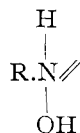


THE RELATIVE MASSES OF PROTEIN ANIONS AND CATIONS¹

BY A. R. C. HAAS

Investigation as to the mode of dissociation of protein compounds with inorganic bases and acids are of interest because they enable us to better understand the structural nature of such compounds. Conclusive evidence brought forward by Robertson² and Pauli³ has shown that the salts which proteins form with inorganic acids and bases do not dissociate at the point of union of the inorganic radical with the protein but at some other place within the protein molecule. The dissociation is shown to yield not an inorganic and a protein ion, but two or more protein ions, in one or more of which the inorganic radical is bound up in a non-dissociable form.

When a direct current of about 1 milli-ampere was passed through a solution of potassium caseinate, it was found by Robertson² that the casein is deposited on the anode and that the amount of casein so deposited is proportional to the quantity of electricity that has passed through the solution. The fact that the deposition of protein in this case takes place on the anode instead of at both electrodes (as his hypothesis of the electrolytic dissociation of protein solutions would seem to require) is explained by Robertson in the following manner:



The anion migrates to the anode where it reacts with water, liberating oxygen and free casein which is eventually precipi-

¹ From the Department of Biochemistry and Pharmacology, Rudolph Spreckels Physiological Laboratory, University of California.

² T. Brailsford Robertson: "Physical Chemistry of the Proteins," New York, 1918, Jour. Phys. Chem., 15, 521 (1911).

³ Wo. Pauli and M. Hirschfeld: Biochem. Zeit., 62, 245 (1914).

tated as the uncombined and, therefore, insoluble protein; the cation $R.CO K^{++}$, on reaching the cathode, reacts with water and liberates KOH, casein, and hydrogen, a condition which prevents the precipitation of free protein because the casein reacts with the excess of KOH to again form potassium caseinate which once more participates in carrying the current in each direction. Robertson concludes that the potassium or other univalent metal, which is carried along with the casein to the cathode, is there converted into the hydrate which then splits the ion, with which it has travelled, into two ions. The resultant cation is presumed to be retained in the cathodal region while the anion migrates back into the anodal region. Each cation which the cathodal region gains, gives up (provided the masses of the cations and anions are in every case equal), one-half its mass to the anodal region again. This theory, therefore, demands that the ratio of the anodal to the cathodal loss should be 2.

The refractive index method used by Robertson¹ for determining the amount of protein in the anodal and cathodal arms, has yielded only approximately the value of 2, the experimental error being quite considerable. At the suggestion of Dr. Robertson, the writer has repeated some of the experiments, using the same method which he used for determining the concentration of protein in solution and also an entirely different method. The results of the investigation confirm very satisfactorily the theory proposed by Robertson.

The apparatus adopted for the electrolysis of potassium caseinate solution was essentially the same as that used by Robertson in his experiments. The casein was prepared by Eimer and Amend as C. P. Casein "nach Hammersten" but was further purified.²

A 15 cc sample of the potassium caseinate solution was used as a control and was kept for 2 hours at 30° C as was the 35 cc of same original solution that was contained in the

¹ T. Brailsford Robertson: *Jour. Biol. Chem.*, **13**, 469 (1909).

² T. Brailsford Robertson: *Jour. Phys. Chem.*, **14**, 528 (1910).

U-tube. The potassium caseinate solution used in each experiment was a 3 percent solution made neutral to litmus. This was prepared by dissolving 3 grams of casein in 100 cc of a solution made by diluting 15 cc of 0.1 *N* KOH to a volume of 100 cc. The solution was stirred in a mortar for $\frac{3}{4}$ to 1 hour and then filtered. Such a solution, neutral to litmus and freshly prepared for each experiment, was used rather than a solution neutral to phenolphthalein because of the possible error arising from hydrolysis and resolution of precipitated casein in the latter solution.

Three experiments were conducted in which the measurements of the amounts of casein in solution were made by the use of the refractometer method, the results of which are as follows:

	Number of experiment		
	1	2	3
Grams of casein lost from the anodal arm	0.2210	0.2763	0.2395
Grams of casein lost from the cathodal arm	0.1105	0.1197	0.1197
Ratio	2.00	2.30	2.00

The value obtained in the second experiment gives only very approximately the value 2 for the ratio. An improvement of technique was, therefore, essential if more conclusive data were to be obtained.

Accordingly, similar experiments were undertaken in which the nitrogen of each arm of the U-tube, as well as of a control, was separately determined at the end of the 2-hour period. The ratio of the grams of casein lost from the anodal arm to that lost from the cathodal arm is the same as the ratio between the nitrogen loss in each arm. In the official Kjeldahl method¹ that was used, a cold water extract of the cochineal insects (freshly made up for each determination) was found to

¹ Bull. Bureau of Chemistry, 107, 5 (1912).

give sharper titration endpoints than the alcoholic cochineal indicator solution.

Since the nitrogen content of each arm was separately determined, it was necessary to ascertain accurately the volume of solution that each arm contained at the close of the electrolysis. The volume or displacement caused by the platinum electrodes and of the precipitated casein on the anode had to be taken into account in the calibration of the arms of the U-tube. Accordingly, the dry U-tube (bearing its dry platinum cathode and the spiral anode with its adhering dry precipitate of casein, in the cathodal and anodal arms, respectively) was calibrated with mercury. In this manner the exact volume of solution in each arm used for analysis after an electrolysis could be accurately determined. Prior to the adoption of this method of calibration, very variable results were obtained.

The results of the final four consecutive experiments with the improved technique, are as follows:

No. of Expt.	N content of 1 cc of control solution of potassium caseinate. No electrolysis. Results in grams	N content of 1 cc of potassium caseinate solution after electrolysis. Results in grams		Difference or N loss of 1 cc of potassium caseinate solution after electrolysis. Results in grams		Ratio of anodal to cathodal loss
		Anodal arm	Cathodal arm	Anodal arm	Cathodal arm	
1	0.00389	0.00315	0.00354	0.00074	0.00035	2.11
2	0.00372	0.00302	0.00337	0.00070	0.00035	2.00
3	0.00332	0.00259	0.00297	0.00073	0.00035	2.08
4	0.00350	0.00290	0.00321	0.00060	0.00029	2.06

It is evident that these results, obtained by a different method than that used by Robertson, fully confirm the theory proposed by him which requires that the ratio of the anodal to the cathodal loss should be 2. The good agreement with the theoretical ratio can be taken as confirmatory of the theory that the protein anions and cations are equal in mass at least so far as solutions which are neutral to litmus are concerned. The results of the investigation moreover afford evidence of

the correctness of the view of Robertson that the protein is transported into the cathodal arm and, therefore, that the current is carried by protein ions in both directions.

I am indebted to Professor T. Brailsford Robertson for the constant interest that he has taken in this work, and to Harvard University for the grant of a Sheldon Traveling Fellowship which has given the financial aid in carrying out the investigation.