The effect on the molecular concentration and electrical conductivity of muscle extracts of removal of the proteids / by G.N. Stewart.

[*Reprinted from the Journal of Physiology.*
Vol. XXIV. No. 6, *August* 21, 1899.]

THE EFFECT ON THE MOLECULAR CONCENTRATION AND ELECTRICAL CONDUCTIVITY OF MUSCLE EXTRACTS OF REMOVAL OF THE PROTEIDS.

By G. N. STEWART, *Western Reserve University, Cleveland, U.S.A.*

(*Preliminary Note.*)

Several investigators have tried to determine whether proteids exert a sensible osmotic pressure by observing the freezing point of solutions of such proteids as egg-albumin before and after heat-precipitation of the proteids. The result in most cases has been negative. In the course of some work on the proteids of muscle, I had the opportunity to determine whether any changes took place in the freezing point and electrical conductivity of various extracts after removal of the proteids. It seemed to me that such observations might throw light on the obscure relations of salts to proteids in solutions where both exist together, and especially on the relations of salts to the globulins which they hold in solution. Further, it suggested itself that information as to the nature of the proteid which is precipitated from a muscle extract at a given temperature might be obtained by observing whether the changes in the freezing point and conductivity after its precipitation indicated that salts had been held in combination with it in the solution or not. For instance, it might be expected that a globulin would hold the salts more firmly than an albumin or hold a larger amount of salts in combination. Different globulins, too, might be combined with different quantities of salts; and from the amount of the change of freezing point or conductivity caused by the precipitation of given amounts of globulins at different temperatures, it seemed possible to learn something as to their chemical or chemico-physical resemblances or differences. If, for example, the precipitation of a certain amount of a globulin coagulating at 47° were found to cause a greater or a smaller change in the freezing point or conductivity than the precipitation of the same

quantity of a globulin coagulating at 56°, an additional proof that the two globulins are chemically different bodies would be afforded; and the same for substances whose coagulation temperatures lie closer together.

It will be seen from the results subjoined that distinct differences were made out in the conductivity after removal of the proteids, the conductivity being increased. In Exp. 3 the increase after removal of ·2378 grm. proteid from 100 c.c. of a magnesium sulphate extract was from 72·49 to 76·41 or 5·4 p.c. (= 22·7 p.c. per grm. of proteid); in Exp. 4, after removal of ·3818 grm. proteid from 100 c.c., the increase was from 113·0 to 117·0 or 3·5 p.c. (= 9·2 p.c. per grm. of proteid); and in Exp. 5, after removal of ·5739 grm. proteid, the increase was 1·4 p.c. (= 2·4 p.c. per grm. of proteid). In the same experiment the removal of ·2033 grm. of proteid coagulating between 60° and 100° caused an increase in the conductivity of 1·9 p.c. (= 9·5 p.c. per grm. of proteid). The removal of all the proteids from the cooled plasma of frog's muscles (Exp. 1) caused an increase of 10·2 p.c. The resistance measurements are too exact to permit us to suppose that the apparent increase is due to errors of measurement. If it is due merely to the elimination of the well-known depressing influence (molecular friction) of dissolved non-conducting substances on the conductivity of electrolytes, apart from chemical or physico-chemical combination with them, the globulins of muscle extracts must depress the conductivity in a much greater degree than the proteids of serum or hæmoglobin.

For Bugarsky and Tangl[1] found that 1 grm. of proteid, prepared by long-continued dialysis from blood-serum, depressed the conductivity of 100 c.c. of serum by only 2·5 p.c.; and I found for 1 grm. of hæmoglobin in 100 c.c. of serum a depressing effect of no more than 1·8 p.c.[2] It seems necessary to suppose then, that a certain portion of the magnesium sulphate is combined with the proteids, or at least is so affected by them that its dissociation coefficient (the number which measures the extent to which it is dissociated) is diminished. Liebermann and Bugarsky[3] have recently stated that egg-albumin, albumose and pepsin do combine with sodium hydroxide, and hydrochloric acid, but not with sodium chloride. It might be expected that the globulins, which are only dissolved by the aid of neutral salts, would

[1] *Pflüger's Archiv*, LXXII. S. 540. 1898.
[2] This *Journal*, XXIV. p. 216. 1899.
[3] *Pflüger's Archiv*, LXXII. S. 51. 1898.

show a different behaviour towards them from the albumins, which are soluble in water.

The number of experiments hitherto made have not been sufficient to disclose any constant difference between the various proteids of muscle extracts.

