded to chew.

Recently VERZÁR (1) in this laboratory carried out an excellent series of observations on the blood gases of the cat's gastrocnemius. His findings were somewhat different from those of CHAUVEAU and KAUFFMANN, for, although he always obtained an increased oxidation as the result of stimulation of the nerve, this increase did not always take place till after the stimulus had ceased; indeed, in several cases, VERZÁR got a decreased coefficient of oxidation whilst the muscle was actually in contraction. It should be observed that VERZÁR's stimulus was usually a faradic current of short duration; in his figure (p. 250) it is given as lasting 6 seconds; in some cases he used single induction shocks as a stimulus.

The Coefficient of Oxidation in Active Muscle.—The following Tables will show that we never obtained a decreased coefficient of contraction as the result of activity of the muscle. The difference between our experiments and VERZÁR's in this respect was no doubt due to the nature and duration of the stimulus which we employed. The reduction in the coefficient of oxidation which he observed was no doubt due to the fact that at times he got almost complete venous stasis (see fig. 6, also p. 251). Our samples collected after the muscle had settled down into its condition of activity always came more quickly during activity than during rest.

Having obtained the qualitative result that the coefficient of oxidation rises with activity, it is difficult to pass from that to any quantitative statement as to how much the oxygen consumption of the muscle increases for a given degree of activity. At present we can only point out some of the difficulties which must be overcome before any such calculation can be made. Perhaps the most fundamental of these centres about the coefficient of oxidation of resting muscles.

In order to estimate the effect of functional activity upon oxidation, two obvious courses present themselves—

(1) To measure the total oxidation actually taking place in the muscle.

(2) To measure excess of oxidation during the stimulation and post-stimulation periods, over that which occurs in the resting muscle.

Whichever of these methods we tried we found that we had no certain base line from which to work. It may here be well to anticipate what we are going to say later so far as to state two facts—

(1) That in common with all other observers we have found that the coefficient of oxidation in resting muscle is very different in different muscles; and

(2) That the increased oxidation caused by fifteen minutes' stimulation such as we have given may last for several hours, perhaps four or five.

Taking these facts together it seems quite probable that the oxidation which observers have been accustomed to regard as that of resting muscle is largely the after-effect of previous contractions, and in order to make any just estimate of

whether the muscle was a fit subject for estimation of the kind, one would have to know its history for some hours before the experiment took place.

To turn now to the facts which have led to the above conclusions. CHAUVEAU and KAUFFMANN obtained figures varying from 0·0082 to 0·0029 c.c. per gramme per minute in three experiments; VERZÁR, in 11 experiments, gives figures from 0·0086 to 0·0023.*

In five experiments on muscle not artificially stimulated we obtained data which varied from 0·055 to 0·0052. These we append, together with some remarks upon the extent to which the muscle could really be regarded as resting, to judge from its present and previous history.

TABLE II.

Experiment.	Muscle.	Oxygen used per gramme per minute.	Remarks.
		c.c.	
8	Digastric	0·030	The muscle had been stimulated a short time before the sample was taken.
9	Digastric	0·055	The muscle from time to time underwent rhythmic movements.
10	Gastrocnemius	0·017	Nerve cut just before.
11	Gastrocnemius	0·0052	Nerve cut 1 h. 40 m. before observation.
12	Gastrocnemius	0·010	Nerve cut 1 h. 5 m. before observation.

It will be clear that those muscles in which the nerve was cut some time previously have a lower coefficient of oxidation than those in which it was intact. We must qualify this statement by noting: (1) that the muscles were not the same—in the one case the gastrocnemius was used, in the other the anterior belly of the digastric—it may be that these two muscles have different coefficients of oxidation in any case; (2) that ZUNTZ describes mere cutting of the nerve as reducing the coefficient of oxidation by removal of the tone from the muscle. The experiments of ZUNTZ on the subject were few, and not altogether concordant. They were never completed, and it is very desirable that in the light of more recent work the matter should be taken up again.

The Extent and Duration of the Hyperoxidation due to Functional Activity.—Table III gives the results of five experiments which we performed on muscle.

The points which the five experiments have in common are:—

(1) The coefficient of oxidation rises during the stimulation.

(2) It remains high for some time after the stimulation.

(3) There is a well marked summit on the curve of oxidation in the period following the stimulation. The time at which this appears is rather variable, but it is often about an hour after the stimulus ends.

* We have excluded Experiment 6 in VERZÁR's paper.

TABLE III.—Coefficients of Oxidation of Stimulated and Unstimulated Muscle.

	Before stimulation.	During stimulation.		After stimulation.					
Experiment 8.									
Time . . .	20 m.	6 m.	—	19 m.	31 m.	1 h. 9 m.	2 h. 13 m.	—	—
Coefficient of oxidation .	0·030	0·13	—	0·040	0·055	0·030	0·013	—	—
Experiment 9.									
Time . . .	4 m.	5 m.	13 m.	20 m.	1 h. 15 m.	—	—	—	—
Coefficient of oxidation .	0·055	0·075	0·067	0·033	0·141	—	—	—	—
Experiment 10.									
Time . . .	10 m.	3 m.	15 m.	16 m.	1 h. 15 m.	3 h. 8 m.	—	—	—
Coefficient of oxidation .	0·017	0·082	0·041	0·022	0·041	0·006	—	—	—
Experiment 11.									
Time . . .	10 m.	3 m.	15 m.	16 m.	52 m.	1 h. 40 m.	2 h. 25 m.	3 h. 30 m.	4 h. 47 m.
Coefficient of oxidation .	0·0052	0·016	0·041	0·012	0·027	0·012	0·013	0·012	0·064
Experiment 12.									
Time . . .	17 m.	3 m.	14 m.	15 m.	1 h. 2 m.	2 h. 6 m.	3 h. 1 m.	4 h. 45 m.	5 h. 33 m.
Coefficient of oxidation .	0·015	0·044	0·049	0·080	0·087	0·13	0·070	0·043	0·050

The points in which the five experiments differ, or appear to do so, are :—

(1) The extent of the hyperoxidation : For instance, in Experiment 11, the oxidation rises during the period of stimulation about eight-fold (from 0·0052 to 0·041), and in the period subsequent to stimulation to about five-fold the resting value (0·0052—0·027) ; whilst in Experiment 9 the coefficient only rises from 0·055 to 0·075 during the stimulation, and it is not higher than 0·041 after the stimulation. I have here chosen the extreme cases partly because they illustrate the difficulty to which allusion has already been made. Though the rise in Experiment 9 is so slight, the actual coefficients of oxidation during the contractile and post-contractile periods are greater in Experiment 9 than in Experiment 11. The difference between the two experiments lies not in deficient oxidation during the contractile period but in the high coefficient which obtained in Experiment 9 during the initial unstimulated period, and this in turn was due, as shown in Table II, to a long maintained but slow rhythm which took place in the muscle whether it were artificially stimulated or not.

(2) The duration of the period of hyperoxidation : It lasted as follows :—

TABLE IV.

Experiment	8.	9.	10.	11.	12.
Time from end of stimulation till return of coefficient to value prior to stimulation	1 h. 9 m.	Under 35 m.	Between 1 h. 15 m. and 3 h. 8 m.	Between 3 h. 38 m. and 4 h. 47 m.	Over 5 h. 33 m.

Here again reference to Table III will show that these great apparent differences are largely due to the variable nature of the initial coefficient of oxidation. The striking fact is that in a muscle which has been prevented from contracting by cutting the nerve some 1 hour and 25 minutes before the estimations commenced, stimulation of a rhythmic nature once a second for 15 minutes produced a hyper-oxidation which persisted for at least five hours and a half.

In this experiment, estimations were made on the gaseous exchange in both gastrocnemii. In both cases the nerves were cut, but that of the left gastrocnemius was not stimulated. The results are given in Table V.

TABLE V.—Oxygen used by Muscle per gramme per minute.

	Time.		Right.	Left.
	h.	m.	c.c.	c.c.
Before cutting nerves	0	20	—	0·018
	0	18	0·017	—
After cutting nerves	1	5	—	0·01
	1	25	0·015	—
During stimulation (time measured from commencement of stimulation)	0	3	0·044	—
	0	15	0·049	—
After stimulation (time measured from end of stimulation)	0	15	0·08	—
	1	2	0·087	—
	1	11	—	0·016
	2	6	0·13	—
	3	1	0·070	—
	3	10	—	0·01
	4	44	0·043	—
	4	55	—	0·013
	5	34	0·050	—
	5	38	—	0·015

The General Form of the Oxidation Curve.—The form of the oxidation curve most commonly met with is that shown in fig. 4, which is a graphic representation of Experiment 11. The curve has two summits, one during and one after the stimulation. The second of these two forms an interesting problem. That the oxidation should go on after the activity ceases is intelligible enough, and is in line with all the work which has been done in Cambridge by FLETCHER (9), HOPKINS (10), HILL (11), WEIZSÄCKER (12), PARNAS (13), and others during the last 15 or 20 years. The view

of this school is that the oxidation which takes place in muscle primarily concerns itself with the replenishing of the store of potential energy caused by the contraction. If the muscle has been much exercised the oxidation does not keep pace with the chemical breakdown in the muscle, and lactic acid is found in the muscle, which, according to PARNAS, is oxidised, the alternative possibility being that it is rebuilt up into the muscle. PARNAS, indeed, calculates that 20 hours are necessary for the lactic acid in completely fatigued frog's muscle to be oxidised.

