

Physics. — *The spreading of ovalbumin.* By E. GORTER, J. VAN ORMONDT and F. J. P. DOM. (Communicated by Prof. P. EHRENFEST.)

(Communicated at the meeting of June 25, 1932).

It has been shown previously¹⁾ by one of us, in collaboration with Dr. F. GREDEL, that proteins can be made to spread in a mono-layer at the air-water interphase of a Langmuir tray.

For casein the influence of the acidity or alkalinity of the water in the tray has been carefully studied, with the result that at the isoelectric point 4.7 and at a great distance from it, in a distinctly acid and a distinctly alkaline medium, maximal spreading was obtained, whereas at intermediate values of the hydrogen ion concentration at a pH 3 and at pH 8.5 minima were seen. We have concluded that the protein itself and its salts spread beautifully, but that protein-ions occupy a much smaller surface area per molecule.

In the experiments to be related, we have studied the spreading of ovalbumin. We have preferred this protein because it is easily soluble in water without the addition of electrolytes.

Moreover it can easily be prepared in a very pure state. We made use of the HOPKINS—PINKUS method²⁾ of preparation as modified by SÖRENSEN.

The ovalbumin was recrystallized 5 to 6 times and then dialyzed against distilled water in the icebox until the sulphate reaction of the water remained negative after 48 hours standing in the dialyzer. Usually several weeks were necessary to reach this state of purity.

The ovalbumin concentration was estimated in two ways:

1. the nitrogen content of the solution was estimated by the TER MEULEN and HESLINGA method³⁾. In this method hydrogen gas is passed over a heated mixture of powdered nickel with the sample in which nitrogen is to be estimated and the ammonia formed titrated directly in a receiving tube with 0.01 *n* hydrochloric acid. The nitrogen found, was multiplied by the factor 7.0 to get the ovalbumin.

2. the ovalbumin as such was determined by BANG's method for the estimation of albumin in urine. The albumin is precipitated by boiling with a mixture of dilute acetic acid and sodium acetate, filtered, washed with

¹⁾ E. GORTER and F. GREDEL. On the Spreading of Proteins. Transactions of the Faraday Society **71**. 1926. p. 477. Biochemische Zeitschrift **201**, 1928, p. 391.

²⁾ F. GOWLAND HOPKINS and S. N. PINKUS. Journal of Physiology. **23**. 1898. p. 130.

³⁾ TER MEULEN and HESLINGA. Nieuwe methoden voor elementaire analyse. Delft 1930. Rec. Trav. Chem. **49** (1930), p. 396.

alcohol and ether and dried to constant weight at 105° . The results of the 2 methods were in good agreement with each other.

These estimations of the ovalbumin content had to be performed fairly often as it appeared to be impossible to keep a solution for an indefinite length of time. Always after some weeks and sometimes even after one week a slight flocculation appeared which made it necessary to filtrate the solution and estimate again. This flocculation had nothing to do with a decay of the protein, but was caused by the instability of the colloidal system when dialysis is prolonged so far that most of the electrolyte is taken away. The stock solutions were always kept in the icebox after adding some preservative, for which thymol was usually taken; chloroform and toluene seemed less satisfactory, as they rather increased the tendency to flocculate. The concentration of the solution was always about 5 milligram per cc; e.g. one of the stock solutions had at the outset a concentration of 5.6 mg per cc and after three months when it had been filtered several times, a concentration of 4.6 mg per cc.

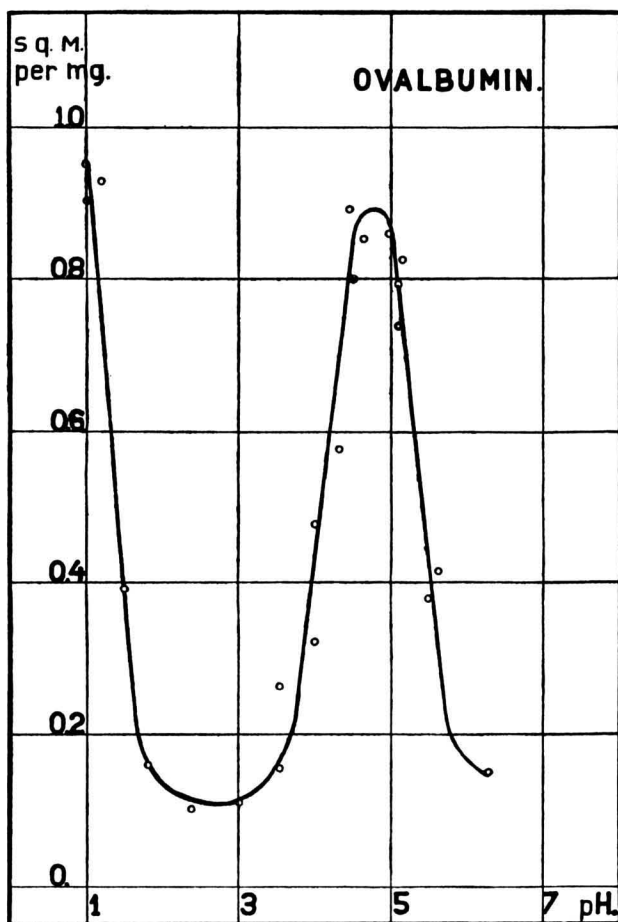


Fig. 1. Influence of pH.

