Medicine. — *Spreading of gliadin.* I. By E. GORTER and P. C. BLOKKER.

(Communicated at the meeting of February 28, 1942.)

Introduction.

In the investigations on the spreading of various proteins made by one of us in collaboration with GRENDEL [1]), a few experiments were also made with gliadin. Of late years several experimenters have investigated the spreading of this remarkable protein more thoroughly [2]), [3]), [4]). Moreover in the last mentioned investigation COCKBAIN and SCHULMAN studied the influence of tannic acid on gliadin films. As the results of the experiments with gliadin show a marked difference with those carried out with most of the other proteins, it seemed to us worthwhile to make a closer study of the properties of gliadin films. Particularly we investigated the influence of the pH on the spreading pressure, the surface potential and the surface viscosity.

Apparatus:

The method of *spreading* invariably used by us consists in carefully blowing a small quantity of protein solution on to the water surface by means of a micropipette.

Experiments.

The gliadin was prepared from wheat gluten according to the method described by DILL and ALSBERG [5]). The gliadin was obtained in a very pure state. The nitrogen content of the dried substance was 17.3 %. The liquid spread was a clear solution of gliadin in 60 per cent (by volume) aqueous ethyl alcohol. The concentration of the solution was invariably 0.58 %, in the viscosity experiments only the concentration was twice as great.

To study the influence of the pH on the properties of gliadin films the spreading was effected on aqueous solutions of different pH. The solutions used for pH 3.5—5.6 were 1/300 mol. sodium acetate-acetic acid buffers. The solutions of higher acidity were made with hydrochloric acid. For pH 5.6—9.0 1/300 mol. veronal-acetate buffer solutions as described by MICHAELIS [6]) were used. The solutions of higher alkalinity were made with sodium carbonate. The pH of the solutions was controlled by measurements with a hydrogen electrode; a sensitive mirror galvanometer served as zero instrument.

In connection with the publication of COCKBAIN and SCHULMAN [4]) the influence of the pH on the properties of gliadin-tannic acid films was also studied. The tannic acid (KAHLBAUM pro analyse) was purified by extraction with petroleum spirit in order to remove traces of fats etc. which might be present. However, the preparation still contained impurities after this treatment. This was shown in blank experiments with solutions of tannic acid in the buffers (about 100 mg. per l. buffer); notwithstanding repeated cleaning of the surface of the solution with a paraffined glass rod, after some time rather high pressures were found when the surface was compressed.

[1]) E. GORTER and F. GRENDEL, Proc. Acad. Sc. A'dam 29, 1262 (1926); Trans. Faraday Soc. 22, 477 (1926).

[2]) J. S. MITCHELL, Trans. Faraday Soc. 33, 1129 (1937).

[3]) A. H. HUGHES and E. K. RIDEAL, Proc. Roy. Soc. A 137, 62 (1932).

[4]) E. G. COCKBAIN and J. H. SCHULMAN, Trans. Faraday Soc. 35, 1266 (1939).

[5]) D. B. DILL and C. L. ALSBERG, J. biol. Chem. 65, 279 (1925).

[6]) L. MICHAELIS, Bioch. Z. 234, 139 (1931).

Pressure-area measurements on gliadin and gliadin-tannic acid films.

In fig. 1 are shown some pressure-area curves of gliadin at different pH values. These curves do not contain a linear part as is the case with most proteins. They satisfy the equation of a rectangular hyperbole, which indicates that the films are of the liquid type.
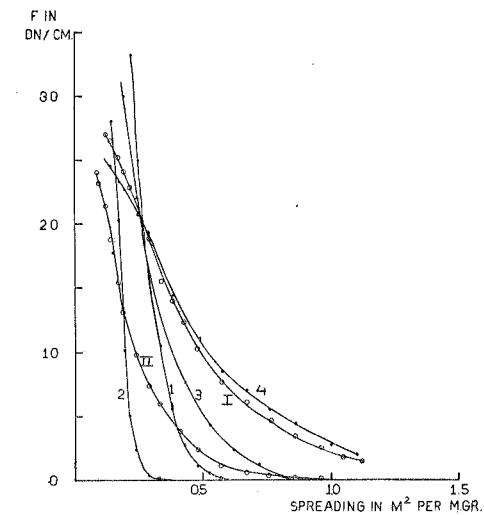

Fig. 1.   Surface pressure as a function of the area occupied by one mg. substance for gliadin and gliadin-tannic acid films.