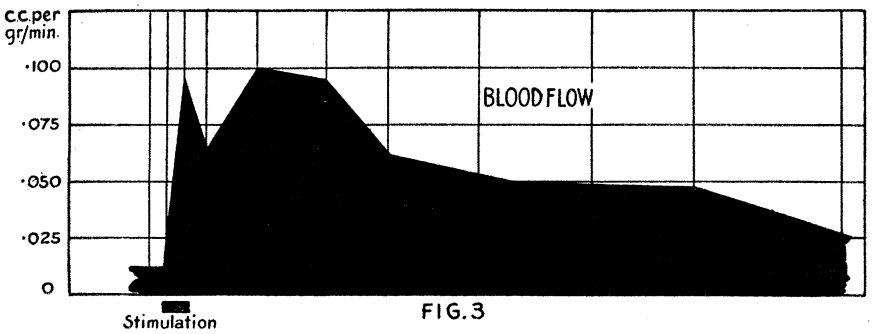

FIG. 3

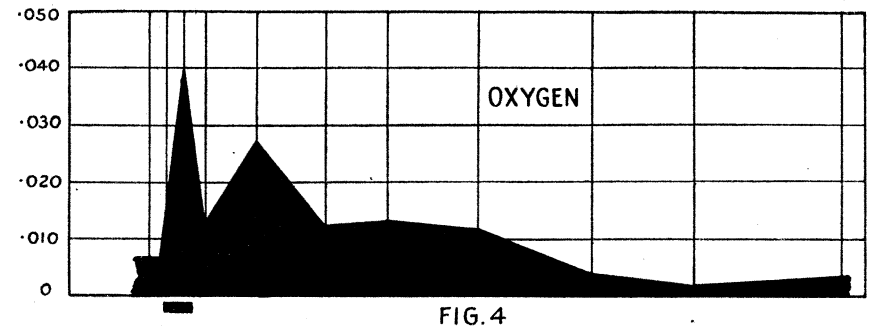

FIG. 4

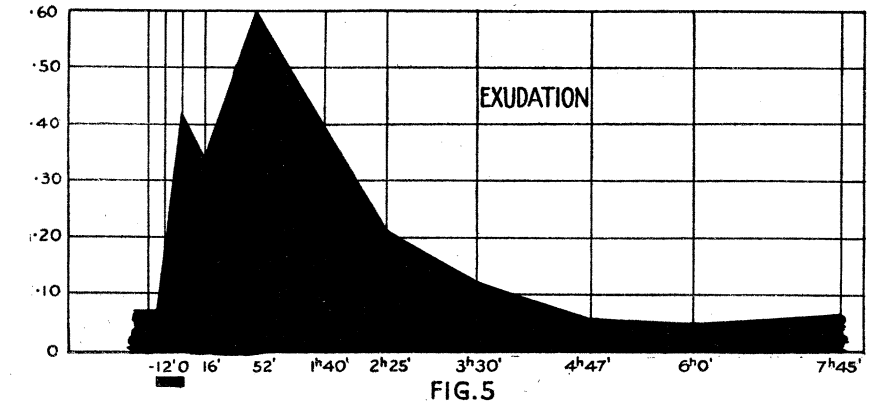

FIG. 5

FIGS. 3, 4, and 5 represent the blood-flow, coefficient of oxidation and exudation in Experiment 11. Ordinate in each case is cubic centimetres per gramme of muscle per minute. Abscissa = time measured relative to the end of the stimulus. The signal represents the period of stimulation.

That the acid is formed in mammalian muscle, even in tetani of a few seconds duration, was demonstrated by VERZÁR, who estimated the change in reaction of the blood which left it.

One must picture the lymph in the muscle and the muscle itself in Experiment 11

as being charged at the end of the stimulation with a store of acid which awaits oxidation. But one might reasonably expect that the oxidation would gradually diminish in degree in proportion as the concentration of acid to be oxidised became reduced. How, then, is the secondary summit to be accounted for? The suggestion which we put forward tentatively is based on the known fact that some oxidising ferments (*e.g.* tyrosinase) are themselves very sensitive to changes in hydrogen ion

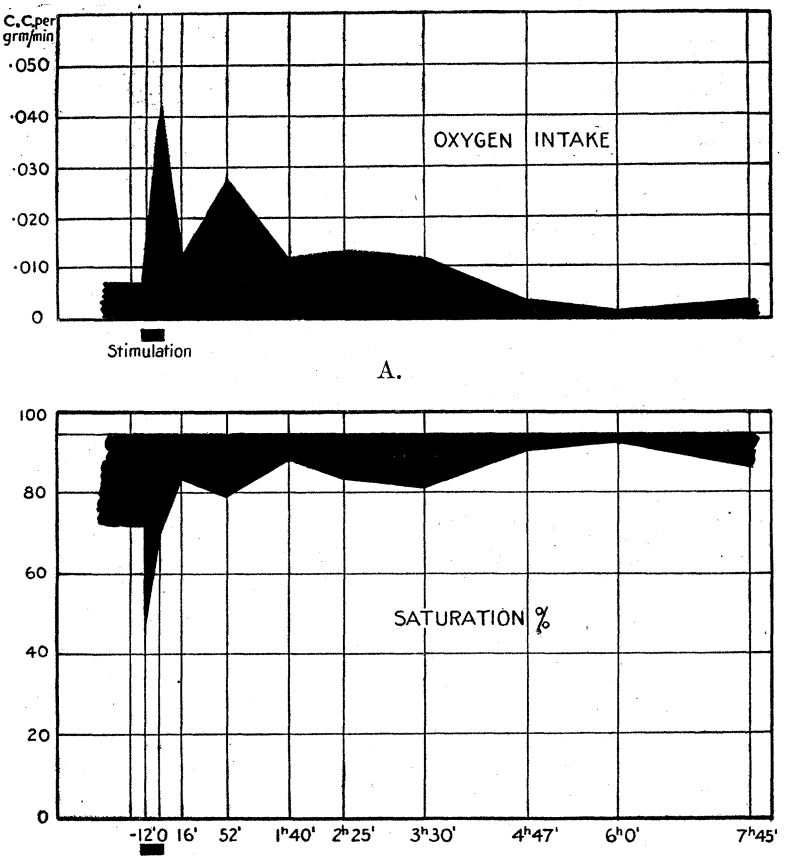

FIG. 6.—A, oxygen intake of muscle in Experiment 11; B, percentage saturation of blood with oxygen, the top of the black area representing the percentage saturation of the arterial blood and the bottom the venous blood. Abscissa, time as in figs. 3, 4, and 5. Signal = period of stimulation.

concentration, being inhibited by increased hydrogen ion concentration and accelerated when the acid is more dilute, and the same may be true of similar ferments in muscle. We suggest, then, that during the contraction the hydrogen ion concentration in the muscle increases, and that immediately after the contraction the oxidising ferments are retarded in their action. The acid now becomes less concentrated, to which end three factors work:—

(1) A portion becomes oxidised.
(2) A portion is removed in the blood (VERZÁR).
(3) The acid is diluted by lymph (see the later portion of this paper).

The decreased concentration of hydrogen ions accelerates the action of the muscle oxidases, even though they have less lactic acid to work upon.

If the line of argument which we have just indicated is correct, it is clear that the more the oxygen supply to the muscle is curtailed the more will the oxidation be deferred, causing the primary summit to bulk smaller relatively to the secondary one, and this seems to be so, as is shown by a comparison of Experiments 11 and 12.