The buffer solutions used were in the earlier measurements acetate buffers as described by WALPOLE¹).

They were diluted to a molarity of $1/300$ and could be used for pH 3.6 to pH 5.6. The more acid solutions were made with hydrochloric acid. The later experiments were made on veronal-acetate buffersolutions as described by MICHAELIS²), also diluted to $1/300$ mol. This latter buffer has several advantages :

- 1st. it covers a large pH range, from pH 2 to pH 10 ;
- 2nd. it contains only monovalent ions ;
- 3rd. its ionic strength can be kept constant over the whole pH range.

The pH of all buffer solutions was controlled by colorimetric and electrometric measurements.

The influence of changes of the acidity of the water on the spreading of ovalbumin was the same as had been observed with casein. (See fig. 1.)

We have now studied the influence of the addition of different salts to the water in the tray upon the spreading of ovalbumin.

It was easy to show that, at the isoelectric point, salts had no influence on the surface occupied by the protein. But when studying the influence of potassiumchloride on the spreading in acid medium, it was possible to discover a very simple relation between the size of the area and the concentration of Cl ions in the fluid. This relation is to be seen from Fig. 2, where the pCl is plotted against the surface area occupied by the protein.

EFFECT OF Cl⁻ ION.

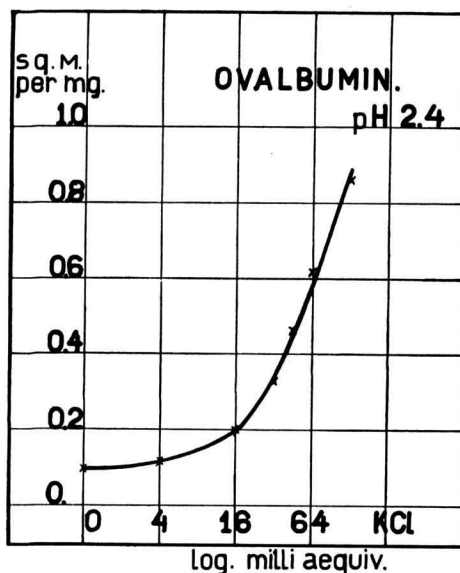


Fig. 2

¹) G. S. WALPOLE. Journ. Chem. Soc. 105. (1914). p. 20001.

²) L. MICHAELIS. Bioch. Ztschr. 234. (1931). p. 139.

The part of the curve between pH 1.2 and pH 3, is explained by the presence of Cl ion in the water, because HCl and KCl have exactly the same influence on the spreading (fig. 3). We may conclude from this observation which can be made as well with other salts (e.g. BaCl₂ fig. 3a) that the addition of

INFLUENCE Cl⁻ IONS.

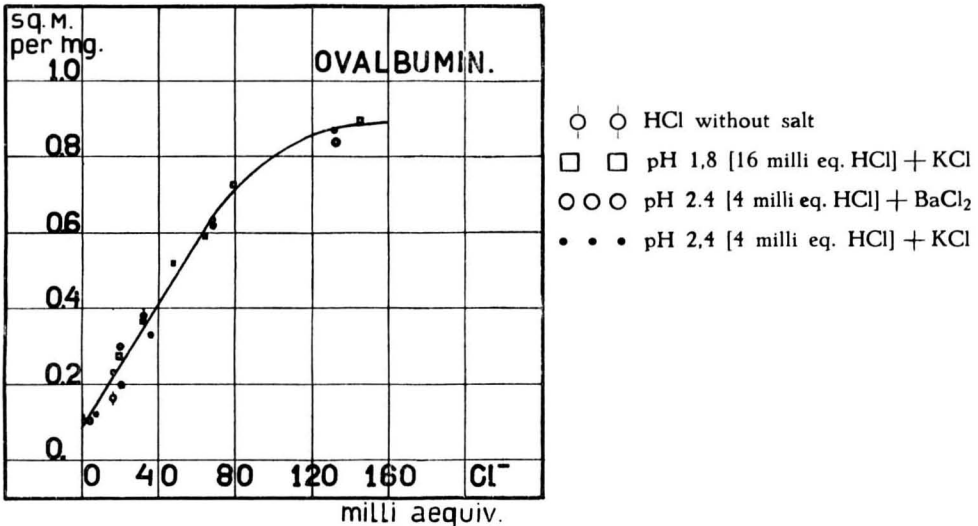


Fig. 3

EFFECT OF Cl⁻ ION.

