'97, Sherrington, C. S., "Further Note on the Sensory Nerves of Muscles," 'Roy. Soc. Proc.,' vol. 61 (1897).
'97a, Sherrington, C. S., "On the Question whether any Fibres of the Mammalian Dorsal (Afferent) Spinal Root are of Intraspinal Origin," 'Journ. Physiol.,' vol. 21 (1897).
'10, Sherrington, C. S., and Tozer, F. M., "Receptors and Afferents of the IIIrd, IVth, and VIth Cranial Nerves," 'Roy. Soc. Proc.,' B, vol. 82 (1910).
'92, Tooth, H. H., "On the Relation of the Posterior Root to the Posterior Horn in the Medulla and Cord," 'Journ. Physiol.,' vol. 13 (1892).
'10, Tozer, F. M. See Sherrington and Tozer.
'12, Tozer, F. M., "On the Presence of Ganglion Cells in the Roots of Third, Fourth, and Sixth Cranial Nerves," 'Physiol. Soc. Proc.,' July 27, 1912, 'Journ. Physiol.,' vol. 45.

The Osmotic Balance of Skeletal Muscle.

By Dorothy Jordan Lloyd.

(Communicated by W. B. Hardy, F.R.S. Received February 24, 1915.)

Fletcher was the first to follow continuously, for any considerable length of time, the change in weight of a muscle immersed in a hypotonic solution.* He found that the muscle at first increased in weight and then decreased. In isotonic solution the muscle "neither gains nor loses weight." This amounts to a definition of an isotonic solution.

The changes in weight of the gastrocnemius or sartorius, the muscles used by Fletcher, are slow, owing to the low value of the ratio of surface to volume. Very early in this work therefore it was decided to use a thin flat muscle sheet. The sternocutaneous muscle of the frog was fixed upon. It reaches its maximal intake from a hypotonic solution in from 5 to 20 minutes according to the concentration of the solution and the state of the muscle. In the case of so small a muscle it is possible that all the fibres are nearly in the same state at the same time. This cannot be the case with larger muscles. The central fibres of, for instance, the sartorius may be irritable whilst the external fibres are in water rigor. Complex physical and physiological reactions between the fibres must occur and complicate the problem. An obvious disadvantage of a muscle with a large surface is the magnitude of the error in weight due to variation in the quantity of moisture adherent to the surface. The surface was always dried quickly by filter-paper before weighing, and the smoothness of the curves of variation of

* 'Journ. Physiol.,' vol. 30, p. 414 (1904).

weight with time is, I think, sufficient proof that the error was reduced to about 1 per cent. of the total weight.

According to the definition of an isotonic solution given above, such a solution does not, strictly speaking, exist. A muscle may remain steady within the limit of error of weighing for periods up to half-an-hour, but sooner or later measurable variations of weight appear. In other words the muscle removed from the body is a changing system. It is a question whether the apparently steady periods are not really periods of very slow change. It is noteworthy that different workers have fixed upon solutions of sodium chloride over such a wide range as from 0·6 to 0·8 per cent. as being isotonic with frog's muscle; and the only curve given by Fletcher of a muscle in an isotonic solution shows a steady rise in weight.

A muscle simply removed from the body is called by Fletcher a resting muscle. The use of the term is inadvisable. Such a muscle has suffered a certain amount of mechanical disturbance, and in the process of pithing the frog it has been thrown into tetanus lasting a minute or more, at a time when the circulation of blood is defective. Such a muscle is best indicated by the term untreated muscle.

The weight changes characteristic of an untreated muscle in solutions of the sugars biose, dextrose, sucrose, and raffinose between the concentrations zero to 0·27 molecular are shown in fig. 1, which shews a curve for