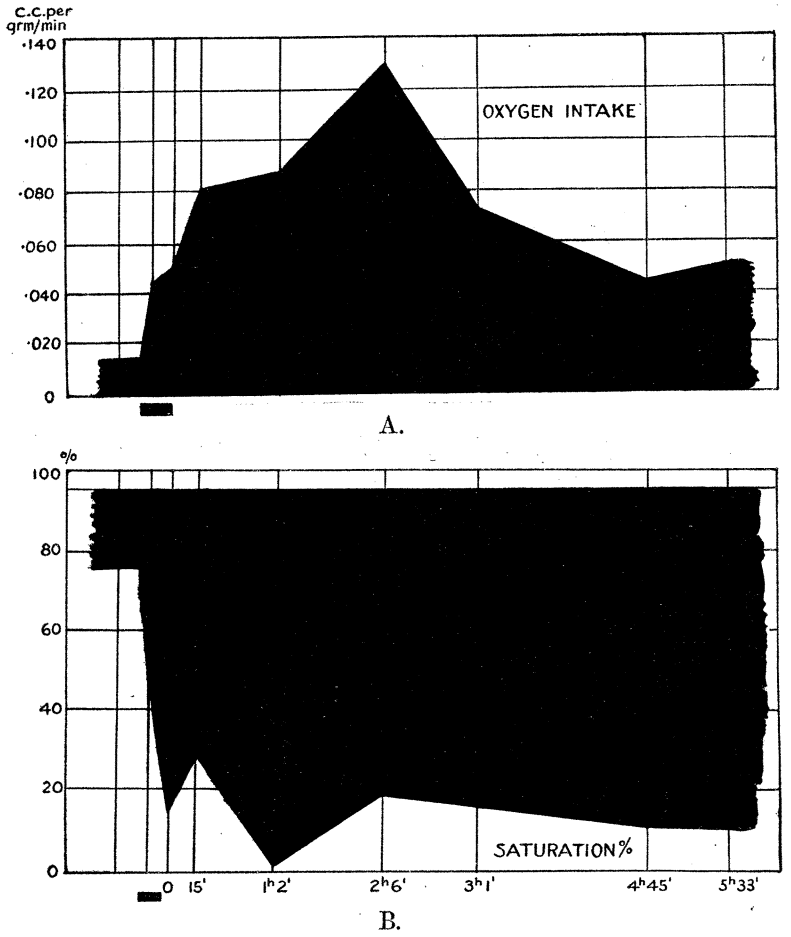

FIG. 7.—Experiment 12, data figured as in fig. 6.

The point is most easily seen in figs. 6 and 7, in which are shown (1) the oxygen used and (2) the percentage saturation of the blood. In Experiment 11, in which the venous blood was red throughout the stimulation, being 70 per cent. saturated with oxygen at the end, the bulk of the oxidation takes place much earlier in the experiment than in Experiment 12, in which the venous blood was black, being about 15 per cent. saturated at the end of stimulation, and the oxidation is much deferred.

The Rate of Blood Flow in Muscle.

In every experiment the rate of blood flow through the muscle has been increased as the result of functional activity. This is so during the period of activity in every

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TABLE VI.—Rate of Blood Flow through Muscle.

	Before stimulation.		During stimulation.		After stimulation.							
Experiment 8.												
Time	20 m.		6 m.	—	19 m.	31 m.	1 h. 9 m.	2 h. 13 m.				
Rate of flow	0·44		1·1	1·1	1·0	0·73	0·57	0·25				
Experiment 9.												
Time	4 m.		5 m.	13 m.	20 m.	1 h. 15 m.						
Rate of flow	0·31		0·35	0·32	0·15	0·18						
Experiment 10.												
Time	10 m.		3 m.	15 m.	16 m.	1 h. 15 m.	—	3 h. 8 m.				
Rate of flow	0·05		0·124	0·093	0·087	0·061	—	0·021				
Experiment 11.												
Time	10 m.		3 m.	15 m.	16 m.	52 m.	1 h. 40 m.	2 h. 25 m.	3 h. 30 m.	4 h. 47 m.	6 h. 0 m.	7 h. 45 m.
Rate of flow	0·014		0·021	0·095	0·063	0·10	0·095	0·063	0·093	0·048	0·048	0·026
Experiment 12.												
Time	2 h. 7 m.	17 m.	3 m.	14 m.	15 m.	1 h. 2 m.	2 h. 6 m.	3 h. 1 m.	4 h. 46 m.	5 h. 33 m.		
Rate of flow	0·03	0·025	0·03	0·025	0·04	0·038	0·069	0·038	0·021	0·025		

experiment but one (in none has there been a falling off during activity as in the shorter experiments of VERZÁR (1)), the rate of flow has usually outlasted the contractile period by a considerable time. This is best seen in those experiments in which the nerve was cut some time previously to the stimulation (Experiments 11 and 12) in which the hyperæmia lasted in the one case for over seven hours, in the other for over three, after the stimulation of 15 minutes had ceased.

It is scarcely necessary now to discuss the question of whether metabolites in an organ produce a sustained dilatation of the vessels. Our first appreciation of their importance was derived from GASKELL, who, in his lectures, urged that such a local chemical mechanism existed. In recent years it has been amply demonstrated in the alimentary canal by BAYLISS and STARLING (14), in the coronary vessels by DIXON and BARCROFT (15 and 16), and by STARLING and MARKWALDER (17), and in the submaxillary gland by BARCROFT and PIPER (16 and 18), but it is doubtful whether up to the present time any such effect, at once so striking and so sustained, has been demonstrated as that which took place in Experiment 11.

The full extent of the dilatation in experiments can best be appreciated when account is taken not only of the magnitude of the blood flow but of the nature of the blood which emerged from the vein. On the assumption that the arterial flow was 95 per cent. saturated, it is possible to calculate from the data which we have the percentage saturations of the venous flow. The data are given in Table VII, from which it appears—

(1) That the percentage saturation, even during the stimulation, when the muscle was using up most oxygen, was never under 50.

(2) That in the subsequent period it was usually over 80 per cent. saturated.

(3) After the hyperoxidation had passed off, it reached 90 per cent.

These figures are of course rough, nevertheless the picture of blood indistinguishable in tint from that in the artery, and rushing out of the muscle at four times its ordinary rate, was a striking one. Occurring as it did 4–5 hours after the exercise had been taken, it was a remarkable tribute to the activity of metabolic products.

This extreme redness of the blood cannot be regarded as a constant phenomenon. Experiment 12, for instance, contrasts strongly with Experiment 11 in this respect (see Table VIII and fig. 7).

The Exudation from the Vessels in Muscle.—The water which disappears from the blood may either accumulate in the muscle and swell it or it may leave the muscle *vid* the lymphatics.

The work of the earlier observers led to the general conclusion that the lymph flow from muscle at rest is very small, if, indeed, it is existent at all, but that it may be either induced or demonstrated by massaging of the muscle. When the muscle undergoes a series of rhythmic contractions the conditions are better calculated to produce a flow. To get at the root of the matter it is necessary to go to the blood

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TABLE VII.

	Before stimulation.	During stimulation.	After stimulation.							
Experiment 11.										
Time	10 m.	3 m.	15 m.	16 m.	52 m.	1 h. 40 m.	2 h. 25 m.	3 h. 30 m.	4 h. 47 m.	6 h. 0 m.	7 h. 45 m.
Blood flow	4·6	6·7	30	20	32	30	20	17	15	15	8·5
Percentage saturation of venous blood with oxygen	74	51	70	84	79	88	83	81	90	93	87
Oxygen in arterial blood	0·190 c.c.										
Oxygen in venous blood	0·147	0·102	0·139	0·168	0·158	0·176	0·165	0·163	0·180	0·186	0·173

TABLE VIII.

	Before stimulation.	During stimulation.	After stimulation.						
Experiment 12.									
Time	17 m.	3 m.	14 m.	13 m.	1 h. 2 m.	2 h. 6 m.	3 h. 1 m.	4 h. 45 m.	5 h. 33 m.
Blood flow	10	12	10	20	15	27·5	15	8·5	10
Percentage saturation of venous blood with oxygen	76	51	15	29	1	19	17	11	11
Oxygen in arterial blood	0·214 c.c.								
Oxygen in venous blood	0·171	0·115	0·034	0·065	0·002	0·043	0·031	0·025	0·025

and to ascertain whether under such circumstances there really is an increased exudation from the vessels during activity, or whether as the alternative there is merely a mechanical propulsion of fluid already in the lymph spaces along the lymphatics.

Our experiments indicate :—(1) A trifling exudation from the vessels of the resting muscle in each case ; reckoned per gramme of muscle it is as follows :—

		Exudation per gramme per minute.	
Experiment	8	0·006 c.c.	nerve intact.
,,	9	0·005 ,,	
,,	10	0·003 ,,	nerve cut.
,,	11	0·004 ,,	nerve cut 1 h. 40 m. previously.

Little stress could be laid on any one of these figures, but taken together they suggest a positive result, being all of the same order of magnitude.

(2) That the exudation from the vessels is increased during contraction of the muscle. It is perhaps worth recording that the exudation rapidly reaches its full value, being as great in the first observation on the stimulated muscle, usually about 3 minutes from the commencement of the stimulus, as later.

(3) Like the oxygen consumption and the blood flow, this hyperexudation continues long after the contractile period has passed off. These facts are shown in fig. 5. The more theoretical discussion of the relation of lymph flow to functional activity may be postponed till we have considered the exudation in the submaxillary gland.

Our experiments are concordant in that they show an increase of exudation in every case accompanying and long outlasting the activity of the muscle.

Having established the fact that there is always an increase of exudation when the muscle contracts, let us return to the question of what becomes of the fluid.

In Experiment 11, of which we have the most complete data, a rough calculation may be made of the total amount of exudation which took place. In the eight hours which elapsed from the commencement of the stimulation till the end of the experiment the exudation amounts to about 100 c.c. of fluid. In order to test whether this had all left by the lymphatics or whether the muscle had become œdematous, we weighed the two muscles of the right and left side, the right side being the one which had been the subject of our experiment, with the following result :—

Experiment 11.—Weight of right gastrocnemius after exercise 7 h. 45 m.	38·2	grm.
Weight of left gastrocnemius unexercised .	31·6	,,
	6·6	,,
Difference per cent. of unexercised muscle .	21	per cent.