EFFECT OF SO₄⁻² ION.

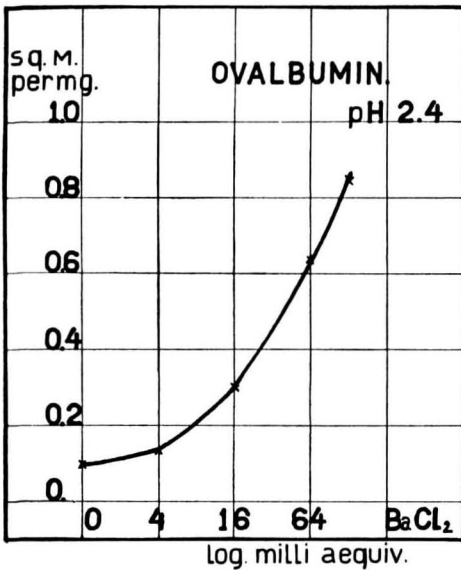


Fig. 3a

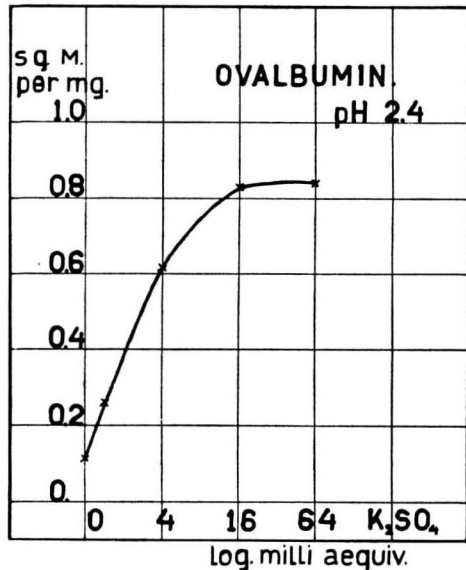


Fig. 4

EFFECT OF MTSK⁺⁺⁺ ION.

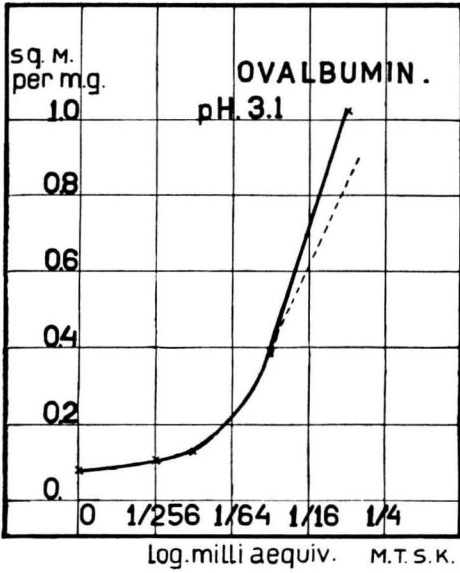


Fig. 5

EFFECT OF K⁺ ION.

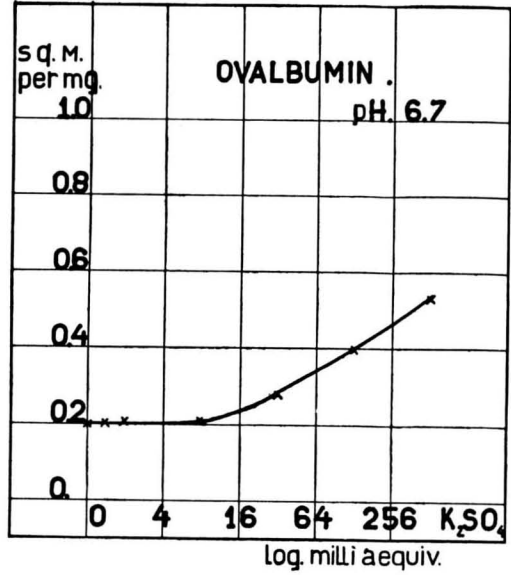


Fig. 6

EFFECT OF Ba⁺⁺ ION.