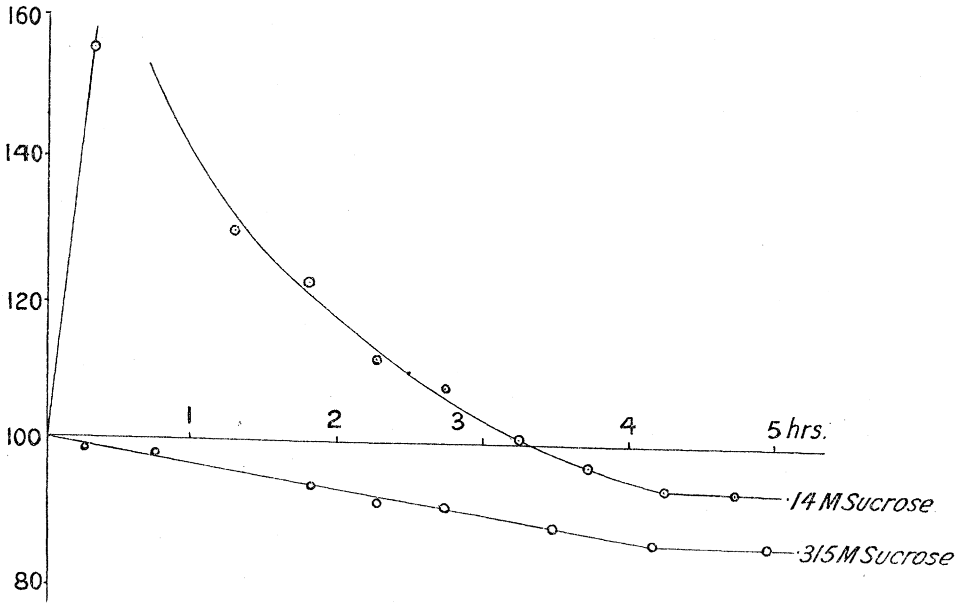

FIG. 1.—Abscissæ = hours from beginning of experiment; Ordinates = weight of muscle expressed in percentage of initial weight.

0·14 molecular sucrose.* There is a rapid intake of water followed by loss, the weight often falling much below the initial weight. This curve should be compared with the curve of change of weight in hypotonic (0·10 molecular) Ringer, given in fig. 2.

The curve characteristic of a concentration higher than 0·27 molecular is

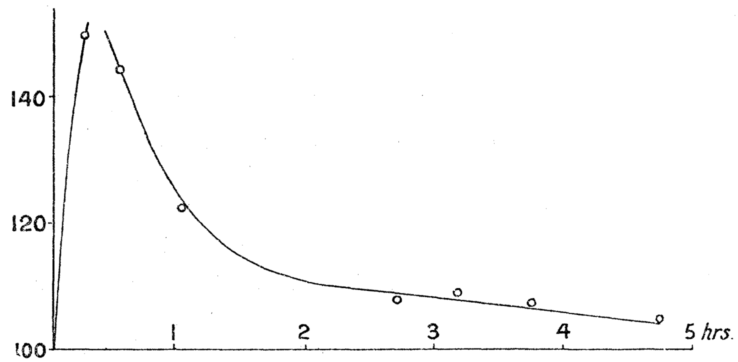

Fig. 2.

also given in fig. 1. The initial intake not only is not present but is not even represented by a variation in the rate of loss. The relation throughout is simply linear.

The fact that the loss of weight in the more concentrated solutions is in linear relation to time is of interest. The osmotic equivalent of an untreated muscle exposed to a solution such that it takes in water, clearly undergoes a change since the intake gives place to a loss in weight usually greater than the previous gain. Does the osmotic equivalent of the surviving muscle spontaneously change or is the change just mentioned due to the exposure to a hypotonic solution? The linear form of the curve of loss in a hypertonic solution suggests that the variation of state is due to the influence of the solution. The linear form of the curve also would imply that the loss is due to a change in the state of the muscle, for if it were merely the establishment of an osmotic balance with a fixed effective mass of solute within the muscle the rate would diminish as the effective concentration within the muscle approached that outside it. Both above and below a certain concentration the progress of change bears the character, not of the simple establishment of osmotic equilibrium between two solutions initially of different concentration, but of the response of a labile system to an external change of state.

* It must be remembered that a 0·125 molecular solution of sodium chloride (taken by most writers as isotonic) is osmotically equivalent to a 0·23 molecular solution of a sugar.