A similar, though less marked, difference of weight occurred in Experiment 12, where at the end of $5\frac{1}{2}$ hours there was a balance in favour of the stimulated muscle

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TABLE IX.

	Before stimulation.	During stimulation.		After stimulation.							
Experiment 8.											
Time	20 m.	6 m.	8 m.	19 m.	31 m.	1 h. 9 m.	2 h. 13 m.				
Exudation per minute .	0·10	0·29	0·27	0·20	0·13	0·13	0·04				
Exudation per gramme per minute	0·006	0·019	0·018	0·013	0·012	0·012	0·003				
Hæmoglobin ratio of venous to arterial blood	1·015	1·017	1·016	1·013	1·012	1·015	1·011				
Experiment 9.											
Time	4 m.	5 m.	13 m.	20 m.	1 h. 15 m.						
Exudation per minute .	0·04	0·097	0·094	0·037	0·051						
Exudation per gramme per minute	0·005	0·012	0·012	0·005	0·006						
Hæmoglobin ratio . .	1·019	1·034	1·034	1·030	1·033						
Experiment 10.											
Time	10 m.	3 m.	14 m.	16 m.	1 h. 15 m.	3 h. 8 m.					
Exudation per minute .	0·28	5·2	3·2	3·2	1·00	0·14					
Exudation per gramme per minute	0·003	0·054	0·033	0·033	0·010	0·015					
Hæmoglobin ratio . .	1·006	1·046	1·036	1·038	1·017	1·007					
Experiment 11.											
Time	10 m.	3 m.	15 m.	16 m.	52 m.	1·40	2·25 m.	3·30 m.	4 h. 47 m.	6 h. 0 m.	7 h. 45 m.
Exudation per minute .	0·06	0·19	0·42	0·34	0·61	—	0·21	0·12	0·06	0·05	0·10
Exudation per gramme per minute	0·002	0·006	0·014	0·011	0·020	—	0·007	0·004	0·002	0·002	0·003
Hæmoglobin ratio . .	1·010	1·028	1·014	1·017	1·019	—	1·012	1·008	1·004	1·003	1·014

of 6 per cent. It is, of course, necessary to discount any possible difference which there might have been before the experiment took place, as the muscles might have been different to start with. This may be accomplished in more than one way. The method which occurred to us on the spot was to test the specific gravities of the muscles. If the right muscle was œdematous it would have a correspondingly less specific gravity; if the difference in size was merely accidental, the specific gravities of the two muscles might reasonably be expected to be the same.

Specific Gravity of Exercised and Unexercised Muscles.—Our method of testing the specific gravity was to weigh the two muscles in mammalian Ringer's solution and to compare their values with the weight in air. The length of time necessary in order to weigh a muscle of 38 grm. in isotonic salt solution is so small and the size of the muscle so great that no appreciable error creeps in from the inhibition of the saline during the process of weighing; to test this the muscle was weighed three times. The specific gravities were as follows:—

Experiment 10.—Specific gravity of muscle exercised 7 h. 45 m. previously	1062
Specific gravity of control unexercised muscle .	1073

We may now calculate how much fluid must be absorbed by a muscle 31·6 grm. in weight to change its specific gravity from 1062 to 1073. This would be 5·3 c.c., and would therefore raise the weight of the muscle from 31·6 to 36·9, a figure fairly close to the actually observed weight of the stimulated muscle.

The œdema which we have just described as resulting from stimulation is so readily observed in frogs, that it seemed simplest to treat its investigation in that way. This work was undertaken by Miss Back and Miss Cogan, of Newnham College, and Miss Towers, of Girton; a preliminary account of their results was published in the 'Proceedings of the Royal Society' (19).

Change in Length and Appearance.—The difference between the exercised and the unexercised muscle is quite evident to the naked eye (fig. 7A); the exercised muscle was shorter (3·75 as opposed to 4·0 inches in length) and of greater circumference, as well as being heavier. Moreover, the feel of the muscle was different, it felt firmer than the control muscle.

Summarising then the changes which are observed, they are that the exercised muscle is (1) heavier, (2) of smaller specific gravity, (3) shorter, (4) of greater girth, (5) stiffer, in the objective sense of the word.

"According to Ranke, the amount of solid matter in muscle undergoes a diminution when muscles are tetanised, so that there appears to be a relative increase in water" (20). Changes in the water content of muscle have been observed in excised frog's muscle, which, after being thrown into tetanic contractions, are then placed in hypotonic salt solution. Until the work of Fletcher (21), opinion was divided as to whether muscle which had been tetanised swelled more or less

than control muscle when placed in hypotonic salt solution. FLETCHER showed, by a continuous series of observations, (1) that the tetanised muscle tended first to swell more than the control, but also it tended to return sooner to its normal size, (2) that this effect was dependent upon exclusion of oxygen from the muscle, for if two gastrocnemii were tetanised, and one exposed to oxygen, it swelled at first more slowly than the anaërobic one, but it continued to swell after its fellow had begun to shrink.

The point to which we wish to draw attention is not that fatigued muscle swells when placed in an isotonic medium, but that muscle swells when stimulated in the body, with a plentiful supply of oxygen, and under the most favourable vascular conditions, and that this swelling may be great seven hours after the stimulation.

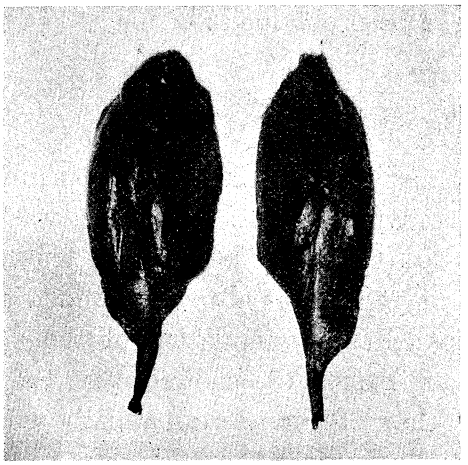

FIG. 7A.—Left (unexercised) and right (exercised) gastrocnemii of dog in Experiment 11.

Moreover, it swells to such an extent as to alter the properties of the muscle, and to give rise to an objective tenseness in the muscle, and probably to a degree of tension sufficient to cause the feeling of discomfort which we call "stiffness." (These properties are at present the subject of observations in this laboratory.)

The vascular conditions in Experiment 11 have already been described, and stress was laid upon the rapid blood flow and the redness of the blood in the veins. It is clear that the muscle was adequately supplied with oxygen throughout the whole experiment subsequent to the stimulation (it is probable that during extensive stimulation muscle always suffers more or less from oxygen want). In this connection it is of great interest to observe that FLETCHER observed the most persistent and extreme œdema, with a rich supply of oxygen, and probably our experiments are somewhat analogous.

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The Submaxillary Gland.

The Oxygen used by the Submaxillary Gland.

In the case of the submaxillary gland we made determinations of—

(1) The oxygen used by the gland.
(2) The rate of flow of blood through the gland.
(3) The exudation from the vessels.
(4) The flow of saliva.

The coefficient of oxidation of the resting submaxillary gland, *i.e.*, the gland with both the chorda tympani and the sympathetic severed, appears to be much more constant than the coefficient of oxidation of uncontracting muscles.

The following figures have been obtained :—

Experiment	2	3	4	5	6	7
	0·023	0·026	0·027	0·01	0·020	0·024

Experiments by one of us showed that the submaxillary gland used up more oxygen when secreting than when at rest. This fact was confirmed for the parotid gland by Moussu and Tissot (6). This is not only true of the gland when under the influence of the chorda tympani but also when under the influence of adrenalin, which presumably is equivalent to stimulation of the sympathetic. The further researches of Barcroft and Piper (18) showed that the duration of the period of oxidation is greater than that of the secretion both when the chorda tympani is stimulated and when adrenalin is given.

In most of the experiments carried out in the present series of observations, pilocarpine was used to cause the flow of saliva, our wish being to maintain as steady a flow as possible over a considerable period of time.

The flow of saliva therefore is not one of a few seconds' or minutes' duration, as in the case of former experiments, but is maintained for some hours, though not at a constant rate.

It is most rapid at first and falls off gradually after the first few minutes, until at last it ceases.

The most marked feature about the oxygen consumption of the gland is that the coefficient of oxidation is maintained not only relatively to the flow of saliva, but absolutely, for some hours, indeed, there may be a summit on the curve of oxygen consumption long after the saliva has reached its maximal rate of flow.

