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Swelling of the Muscles of Adrenalectomised Rats.*

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Following a suggestion of Viale and Bruno,[1] that the changes in water content of the tissues of adrenalectomised animals may be due to an increase in permeability, Winter and Hartman[2] carried out experiments on the swelling and shrinking of muscles from normal and adrenalectomised rats in balanced salt solutions of various strengths. They conclude that water enters or leaves muscles from adrenalectomised animals more readily than it leaves or enters normal muscles, and regard this as evidence of an increased permeability following the adrenalectomy. Since a hormonal control of tissue permeability, if demonstrated, would be of extreme importance in general physiology, we have attempted to reproduce Winter and Hartman's results.

The method used was essentially that of Winter and Hartman, except that we confined ourselves to determining the course of the swelling curves of muscles in a hypotonic solution, instead of investigating shrinking curves in hypertonic solutions as well. The latter are notoriously irregular, and are not suitable for analysis. The rats used were adrenalectomised at 30 days of age, and in all but a few cases were killed in the terminal stages of adrenal insufficiency, as indicated by a fall in weight and body temperature, asthenia and oftentimes prostration. In a few cases the rats were killed in slightly earlier stages of adrenal insufficiency. The animals survived from 3 to 32 days after the operation, the average time of survival being 9 days. The average weight at the time they were killed was 63 gm. The control rats were from the same stock, and as nearly as possible of the same age.

The animals were killed by a blow on the head, and both gastrocnemii dissected out entire. The muscles were immersed in the Locke's solution described by Winter and Hartman (pH = 7.3), diluted so as to be hypotonic (70 parts solution plus 30 parts of

* We are indebted to Mr. C. E. Tobin for assistance in the preparation of animals.

1 Viale, G., and Bruno, A., *Compt. Rend. Soc. de Biol.*, 1927, **97**, 261.

2 Winter, C. A., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 207.

water). They were weighed before immersion and after intervals of 3, 6, 10, 15, 25, 40, and 60 minutes on a torsion balance, which allows very rapid weighing. There is little point in continuing observations beyond about 60 minutes, for the swelling curves become very irregular.

The average results for 40 muscles from 20 adrenalectomised animals and for 40 muscles from 20 controls are shown in Table I.

TABLE I.

	% gain in weight						
	3′	6′	10′	15′	25′	40′	60′
Normals	106.1	109.1	111.8	114.7	118.3	122.8	127.1
Adrenalectomised	104.1	106.9	109.1	112.1	114.9	118.4	122.3
Difference	2.0	2.2	2.7	2.6	3.4	4.4	4.8
S.E. of difference	0.57	0.68	0.87	1.10	1.28	1.37	1.43

The table shows that the muscles of the adrenalectomised rats take in water at a slower rate than do those of the control animals, a conclusion which is exactly the opposite from that of Winter and Hartman. Winter and Hartman do not give figures to show that the differences in swelling rate which they observed (in the opposite direction) were statistically significant, but there is no doubt about the differences shown above. If differences in the form of the swelling curves obtained in experiments such as these are to be taken as indicating differences in permeability, our conclusion would have to be that the muscles of adrenalectomised animals are less permeable to water than those of normal animals: there are, however, several reasons for attributing the differences to other than permeability changes.

1. Throughout the experiments we observed that heavier muscles tended to swell less rapidly than lighter ones, even when the swelling was expressed in each case as a percentage of the initial weight. This is to be expected on the grounds that the heavier muscles have a smaller surface/volume ratio. It so happened that our control muscles were somewhat lighter on the average than the muscles from the adrenalectomised animals, and this difference in weight is probably partly responsible for the slower swelling of the latter.

2. When muscles of equal weight from normal and adrenalectomised animals were compared, the rate of swelling of the control muscles was again found to be greater than that for the muscles from the adrenalectomised rats, although the differences were smaller than before. Taken individually, indeed, (as in the table

above) only one such difference exceeded twice its standard error, but all the differences were in the same direction, and the odds against this occurring by pure chance are enormous. A simple explanation exists, however, for this greater rate of swelling in the case of the controls, for the muscles from the control animals contained a smaller percentage of water than those of the operated animals.[3-5] If we suppose that the initial tonicity of the control muscles was higher than that of the muscles of the adrenalectomised animals, the former would be expected to swell more rapidly than the latter in any given hypotonic solution. The average water content of our control muscles was 76.6%, and that of our muscles from the operated animals 78.3%; this difference is about of the right order, if reflected in a tonicity change, to account for the different swelling rates observed.

3. While these are the average results for all the muscles used, the swelling curves of individual muscles vary so much that we are very doubtful if this type of experiment can be relied upon to supply information regarding permeability of tissues. For instance, the muscles of the right and left legs of the same animal do not always show the same swelling curve, even when the 2 muscles are of the same initial weight, and we have met with as great differences between the behavior of the paired muscles as between the behavior of normal muscles and those from the adrenalectomised animals.

Finally, it ought to be pointed out that an increase in the permeability of the tissues to water, and this alone, could not account for the increased water content of the tissues after adrenalectomy. The water content is determined, not by the rate of entry of water, but by those conditions which control the final equilibrium, and these might well change without any alteration in permeability being involved.

Conclusions. The conclusion of Winter and Hartman, that adrenalectomy in the rat is followed by an increase in the rate at which water enters the muscles, *i. e.*, by an increase in permeability, is not confirmed. The observation that the muscles of the operated animals contain a greater percentage of water is confirmed. It is pointed out that an increase in permeability to water, even if it were to exist, would not account for the increased water content.

[3] Hartman, F. A., Brownell, K. A., and Lockwood, J. E., *Endocrinology*, 1932, **16**, 521.

[4] Silvette, H., and Britton, S. W., *Am. J. Physiol.*, 1933, **104**, 399.

[5] Hartman, F. A., *Annals Int. Med.*, 1933, **7**, 6.