The region between 0·21 molecular and 0·27 molecular for the sugars is one in which the initial intake may or may not appear. The muscle either at once loses weight after a short period of slight change, or shows a typical initial intake followed by a typical loss. The variation over the region may provisionally be attributed to a variation in the state of the muscle due to mechanical disturbance. For instance, in dissecting the muscle out it is subjected to a varying amount of tension, and this will tend to produce passage of fluid from the interior of the fibre to the lymph space or *vice versâ*, and pithing the frog causes fairly prolonged twitching when the blood flow is poor. If it were possible to secure muscles in a definite physiological state this diffuse zone would probably narrow to a critical concentration, below which the initial intake would occur and above which it would vanish. Some part of the initial intake of water from hypotonic solution is unquestionably due to the mechanical disturbance of the muscle.

Effect of Oxygen.—Fletcher found that the osmotic changes induced in a muscle by activity were removed by exposure to oxygen. The muscle was put back into the "resting" state, which was characterised by a large intake of water from hypotonic solutions. My results with the sterno-cutaneous do not readily harmonise with those of Fletcher. The effect of previous exposure to oxygen is to reduce, and finally to obliterate, the initial intake of fluid even from distilled water. In fig. 3 are two curves, (*a*) from a muscle put directly after removal from the body into distilled water, (*b*) from a muscle placed in distilled water after three hours' exposure to moist oxygen.

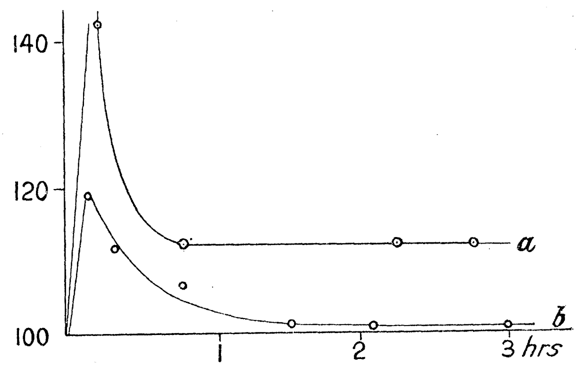

Fig. 3.

It might be urged that the intake has already taken place from the water vapour during exposure to oxygen. This is not so. Muscles in oxygen and water vapour always tend to lose weight. If any intake does occur it must be so transitory and slight as to have escaped detection.

Exposure to Water Vapour.—The behaviour of a muscle immersed in a

solution must always be open to a variety of interpretations. The course of events depends not only upon the initial state of the muscle but also upon properties of the surfaces of muscle and muscle fibre considered as semi-permeable membranes. Further, osmotic relations are complicated by change in the muscle due to the chemical nature of the solution. Thus, to take salts as examples, the sternocutaneous muscle in $\frac{1}{8}$ molecular solution of sodium chloride usually remains of constant weight for some time, and then loses weight; in a similar solution of potassium chloride there is an immediate and prolonged rise in weight followed by a fall; in an isosmotic solution of calcium chloride there is a very small and fleeting initial rise in weight followed by a long and steady fall which may reduce the muscle to 60 per cent. of its original weight.

It is possible, however, to examine the osmotic balance of a muscle by exposing it to water vapour of varying pressure. The gas space round the muscle then acts as a theoretically perfect semi-permeable membrane so far as non-volatile solutes are concerned. The method adopted was to suspend the muscle in a flask over a solution of known concentration, the gas in the flask first having been shaken thoroughly with the solution and then left at the desired temperature for some hours in order to attain equilibrium. The muscle was removed for each weighing, and results therefore are affected by an error due to loss of water during weighing, and loss of vapour from the flask during removal and replacement. Control experiments with fine plates of agar saturated with water gave a maximal loss of 3 per cent. in four hours.

In fig. 4 are shown curves of the weight changes of muscles suspended in oxygen above the plane surface of (*b*) distilled water, (*c*) 0·06 molecular