The following data show the duration of a high coefficient of oxidation :—

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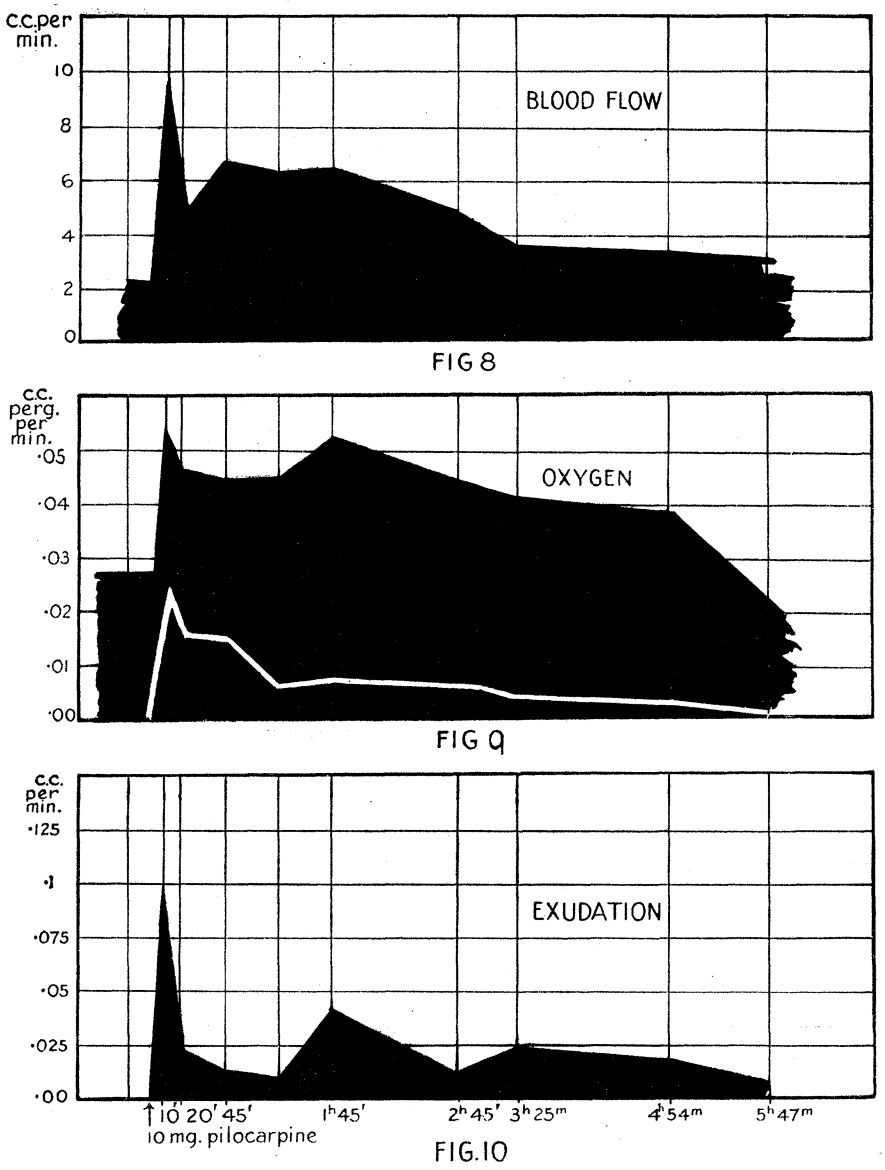
TABLE X.

	Before.	After.							
Experiment 3, dog, 18 kgrm.									
Time relative to injection of 10 mgrm. of pilocarpine	15 m.	5 m.	15 m.	40 m.	1 h. 10 m.	1 h. 40 m.	2 h. 40 m.	3 h. 25 m.	4 h. 49 m.	5 h. 52 m.
Coefficient of oxidation . .	0·026	0·054	0·045	0·044	0·044	0·052	—	0·42	0·038	0·022
Experiment 4, dog, 8·5 kgrm.										
Time relative to injection of 5 mgrm. of pilocarpine	10 m.	5 m.	16 m.	50 m.	1 h. 20 m.	2 h. 12 m.	2 h. 50 m.			
Coefficient of oxidation . .	0·027	0·063	0·063	0·051	0·033	0·039	0·015			
Experiment 5, dog, 5 kgrm.										
Time relative to injection of 3 mgrm. of pilocarpine	8 m.	—	18 m.	36 m.	55 m.	1 h. 18 m.	1 h. 43 m.			
Coefficient of oxidation . .	0·010	—	0·047	0·045	0·078	0·040	0·020			
Experiment 6, dog, 20 kgrm.										
Time relative to injection of 10 mgrm. of pilocarpine	25 m.	6 min.	27 m.	—	56 m.	1 h. 20 m.	2 h. 20 m.	3 h. 1 m.	4 h. 38 m.	
Coefficient of oxidation . .	0·020	0·082	0·063	—	0·063	0·063	0·058	0·043	0·036	

The curve of oxidation in Experiment 3 is shown in fig. 9, together with the rate of flow of saliva. It will be seen to have two maxima, one shortly after (within 10 minutes of the injection of the pilocarpine), the other about an hour and a half



FIGS. 8, 9, 10.—Blood Flow, Coefficient of Oxidation, and Exudation respectively; the white line in fig. 9 shows the saliva, to which corresponds the scale on the right-hand side.

subsequently. The actual presence of the second summit, whilst interesting enough in itself, is less important than the fact that the coefficient of oxidation rises steadily as compared to the amount of saliva secreted per minute, throughout the whole course of the experiment, as is shown in fig. 11.

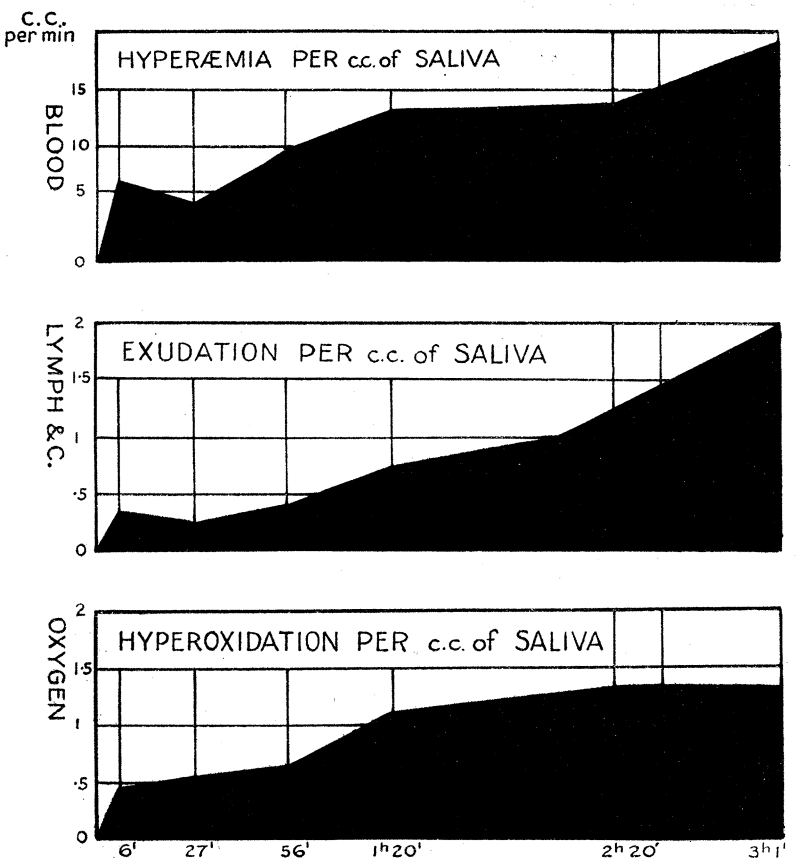


FIG. 11.—Showing Relation of Hyperæmia, Exudation, and Oxidation to Saliva in Experiment 3.

The Rate of Blood Flow through the Gland.

The quantities of blood which emerge from the gland per minute are given in Table XI. It must be borne in mind that these are not the quantities which pass through the submaxillary artery. In order to get a more adequate idea of the quantities of blood which enter the gland it is necessary to correct these figures for the water which leaves the blood by other channels: saliva, lymph, etc. This of course is done, as in the case of muscle, by hæmoglobin determinations.

Table XII contains: (1) the quantity of venous blood which leaves the gland, (2) the ratio of the hæmoglobin values of the arterial and venous bloods, and (3) the deduced volume of arterial blood which enters the gland.

The figures, whether for the arterial or venous blood, indicate an immediate and great increase in the blood flow (shown in figs. 8 and 12) about five minutes after the pilocarpine is given, followed by a second summit about an hour after the drug, and that the dilatation is maintained at a high level for a number of hours.

If this dilatation be compared to the saliva (fig. 12), it will be seen that the first summit corresponds to the quickest flow of saliva, but there is no summit on the salivary curve corresponding to the second summit on the curve of blood flow. In

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TABLE XI.—Blood Flow, in cubic centimetres, emerging from the Vein per gramme per minute.