The conductivity of the muscle extract is less than that of the magnesium sulphate solution used in making it. This is probably not due to the dilution of the solution by water passing into it from the muscle, since the molecular concentration of the muscle extract is greater than that of the solution both before and after the removal of the proteids. And indeed the magnesium sulphate solution being hypoisotonic to serum, and therefore presumably to the contents of the muscle fibres (which, according to Loeb are isotonic with a 0·7 p.c. solution of NaCl), water might rather be expected to pass into the muscle from the solution.

The fact that Δ is uniformly greater for the muscle extracts than for the magnesium sulphate solutions used, while the conductivity of the extracts is less than that of the solutions, is accounted for by the passage from the muscle into the extract of non-conducting substances, not only proteids but glycogen, glucose, etc.

As regards the effect of removal of the proteids on the freezing point the results are somewhat variable, and that to an extent greater than can be accounted for by errors in the freezing point determinations. Thus in Exp. 3 the removal of ·1132 grm. paramyosinogen from 100 c.c. of extract diminished Δ by ·015°, while subsequent removal of ·1246 grm. myosinogen increased it by the same amount, bringing it back exactly to its original value. In Exp. 4 Δ remained constant within the limits of error of the method, while in Exp. 5 the removal of the paramyosinogen and myosinogen increased Δ by ·004°, and the removal of all the proteids increased it by ·028°. These irregularities I am not at present in a position to explain, but hope that further experiments may discover their cause. I suspect that they may be due, in part at any rate, to changes taking place in the proteids during the heating, for example hydrolysis.

In Exp. 2 the effect of dialysis on the conductivity of a 5 p.c. magnesium sulphate extract of muscle is shown. After several days it was reduced to one-seventh of its original value, but later on began to increase again. This increase is doubtless associated with the development of acid which constantly accompanies the precipitation of the muscle proteids, and with putrefactive changes.

No. of Exp.		$\lambda(5°)\times10^8$
1	Frog's muscle plasma, extracted by freezing and pressure	82·84
	+1 vol. water	48·19
	Another specimen of the plasma (not from the same frogs)	78·97
	Second specimen after removal of large precipitate of proteids coagulating at 30°—35° in bath	85·10
	After removal of the rest of the proteids by boiling	91·20
2	Extract of dog's muscle in 5 % $MgSO_4$ solution	108·5
	After dialysis for several days in running tap-water*, and filtering off globulin precipitate	15·77
	After further dialysis in distilled water for 2 days	14·64
	After further dialysis in distilled water for 8 days	54·75†
	* Tap-water itself.	1·64

† Nothing was added to the solution to prevent decomposition.

		Δ	$\lambda(5°)\times10^8$	sp. gr.	wt. in 100 c.c. in gr.
3	Muscle extract in about 3 % $MgSO_4$ solution	·331	72·49	1·0154	1·0092
	Dog's thigh muscles. The $MgSO_4$ was added in substance after washing the muscle with water.				
	After removal of 0·1132 grm. paramyosinogen from 100 c.c.	·316	72·90	1·0163	1·1924
	After removal of 0·1246 grm. myosin. from 100 c.c.	·331	76·41	1·0158	1·2804
4	Muscle extract (made with 5 % $MgSO_4$ solution from leg muscles of dog)	·509	113·00	1·0271	2·3498
	After removal of 0·1205 grm. paramyosinogen* from 100 c.c.	·507	115·2	1·0266	2·3100
	After removal of 0·1348 grm. myosin. from 100 c.c.	·509	117·2	1·0263	2·3420
	After removal of 0·0684 grm. myoglobulin and of 0·0581 grm. of remaining coagulable proteids	·508	117·0	1·0263	2·4012
	The 5 % $MgSO_4$ solution	·463	114·6		

Total solids in 100 c.c. of original muscle extract 3·4056 grm.

* The amount of paramyosinogen is probably a little too low.

5	Muscle extract (made with 5 % $MgSO_4$ solution from thigh muscles of dog)	·454	101·69	1·0244	1·7440
	After removal of 0·2627 grm. paramyosinogen from 100 c.c.	—	—	1·0235	1·8240
	After removal of 0·1079 grm. myosinogen	·458	101·24	1·0233	2·0094
	After removal of 0·1079 grm. myoglobulin and 0·0954 grm. remaining coagulable proteid	·482	103·17	1·0232	2·0525
	The 5 % $MgSO_4$ solution	·433	104·32	1·0226	2·2812

Total solids in 100 c.c. of original muscle extract 3·0134.