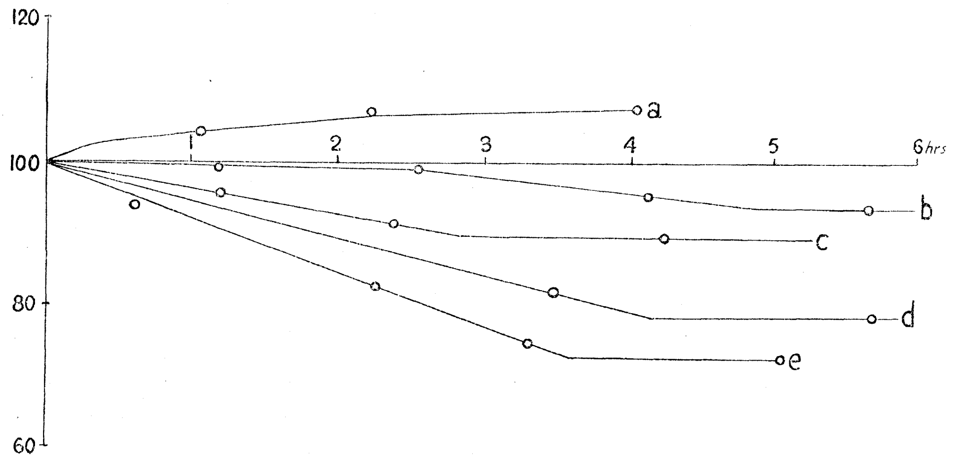

FIG. 4.

Ringer's fluid, (*d*) 0·125 molecular Ringer's fluid, and (*e*) in air above 0·13 molecular Ringer's fluid, and (*a*) in hydrogen over distilled water.

In oxygen saturated with aqueous vapour over distilled water the weight may either remain constant for a variable period and then fall, or start falling at once. That is to say, oxygen obliterates the intake from vapour which occurs in air or hydrogen. There is here at first sight a contradiction. If the untreated muscle has a vapour pressure less than that of water—as would appear from the curve *b*, fig. 4—why does it not at first condense vapour when in oxygen? Oxygen cannot instantly remove the condition which leads to the intake.

A reason may probably be found in the nature of the diffusion column which must be formed at the free surface of the muscle and of each fibre. At the free surface are water vapour and oxygen. Consider a superficial shell at the surface. If the presence of free oxygen within this shell either raises its vapour pressure to that of water, or maintains its vapour pressure at that level, the quantity of water vapour taken up by the shell will depend upon the ratio of the rate of diffusion of water vapour to that of oxygen. The diffusion column of oxygen progressively retards the diffusion of water vapour, so that, even if their diffusion rates initially were equal, that of the water vapour would rapidly tend to vanish. An analogous retardation is that seen when a retardation in the dissipation of heat or diffusion of impurities from the face of the solid arrests the solidification of over-cooled liquids.*

In hydrogen saturated with water vapour the muscle gains in weight. Strictly speaking, the gas was air very much diluted with hydrogen and saturated with water vapour. In this the sternocutaneous maintains its irritability for about six hours.

What change in the muscle is it which is caused by excess oxygen and which raises the vapour pressure? The work of Fletcher† and of Fletcher and Hopkins‡ suggests that exposure to oxygen reduces the concentration of metabolites (such as, for instance, lactic acid), and so raises the vapour pressure. This would accord with the view of Ranke and of all who have followed him, that the intake of water by a fatigued muscle is due to the production during activity of chemical substances of low molecular weight.

The secondary fall in weight of muscles due to loss of water was ascribed by Fletcher to a "loss of the semi-permeable character of the fibres," just as an ordinary osmometer whose membrane is not completely impermeable

* Wilson, 'Phil. Mag.,' (5), vol. 50, p. 238.

† 'Journ. Phys.,' vols. 23 and 28.

‡ 'Journ. Phys.,' vol. 35, p. 247 (1906–7).

to a solute shows first an intake of solvent and later, as the solute escapes, a loss. If this were the sole cause, then the secondary loss of water would be due, either directly or indirectly, to loss of carbonic acid, for this is the only known solute which can escape into the vapour.

It must be pointed out that these experiments cannot be used to calculate the vapour pressure of the muscle substance because of the error in weighing mentioned above, and because the rise of the vapour pressure of the muscle due to curvature of the surfaces is included.

Summary.

1. The sternocutaneous muscle of the frog, immersed in a hypotonic solution of Ringer's fluid, of biose, dextrose, sucrose, raffinose, or of NaCl undergoes first a gain in weight and later a loss.

In a hypertonic solution the weight falls from the start.

2. The initial gain in weight in hypotonic solutions or even in distilled water can be reduced and finally suppressed by previously exposing the muscle to wet oxygen.

3. Muscles absorb water from an atmosphere of hydrogen and water vapour, but not from one of oxygen and water vapour. In the latter a fall in weight was observed.