	Before injection.	After injection of pilocarpine.							
Experiment 3.									
Time relative to injection of 10 mgrm. of pilocarpine	15 m.	5 m.	15 m.	40 m.	1 h. 20 m.	1 h. 40 m.	2 h. 40 m.	3 h. 25 m.	4 h. 49 m.	5 h. 52 m.
Blood flow per gramme . .	0·22	0·97	0·47	0·65	0·58	0·58	0·47	0·36	0·33	0·33
Experiment 4. Dog, 8·5 kgrm.										
Time relative to injection of 5 mgrm. of pilocarpine	10 m.	5 m.	16 m.	50 m.	1 h. 20 m.	2 h. 12 m.	2 h. 50 m.			
Blood flow per gramme . .	0·27	0·77	0·29	0·41	0·54	0·38	0·22			
Experiment 5. Dog, 5 kgrm.										
Time relative to injection of 3 mgrm. of pilocarpine	8 m.	—	18 m.	36 m.	55 m.	1 h. 18 m.	1 h. 45 m.			
Blood flow per gramme . .	0·15	—	0·28	0·28	0·36	0·30	0·34			
Experiment 6. Dog, 20 kgrm.										
Time relative to injection of 10 mgrm. of pilocarpine	25 m.	6 m.	27 m.	—	56 m.	1 h. 20 m.	2 h. 20 m.	3 h. 1 m.	4 h. 38 m.	
Blood flow per gramme . .	0·17	0·92	0·46	—	0·73	0·62	0·515	0·474	0·245	

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TABLE XII.—Data of Arterial and Venous Blood Flows, in cubic centimetres per minute.

	Before injection.	After injection of pilocarpine.								
Experiment 3.										
Time	15 m.	5 m.	15 m.	40 m.	1 h. 20 m.	1 h. 40 m.	2 h. 40 m.	3 h. 25 m.	4 h. 49 m.	5 h. 52 m.
Venous blood	2·3	10·0	4·8	6·7	6·0	6·0	4·8	3·7	3·4	3·4
Hæmoglobin ratio	1·0	1·26	1·156	1·091	10·62	1·113	1·076	1·104	1·100	1·002
Arterial blood	2·3	12·6	5·69	7·31	6·37	7·41	5·16	4·08	3·74	3·41
Experiment 4.										
Time	10 m.	5 m.	16 m.	50 m.	1 h. 20 m.	2 h. 12 m.	2 h. 50 m.			
Venous blood	0·27	0·77	0·29	0·41	0·54	0·38	0·22			
Experiment 5.										
Time	8 m.	—	18 m.	36 m.	55 m.	1 h. 18 m.	1 h. 43 m.			
Venous blood	0·5	—	0·93	0·93	1·2	1·0	1·1			
Hæmoglobin ratio	0·998	—	1·401	1·309	1·235	1·116	1·020			
Arterial blood	0·5	—	1·29	1·20	1·52	1·12	1·14			
Experiment 6.										
Time	25 m.	6 m.	—	27 m.	56 m.	1 h. 20 m.	2 h. 20 m.	5 h. 1 m.	4 h. 38 m.	
Venous blood	1·4	7·5	—	6·8	6·0	4·6	3·8	3·5	2·0	
Hæmoglobin ratio	—	1·118	—	1·205	1·134	1·104	1·123	1·112	1·027	
Arterial blood	—	9·3	—	4·59	6·80	5·08	4·25	3·9	2·0	

fact the blood flow, like the coefficient of oxidation, rises relatively to the flow of saliva up to the end of the experiment.

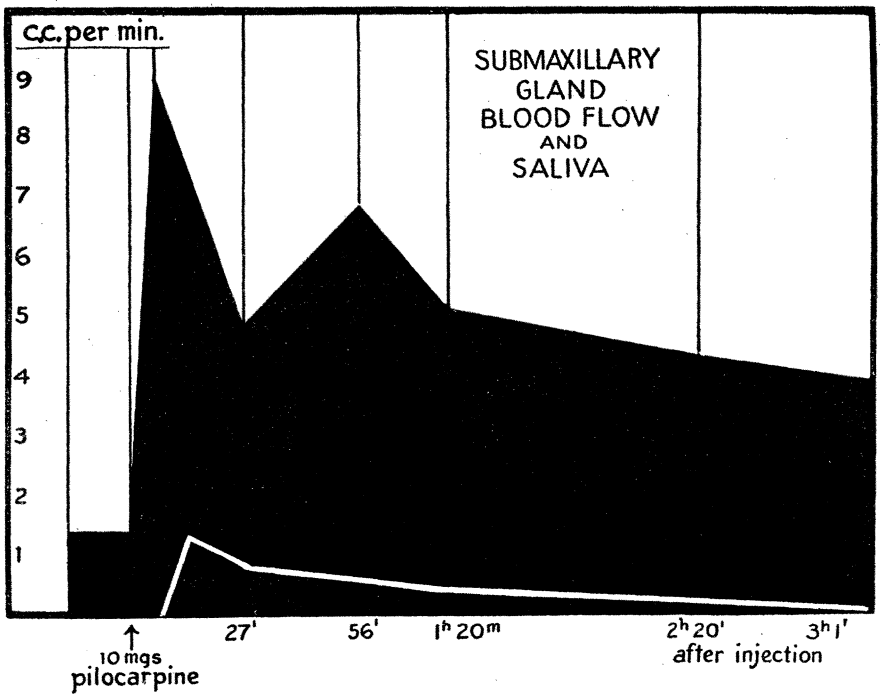

FIG. 12.—Comparison of blood flow and saliva, both plotted on the same scale, in Experiment 6.

The Exudation from the Vessels.

The amount of water which leaves the blood through the vessel walls may be deduced directly from Table XII.

It is only necessary to subtract the volume of venous blood from that of the arterial blood. This is done in Table XIII.

The total exudation consists in three things: (1) The water of the saliva, (2) the lymph, (3) any water which may cause swelling in the gland. We have made no attempt in the case of the salivary gland to judge of the change in size of the gland which results from stimulation of the chorda nerve-ending with pilocarpine. That some change takes place is probable from the experiments of BUNCH, but we have assumed that it is small as compared with the lymph flow, and therefore provisionally we may regard the water which does not go to form saliva as going to form lymph.

The quantity of lymph secreted by the glands in the resting condition per gramme of gland per minute is as follows:—

Experiment	1	2	3	4	5	6	7
Lymph per gramme per minute .	—	0·001	0·000	0·000	0·000	—	0·01

These results are just on the verge of what is discernible, taken together they seem to justify us in the view that the submaxillary gland, with both its nerves cut

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TABLE XIII.—Data of Lymph and Saliva leaving Gland, in cubic centimetres per minute.

	Before injection.	After injection.								
Experiment 3.										
Time	15 m.	5 m.	15 m.	40 m.	1 h. 20 m.	1 h. 40 m.	2 h. 40 m.	3 h. 25 m.	4 h. 49 m.	5 h. 52 m.
Total exudation	0	2·6	0·89	0·61	0·37	0·41	0·36	0·38	0·34	0·01
Saliva	0	1·37	0·68	0·63	0·30	0·35	0·32	0·21	0·19	0·09
Lymph	0	1·23	0·21	−0·02	0·07	0·06	0·04	0·17	0·15	—
Experiment 5.										
Time	8 m.	—	18 m.	36 m.	55 m.	1 h. 18 m.	1 h. 43 m.			
Total exudation	0	—	0·36	0·27	0·32	0·12	0·04			
Saliva	0	—	0·26	0·19	0·10	0·01	0·00			
Lymph, etc.	0	—	0·10	0·08	0·22	0·11	0·04			
Experiment 6.										
Time	25 m.	6 m.	—	27 m.	56 m.	1 h. 20 m.	2 h. 20 m.	3 h. 1 m.	4 h. 38 m.	
Total exudation	0	1·8	—	0·79	0·80	0·48	0·45	0·4	0	
Saliva	0	1·0	—	0·63	0·54	0·28	0·21	0·13	0·05	
Lymph, etc.	0	0·8	—	0·16	0·26	0·20	0·23	0·27	0	
Experiment 7.										
Time	25 m.	—	15 m.	28 m.	48 m.	1 h. 45 m.	2 h. 56 m.			
Total exudation	0·06	—	0·09	0·33	0·57	0·39	0·30			
Saliva	—	—	0·24	0·18	0·13	0·06	0·03			
Lymph, etc.	0·06	—	0·13	0·15	0·44	0·33	0·27			

and unstimulated, gives rise to no appreciable secretion of lymph. This result is in accordance with the observations of some previous workers.

Starting then with the observation that the lymph flow at rest is immeasurably small, within the first five minutes after the administration of the pilocarpine it reaches its maximal value. In the case of Experiment 3 this amounted to 1·23 c.c. per minute, in Experiment 6 to 0·8 c.c., or approximately 0·12 and 0·10 c.c. per gramme of gland per minute.

But the more interesting feature is that, whilst the lymph flow falls off considerably at the end of 15 minutes, it rises again and reaches a second summit, which is not so high as the first, at the end of about an hour and is well maintained, so that the relation of the volume of lymph secreted to that of the saliva secreted is continually rising (see fig. 11). Thus the lymph flow, which at the commencement of the secretion is considerably smaller in quantity than the salivary flow, being in general about one-third in the first 15 minutes, exceeds the salivary flow in Experiment 3 at the end of 80 minutes; Experiment 5, 55 minutes; Experiment 6, 140 minutes; and Experiment 7, 48 minutes.

The data which have been given above show, as is well seen in figs. 8, 9, 10 and 11, that the time relations of hyperæmia, the oxidation, and the lymph formation have certain properties in common as compared with the time relations of the salivary flow. We may now attempt some discussion of the relation of these factors to one another.