I = curve for gliadin film at pH = 2.0
II = ,, ,, ,, ,, ,, pH = 3.6
1 = curve for gliadin-tannic acid film at pH = 2.0 and 5.9
2 = ,, ,, ,, ,, ,, ,, ,, pH = 2.9
3 = ,, ,, ,, ,, ,, ,, ,, pH = 7.5
4 = ,, ,, ,, ,, ,, ,, ,, pH = 11.1

For such expanded films LANGMUIR [7]) derived the equation:

$$(F—F_s) \ (a—a_0) = kT$$

where F = pressure of the film, a = area of spread, T = abs. temperature and F_s, a_0 and k are constants; a_0 is analogous to the b-term in the VAN DER WAALS equation. However, with gliadin in most cases negative a_0 values were found and only in a few cases very small positive a_0 values, so that the equation looses much of its physical meaning in the case of gliadin. For that reason we have refrained from characterizing our curves by this equation. The negative a_0 values may be explained as follows: the measurements showed that in the case of rather high pressures after compressing the film the pressure decreased slowly (sometimes 10 %) and after some time became constant. These final pressures could be reproduced very well. Probably this phenomenon is caused by squeezing out hydration water from between the micelles of the film. On diminishing the pressure the reverse phenomenon was observed.

On account of the lack of a linear part in the pressure-area curves, it is not possible to extrapolate the curves in the usual way to zero-force. COCKBAIN and SCHULMAN's curves do not contain a linear part either; their curves, however, are less bent than ours. In a former publication [8]) SCHULMAN and RIDEAL plotted the pressure against the

[7]) I. LANGMUIR, J. chem. Physics 1, 756 (1933).

[8]) J. H. SCHULMAN and E. K. RIDEAL, Proc. Roy. Soc. B 122, 29 and 46 (1937).

surface concentration. The curves obtained contain a considerable linear part. In extrapolating these curves to zero-force one finds a concentration of about 0.8 and 0.6×10^{-7} g cm² at pH respectively 2.0 and 5.9 which corresponds to an area of about 1.3 resp. 1.6 m²/mg. In extrapolating our curves in this way we find an area of 1.5 resp. 1.3 m²/mg. Finally, it may be stated that GORTER and GRENDEL[1]) neither found a linear part in the pressure-area curves at room temperature, but found one at 40° C. We could not reproduce this latter observation; at 40° C. we found the same type of curves as at room temperature.

The variations in the curves found by different authors may be explained by variations in the manner of preparation of the gliadin, which may influence the character of the protein and therefore also the pressure-area relations obtained.

As extrapolating the pressure-concentration curves to zero pressure in our case has little physical meaning and, moreover, confusion may occur with values for other proteins, obtained in the usual way of extrapolating pressure-area curves, we dropped the first mentioned extrapolation; therefore we only give the area for some arbitrary pressures (see fig. 2).
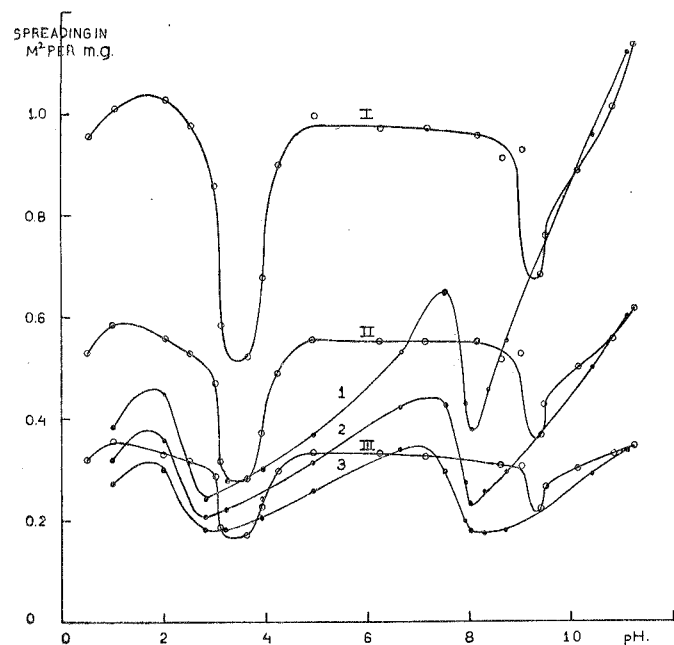

Fig. 2. Spreading of gliadin and gliadin-tannic acid films as a function of the pH.
I, II and III curves for gliadin at surface pressures of resp. 2, 8 and 16 dn/cm.
1, 2 and 3 curves for gliadin-tannic acid at the same surface pressures.