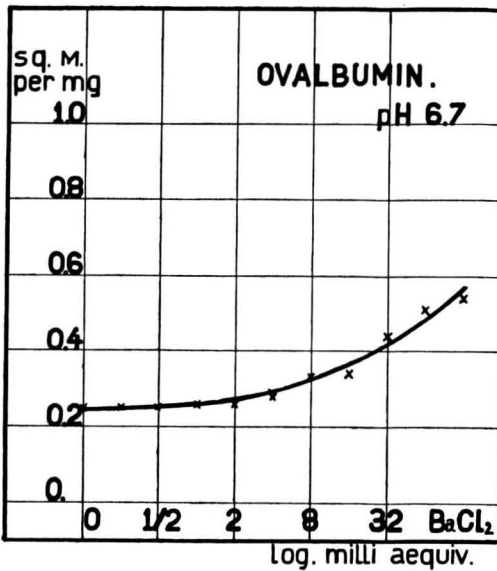


Fig. 7

EFFECT OF LUTEO⁺⁺⁺ ION.

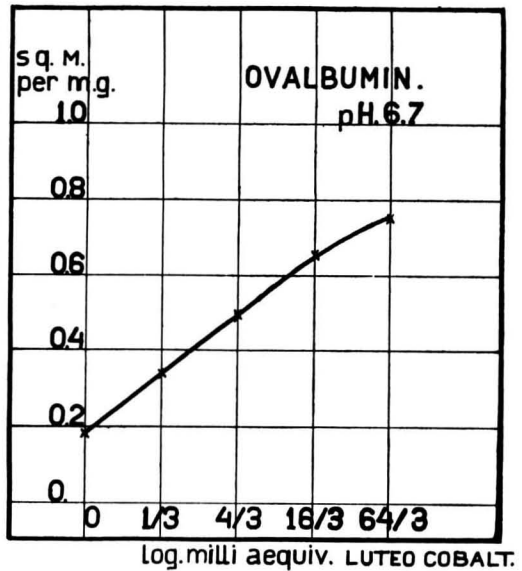
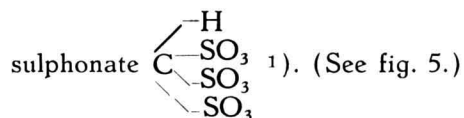


Fig. 8

Cl ions has the tendency to increase the area of spread of a protein salt, that is completely ionized. It has a disionizing effect. The relation between pCl and the area is linear (See already Bioch. Zeitschr. 1. c. p. 404). Now if this interpretation is the right one, bi-valent and tri-valent negative ions should have an identical effect in much lower concentration. This is indeed what had been observed.

We found that SO_4 ions have in much smaller concentration the same discharging effect as the monovalent (See fig. 4). And the tri-valent have an effect, that is identical, but can be observed when making use of still lower concentrations. The tri-valent negative ion was methane tri-



It was interesting to see what would happen on the alkaline side of the iso-electric point after addition of positive ions.

It is hardly necessary to say that KCl has also an influence on the minimum spreading at the alkaline side (fig. 6), but that K_2SO_4 and the methane trisulphonate have no effect in the small concentrations used.

On the contrary the bi-valent positive ions Ba^{++} and Ca^{++} have the same effect on the spreading at pH 8 as the SO_4^{--} at pH 3 (fig. 7) and we were also able to observe a very strong influence of luteocobaltchloride $\text{CO}(\text{NH}_3)_6\text{Cl}_3$ on the spreading at pH 8 (see fig. 8). It is obvious, that this adds convincing arguments to the interpretation given before: at the isoelectric point the neutral protein-molecule spreads in a thin layer; the addition of H^+ (and OH^-) ions diminishes the area occupied by the protein, so much so that it occupies only $1/9$ of the surface. Further addition of negative ions at the acid side of the isoelectric point (from pH 3 downwards) increases the surface by eliminating the charge of the protein ions. The same holds good for the addition of positive ions in alkaline medium. Valency has a very striking influence: tri-valent ions acting more strongly than bi-valent and mono-valent ions having a much smaller influence than bi-valent.

¹⁾ We made use of this salt following the advise of Prof. BUNGENBERG DE JONG. It was kindly given to us by Prof. BACKER.

Mathematics. — *Zur generellen Feldtheorie. DIRACsche Gleichungen und HAMILTONSche Funktion.* Von J. A. SCHOUTEN und D. VAN DANTZIG. (Communicated by Prof. P. EHRENFEST).

(Communicated at the meeting of May 28, 1932).

In der vorigen Mitteilung¹⁾ haben wir u. a. gezeigt dass in der

¹⁾ J. A. SCHOUTEN und D. VAN DANTZIG, Zum Unifizierungsproblem der Physik. Skizze einer generellen Feldtheorie, Proc. Kon. Akad. **35** (1932) 642—655. zitiert mit G. F. I.