The Relation of the Increased Oxidation to the Salivary Flow.

So far as the actual stimulation of the gland by the pilocarpine is concerned, there is every reason to believe that this gradually diminishes in extent as time goes on, and there is no reason therefore to suppose that the increased oxidation, say an hour after the drug is given, is due to a recrudescence of the stimulation. Were such the case it would probably be accompanied by an increased flow of saliva.

Nor can the increased oxidation be attributed to increased blood flow, for it has been shown by Barcroft and Franz Müller (23) that the blood stream may be augmented within very wide limits without any measurable increase in the amount of oxygen taken up by the gland.

When we discussed the kindred problem with regard to muscle we attributed the delayed oxidation in muscle to the necessity of restoring the potential energy in the muscle to its original level. No doubt the same is true of the submaxillary gland, for it has been shown, both in the case of adrenalin secretion and direct stimulation of the chorda tympani, that for some time after the flow of saliva has ceased, the oxidation is maintained. Nevertheless it is possible that in the present case, where we are dealing with a gradually diminishing flow, that this is not the whole story.

It was shown by Heidenhain and others (24) that the more rapid the secretion

of saliva the richer is the saliva in salts. As the saliva is always poorer in salts than the blood, this amounts to a statement that the more rapid the flow of saliva the more closely does it approximate to the blood in saline concentration, and the less work therefore has to be performed in the secretion of each cubic centimetre of the fluid so far as this particular factor is concerned.*

We may therefore attribute the relatively heightened oxidation (1) to a true hysteresis, and (2) to an increased work done per cubic centimetre of saliva in the later periods.

The Cause of the Vascular Changes.—The feature of most interest on the curve of blood flow is the secondary maximum which appears, usually at its height about an hour after the injection of the pilocarpine. Our first idea about this was that it was due to a change in the general arterial pressure. This turned out to be quite erroneous, as was shown by Experiment 4. Fig. 13 shows portions of the tracing

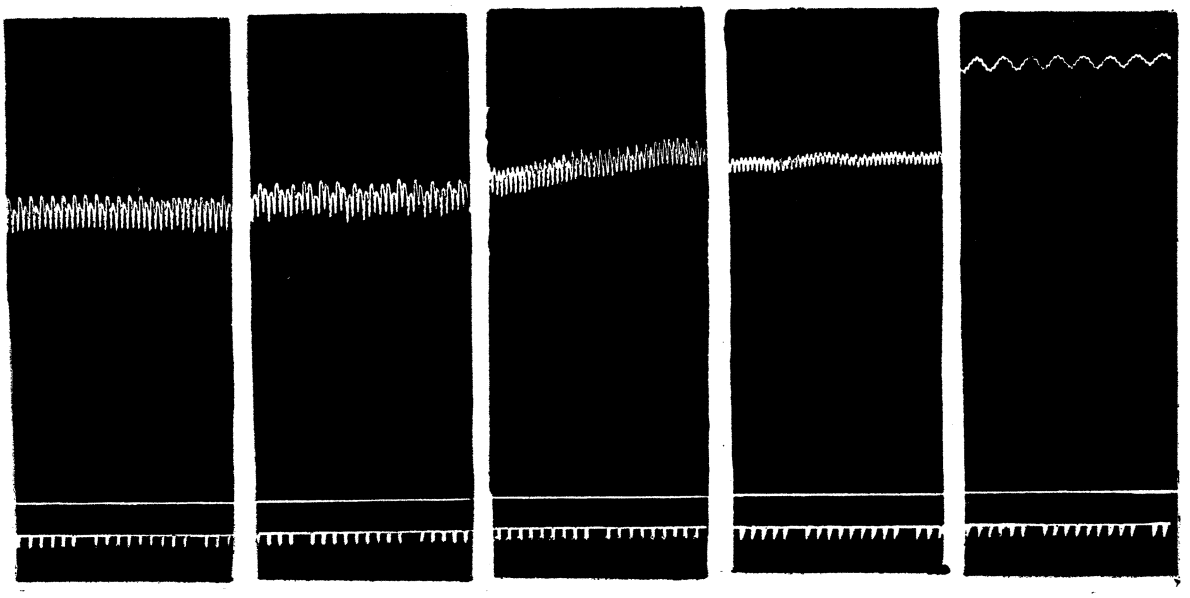

FIG. 13.—Experiment 4—Tracing of arterial pressure, taken respectively 5 m., 16 m., 50 m., 1 h. 20 m., and 2 h. 12 m. after injection of pilocarpine.

of general arterial pressure taken at the times at which the samples of blood were collected. The arterial pressure showed a general inclination to rise, but there is nothing in the tracing to suggest that; the rates of flow after injection of the pilocarpine are :—

5 m.	16 m.	50 m.	1 h. 20 m.	2 h. 12 m.	2 h. 50 m.
3·3	1·3	1·9	2·4	1·7	1·0

* HILL (25) has shown that if arterial blood is changed into two other fluids, venous blood and a secretion, the work done must always be positive, whether the secretion is richer or poorer in salt than the blood.

We attribute the postponed dilatation of the vessels to the gradual accumulation in the gland of the products of dilatation of the vessels, for there is no reason to suppose that the direct action of pilocarpine on the vessels is stronger after an hour than after 15 minutes.

We do not propose to enter here into the cause of the first summit; there are two possible views—one is that it too is due to the liberation of toxic products in the gland, the other that it is the direct effect of pilocarpine on the nerve endings of the chorda tympani in the vessels. The general effect of pilocarpine on vessels is, of course, constriction.

The Cause of the Exudation.—We have now to discuss the cause of the hyper-exudation from the vessels during functional activity.

The historical treatment of this subject would be to suggest two possible causes: (1) the hyperæmia, (2) the increased glandular metabolism. These two suggestions would follow the general lines of thought, on the one hand, of the Ludwig school, set on a sound basis by the work of STARLING and BAYLISS in the early nineties, the pivot of which was that the lymph flow varied with the capillary pressure. The second explanation would be along the line advocated somewhat more recently by ASHER and BARBÈRA (26), whose position was that lymph flow varied with the functional activity of the organ.

It has often seemed to us that these two points of view are too frequently represented as antagonistic. So far from there being any real conflict between them, it becomes more and more difficult to separate them the one from the other. Let us grant that increased capillary pressure causes increased lymph flow. We have seen reason to believe that functional activity by the action of metabolic products causes dilatation of the arterioles in the active organ, and thus must cause also local increase of capillary pressure and increased lymph flow. But it is also reasonable to suppose that functional activity directly causes increased exudation by raising the osmotic pressure in the tissue spaces, and possibly by increasing the permeability of the vessels. In spite of the close association of the two factors, capillary pressure and metabolic products, we have endeavoured to make some analysis of their separate effects, and in this we have met with some trifling success.

In the submaxillary gland we have been able to observe cases in which the metabolism has remained steady, whilst the blood flow from external causes has varied, and other cases where the blood flow has remained constant and the metabolism has varied. Here, then, there seems to be an issue. With which does the exudation keep company? The answer, so far as we have observed in the limited number of experiments which we have performed, is that it varies with both. Given a constant blood flow and variable metabolism, the exudation varies with the metabolism. Given a constant metabolism and a variable blood flow, the exudation varies with the blood flow.

The data on which this conclusion is based are the following:—

In Experiment 6 the oxidation remained constant for a considerable time.

Time	12.49	1.08	1.42	2.42
Coefficient of oxidation .	0·063	0·063	0·063	0·058

These figures are all within the limits of experimental error of one another; in comparison may be given the exudation and the blood flow :—

Time	12.49	1.08	1.42	2.42
Exudation	0·16	0·16	0·20	0·23
Blood flow	3·8	6·0	4·6	3·8
Exudation expressed as a percentage of blood flow	4	4	4	6

Again, in Experiment 5 the blood flow was maintained at a pretty constant rate for some time.

Time	11.58	12.16	12.35	12.58
Blood flow	0·93	0·93	1·2	1·0

The following comparison may then be made between the oxidation and the exudation :—

Time	11.58	12.16	12.35	12.58
Exudation	0·104	0·095	0·192	0·099
Oxidation used by gland .	0·156	0·151	0·260	0·135
Cubic centimetres of oxygen per cubic centimetre of exudation	1·5	1·6	1·3	1·4

These two experiments are shown in figs. 14 and 15.

No doubt many more experiments would be necessary in order to prove the point; all we would say at present is that the two instances we have been able to produce do not violate the position which on other grounds seems to be probably correct.