The area of spread for gliadin at different pH values for three arbitrary pressures is shown in fig. 2. The maximum spreading occurs in the vicinity of the isoelectric point (pH about 6.5). Contrary to other proteins this maximum is not a sharp one; moreover in the minima the spreading is rather great. Possibly both phenomena are due to the solvent, as alcohols often increase the spreading of proteins[9]). With respect to the influence of electrolytes on the area of spread gliadin behaves as the other proteins. In consequence of the high concentration of H^+ or OH^- ions the area occupied by

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[9]) L. FOURT and A. PERLEY, Proc. Soc. exptl. Biol. Med. **33**, 201 (1935);
    S. STÄLLBERG and T. TEORELL, Trans. Faraday Soc. **35**, 1413 II (1939).

gliadin at very low or high pH values is great; addition of 0.01 gr. eq. SO_4^{--} to buffer solutions that give minimal spreading at the acid side or 0.01 gr. eq. Ca^{++} to those on the alkaline side, strongly enlarges the area of spread.

The effect of tannic acid on gliadin films was studied by spreading the gliadin solution on buffers containing 100 mg tannic acid per l.. Higher concentrations of tannic acid only slightly increased the effect found. On the acid side the type of curves has altered entirely; the steep linear part (see fig. 1) indicates that the films are solid. COCKBAIN and SCHULMAN who injected tannic acid underneath gliadin films already present[4]) found that the films obtained collapsed at pressures greater than about 0.5 dn/cm. In our experiments we stated a similar phenomenon. We doubt, however, if this is to be considered as collapsing; after each compression the pressure rises very much and then decreases considerably, but after some time the pressure becomes practically constant at a pressure yet much higher than before. So the curves shown in fig. 1 are obtained by waiting after each compression till the pressure was practically constant.

The reproducibility of the area at which the first measurable pressure occurred was rather bad. This is not to be wondered at as the area occupied by the protein will be mainly determined by the velocity of spreading of the gliadin solution and by the velocity of reaction between gliadin and tannic acid. The gliadin-tannic acid complex itself only spreads slowly or not at all; this was shown by the fact that a compressed gliadin-tannic acid film, even after waiting a long time, exerted no or only a small force on the differential balance when the surface was enlarged. It is even possible that the complex films obtained with our method were not monomolecular in all places and had no uniform thickness. Therefore one should not attach much importance to the areas obtained by extrapolating the curves to zero-pressure.
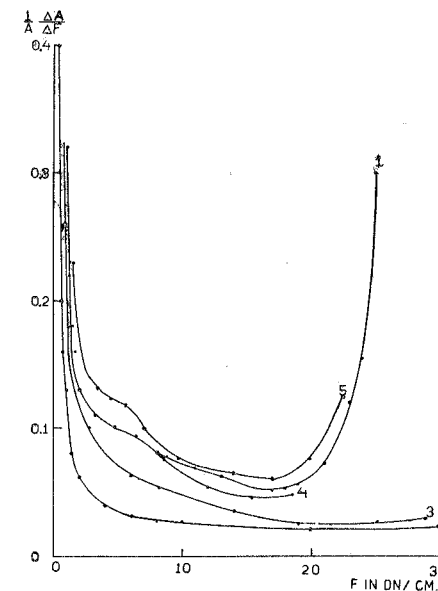

Fig. 3. Compressibility of gliadin films and of gliadin-tannic acid films at different surface pressures F.

1 = gliadin film for pH = 1—11
2 = gliadin-tannic acid film for pH = 1—6.6
3 =   „   „   „   „   „ pH = 7.5—7.9
4 =   „   „   „   „   „ pH = 8.7
5 =   „   „   „   „   „ pH = 10.4

Fig. 1 and 2, and also all further observations with potential- and viscosity measurements, show that above pH 6—7 the complex films behave more and more as gliadin films when the pH increases. At pH = 11 the influence of tannic acid has disappeared.

An interesting result is obtained when we calculate the *compressibility* $-\dfrac{1}{A}\dfrac{dA}{dF}$ (the reverse of surface elasticity). Fig. 3 shows that the compressibility of gliadin is equal at all pH values. Therefore it is very likely that the pH has no influence on the character of the film, but only affects the amount of gliadin that collects in the surface.

Above a pressure of about 20 dn/cm the compressibility strongly increases. Evidently the molecules are pressed out of the film at high pressures and are pushed over each other.