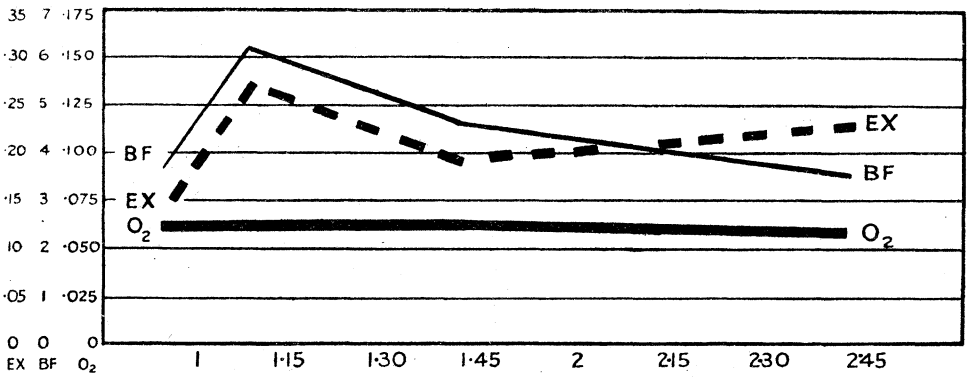

FIG. 14.—Coefficient of oxidation constant, exudation and blood flow variable.

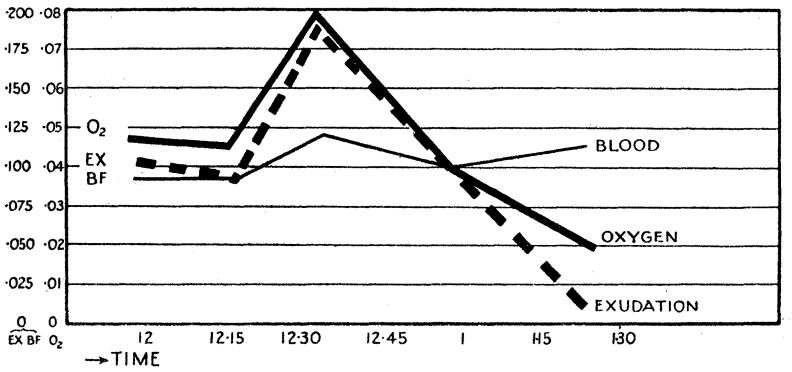

FIG. 15.—Blood flow nearly constant, coefficient of oxidation and exudation variable.

It may be considered, as indeed ASHER pointed out, and before him HEIDENHAIN, that the capillary pressure as an initial cause of lymph flow in the submaxillary gland is ruled out by the atropine experiment. HEIDENHAIN's observation was that the atropinised submaxillary gland did not become œdematous when the chorda tympani was stimulated, whilst the normal gland did. The conceptions which underlie this experiment are :—

(1) That there is no greater lymph flow from the atropinised gland when the chorda tympani nerve is stimulated than when it is not.

(2) That stimulation of the chorda tympani in the atropinised gland leads to no increase in the metabolism of the gland.

(3) That the vascular dilatation induced by stimulation of the chorda tympani is of the same order in the atropinised as in the normal gland.

None of these three conceptions seems to be strictly accurate.

(1) BAINBRIDGE (27) measured the lymph flow from the gland; out of six cases he found the lymph flow in the atropinised gland increased on three occasions, on one of which it was doubled, on another it was increased as 11 : 17.

(2) Experiments by one of us (28) show that there is a small rise in the coefficient of oxidation when the chorda is stimulated.

(3) The vascular dilatation caused by stimulation of the chorda, though as great as in the unatropinised gland for a short period of time, is not maintained.

If then we are to state what we conceive to be ASHER's fundamental idea, in the terms in which it would appear in the light of our investigations, it would be as follows :—

(1) Increased functional activity occasions increased lymph flow.

(2) The two are not altogether synchronous, the lymph flow following on the activity.

(3) The lymph flow is nearly synchronous with the metabolism in the organ caused by the activity.

(4) The metabolic products cause the flow of lymph—

(a) directly by altering the osmotic properties of the system, the permeability of the vessel walls, and possibly the condition of the proteins (Fischer (29)).

(b) indirectly by inducing vaso-dilatation and increased capillary pressure.

Summary.

(1) When skeletal muscle is stimulated rhythmically for 15 minutes, the following effects may be observed :—

(1) The quantity of oxygen taken up is increased both during and after the stimulus, and five hours may elapse before the coefficient of oxidation returns to its former value.

(2) The blood flow is increased, as also is the exudation from the blood vessels. These, like the coefficient of oxidation, may remain abnormally great for several hours.

(3) The exercised muscle, five or six hours after the stimulation, may be heavier, shorter, and of lower specific gravity than the unexercised one. Indeed, its properties may be so altered as to suggest the physical basis of the sensation of "stiffness."

(2) The length of time which is required for the products of activity to become oxidised suggests that special precautions are necessary to insure a condition of rest in which the basal metabolism of the muscle is obtained.

(3) The exudation from the vessels of resting muscle is just appreciable, that from the vessels of the resting submaxillary gland is inappreciable.

(4) When the submaxillary gland is under the influence of pilocarpine, the oxygen consumption, the exudation, and the hyperæmia increase, and for several hours do so relatively to the volume of saliva that is being excreted.

(5) Both in gland and muscle there tend to be two maxima on the curves of oxidation, hyperæmia and exudation, one when the activity is at its height, the other about an hour subsequently.

(6) The sustained increase of blood flow appears to be due to metabolic products. To them also is due the increased lymph flow ; their action may be partly direct and partly indirect, inasmuch as they produce a rise in local and capillary pressure.

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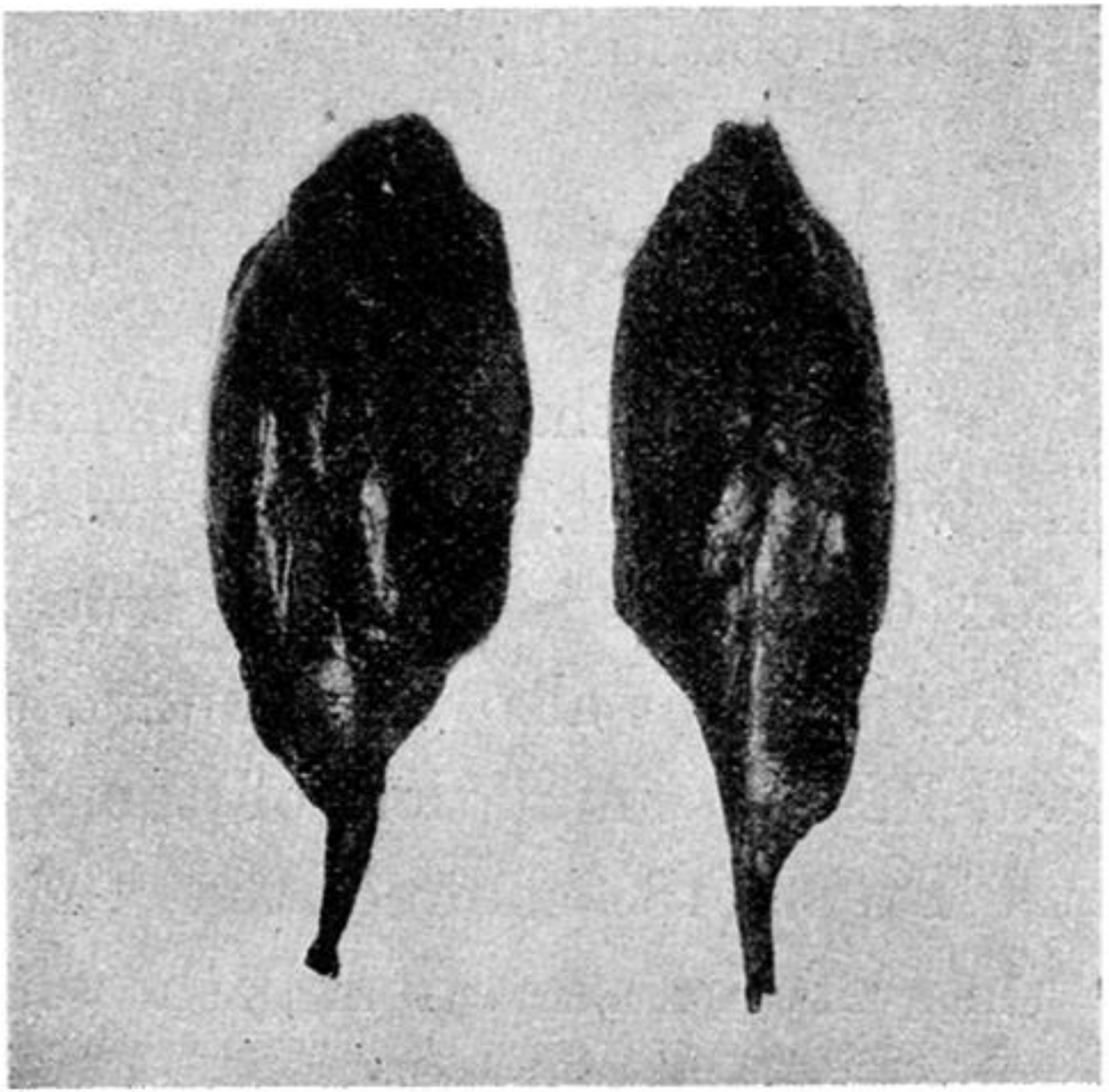

IG. 7A.—Left (unexercised) and right (exercised) gastrocnemii of dog in Experiment 11.