The curves for gliadin show a small bend at a pressure of about 6 dn/cm. This is probably connected with initial gelation (see f.i. 4) at this pressure, a phenomenon that will be dealt with in more detail in treating the viscosity measurements. As the deviation is of the same order as the error made in deriving the compressibility ($\pm$ 0.01) it is not to be excluded, however, that the bend has to be ascribed to accidental causes.

Finally the compressibility curves show clearly that the tannic acid diminishes the compressibility of gliadin films considerably at pH values below 7 and that this influence strongly decreases at higher pH.

(To be continued.)
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Applied Mechanics. — *On the state of stress in perforated strips and plates.* By K. J. SCHULZ. (Communicated by Prof. C. B. BIEZENO.)

(Communicated at the meeting of February 28, 1942.)

1. *Introduction.* In a former treatise (which in this paper will be indicated by the letter "A") the stress-problem of infinite plates containing circular holes of arbitrary position and arbitrary radii has been studied at great length. Special attention was drawn to such problems in which equal holes were arranged in one or more infinite rows of constant pitch and in which the states of stress and strain showed periodicity with a period equal to that pitch. Further investigation of these problems led to the result that by renewed use of this periodicity a whole class of other problems can be made accessible by the same method, namely those problems which bear upon the semi-infinite plate containing one or more rows of equal holes parallel to the edge of the plate, respectively upon a strip of infinite width with one or more rows of holes parallel to its edges. Though the holes of each row must be of equal radius, it is not necessary that this equality holds for the holes of different rows. On the other hand it is required, that the geometrical configuration of the holes and the loadsystem of plate or strip possess a common period. As to the strip, the latter condition is fulfilled in the cases of pure tension and pure bending; bending accompanied with shear requires a separate treatment.

2. *The method of investigation.* The starting point of our investigation is the well known stress function F of Airy from which the stress-components in Cartesian coordinates y, z (conceived as the average over the thickness of the plate, which furthermore will be put equal to unity) are to be derived by

$$\sigma_y = \frac{\partial^2 F}{\partial z^2}, \qquad \sigma_z = \frac{\partial^2 F}{\partial y^2}, \qquad \tau_{yz} = -\frac{\partial^2 F}{\partial y\,\partial z}. \quad . \quad . \quad (1)$$

The function itself is defined by the bi-harmonic equation

$$\triangle' \triangle' F = 0 \qquad \left(\triangle' = \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}\right). \quad . \quad . \quad . \quad (2)$$

and the stress-conditions which relate to the inner and outer boundaries of the plate.

In polar coordinates, r, φ

$$y = r \cos \varphi, \qquad z = r \sin \varphi, \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

the stress-components are expressed by

$$\sigma_r = \frac{1}{r^2} \frac{\partial^2 F}{\partial \varphi^2} + \frac{1}{r} \frac{\partial F}{\partial r}, \quad \sigma_\varphi = \frac{\partial^2 F}{\partial r^2}, \quad \tau_{r\varphi} = -\frac{\partial}{\partial r}\left(\frac{1}{r}\frac{\partial F}{\partial \varphi}\right), \quad . \quad (4)$$

whereas the bi-harmonic equation takes the form

$$\triangle' \triangle' F = 0 \qquad \left(\triangle' = \frac{\partial^2}{\partial r^2} + \frac{1}{r}\frac{\partial}{\partial r} + \frac{1}{r^2}\frac{\partial^2}{\partial \varphi^2}\right). \quad . \quad . \quad (5)$$

The relations between the sets of stresses $\sigma_y, \sigma_z, \tau_{yz}$ and $\sigma_r, \sigma_\varphi, \tau_{r\varphi}$ are given by

$$\left.\begin{aligned}
\sigma_r &= \tfrac{1}{2}(\sigma_y + \sigma_z) + \tfrac{1}{2}(\sigma_y - \sigma_z)\cos 2\varphi + \tau_{yz}\sin 2\varphi, \\
\sigma_\varphi &= \tfrac{1}{2}(\sigma_y + \sigma_z) - \tfrac{1}{2}(\sigma_y - \sigma_z)\cos 2\varphi - \tau_{yz}\sin 2\varphi, \\
\tau_{r\varphi} &= \qquad\qquad -\tfrac{1}{2}(\sigma_y - \sigma_z)\sin 2\varphi + \tau_{yz}\cos 2\varphi.
\end{aligned}\right\} \quad . \quad . \quad (6)$$