and —229° C. We have not yet been able to complete the structure analysis of these modifications, but there are certain facts which indicate that the molecular elements rotate in form I.

In conclusion I wish to express my sincere thanks to Mr. S. Stensholt and Mr. Th. Ringdal for their most valuable assistance in connection with the investigations described in this paper.

*Physical Institute, Oslo. March 1933.*

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(Communicated at the meeting of November 24, 1934).

The method for forming protein films on the surface of a Langmuir tray, used by us, consists in blowing out over the water surface a 0.5% solution from a calibrated pipette containing 0.005 cc. The pipette is held in a horizontal position, so that the sharp point is above the water surface.

If the spreading of proteins is studied, results differ according to the time elapsing between the moment the micropipette, containing the protein solution is blown out at the surface and the beginning of the reading of the surface-area at different pressures.

Consistent results are easily obtained, if all measurements are made after a constant time, usually one or two minutes, calculated from the moment the pipette has been blown out until the beginning of the compression of the film.

When applying this technique (fig. 1) curves as have been published by us (1) are obtained.

Now it can be shown, that time has a very distinct influence on the results. Maximal spreading (the spreading number or spreading value is found by extrapolating the surface-pressure curve to zero pressure) is obtained within one or two minutes, if the conditions, which produce spreading, are optimal. On the other hand spreading over a smaller surface than the maximal one can often be shown to become maximal, if sufficient time is allowed. On the minima of the curve of ovalbumin (at pH = 2.5 and 6.5 in fig. 1) time again has a very slight influence.

The influence of time was studied separately by measuring surface potentials.

By this method the spreading process can be studied directly (2), the
presence of the protein on the surface giving rise to a very distinct change of the surface potential.

![Graph](image)

On the abscissa are plotted the pH values of the solutions on which the protein was spread, below pH 3.6 HCl-solutions, above pH 3.6 buffers 1/300 N were used. The dotted part of the curve has not yet been measured in detail. On the ordinates are plotted the area of the protein films, found by extrapolation surface-pressure curves to zero-pressure.

It was found that, just after the pipette has been blown out, the protein film is limited to a small circle around the pipette, the boundary B of which is wandering over the surface with a certain velocity (fig. 2), which could be measured by following this boundary with the air-electrode Po (fig. 3).

It turned out that this time is a minimum at pH 1.0, at pH 13.0 and at the iso-electric point of the protein and is raised to high values on both sides of this point.

It will be clear at once, that when compression of the protein film is started before spreading is complete, the spreading number is smaller.

This is the explanation of the typical $W$ curve, indicating the dependance of protein spreading on pH. In all the earlier experiments, when a longer
time was not specially indicated, compression was started one or two minutes after spreading. Therefore not in all cases the spreading process

![Graph showing spreading process](image)

Here the protein was spread on acetate buffer 1/300 N. On the ordinates are plotted the areas of that part of the surface, as indicated in figure 2, that is covered by the protein film. These areas are measured with the movable Polonium electrode Po. The boundary B of the film is easily found, because there is a sharp difference in surface potential between the part of the surface which is covered by the film and that part which is not covered. On the abscissa are indicated the time values calculated from the moment the pipette has been blown out. The film at pH 4.90 is homogeneous as the variations of the surface-potentials are only 2-3 millivolt. The film at pH 3.94 is not homogeneous: here the variations amount to 20 millivolt.

came to an end. When one waits longer the tops of the W curve become more flat as can be seen from figure 4.

The time factor, the time in which equilibrium is reached, indicates a more or less stronger tendency to spread. 1)

Near the minima very long exposures are necessary to reach equilibrium in the case of ovalbumin. They are outside the experimental possibilities, because of the danger of the contamination of the surface. It is hardly possible to believe that in these cases even when measuring some hours after having brought the protein on the surface, true equilibria are formed.

1) Compare E. GORTER en F. GRENDEN: De spreiding van oxy-haemoglobine. These Proceedings, 34, 1925, 1257.
TABLE I.

<table>
<thead>
<tr>
<th>PH of acetate buffer 1/300 N</th>
<th>Time required for boundary B to reach its final position</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.80</td>
<td>&lt; 2 hours</td>
</tr>
<tr>
<td>3.94</td>
<td>&lt; 1 hour</td>
</tr>
<tr>
<td>4.35</td>
<td>&lt; 5'</td>
</tr>
<tr>
<td>4.61</td>
<td>&lt; 3'</td>
</tr>
<tr>
<td>4.90</td>
<td>&lt; 3'</td>
</tr>
<tr>
<td>5.05</td>
<td>&lt; 10'</td>
</tr>
<tr>
<td>5.30</td>
<td>&lt; 15'</td>
</tr>
<tr>
<td>5.72</td>
<td>&lt; 9 hours</td>
</tr>
<tr>
<td>6.00</td>
<td>&lt; 12 hours</td>
</tr>
</tbody>
</table>

PH of HCl solution

<table>
<thead>
<tr>
<th>PH of HCl solution</th>
<th>Time required</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.07</td>
<td>&lt; 10'</td>
</tr>
<tr>
<td>1.33</td>
<td>&lt; 20'</td>
</tr>
<tr>
<td>1.62</td>
<td>&lt; 30'</td>
</tr>
<tr>
<td>1.92</td>
<td>&lt; 40'</td>
</tr>
</tbody>
</table>

Films compressed: Fig. 4.

... 1 minute
××××× 4 minutes
after the pipette containing the protein solution was blown out. Ordinates and abscissa as in figure 1: the same solutions were used.
If maxima are obtained by the addition of positive or negative ions at a pH, at which a maximal spreading in 1 or 2 minutes cannot be produced, the result is as rapidly obtained as in the case mentioned above, in which pH is the factor, that favours spreading.

Adding less of these ions gives intermediate values for the spreading area, which also tend to increase if time is allowed after the protein has been brought on the surface.

If one measures the potential differences over compressed proteinfilms at the point at which maximal spreading is seen on account of a suitable pH or ionic concentration, there are no larger variations observed between different spots of the surface than of 2—3 millivolt.

If, however, smaller areas than the maximal are studied by this method, inhomogenities are often found (2). This is in agreement with the results obtained by Zocher and Stiebel (3), who have shown by ultramicroscopical investigation, that sometimes the films of proteins are not homogeneous.

It is therefore not permissible to calculate a thickness of the films near or at the minima of the curves. From the maximal spreading a thickness of 6—7.5 Å can be derived.

These minima occur when the protein has little spreading tendency and this can be due to either a too great solubility or to the reverse, a too small solubility. This is what occurs in the series of fatty acids, where the lower members are too soluble (up to C_{12}) for showing any spreading and the highest members (C_{60}) have too little spreading tendency owing to the fact, that they resemble too much a paraffin.

An example of a minimum due to small solubility is myosin (4); whereas gelatin exemplifies the minima due to a great solubility.

It is now easy to understand why the method used by Lecomte du Nouy (5) gives results which are comparable to ours if time is allowed in order to obtain equilibrium. From a solution the protein must move to the surface and it depends from the thickness of the waterlayer, the spreading tendency of the protein, and its diffusion coefficient how long this will take. The results obtained by Wu (6) which differ from ours with regard to the time-factor only, are easily explained by a difference in technique. He makes use of dilute solutions of protein and of larger pipettes, so that a great part of the solution is blown under the surface and has to come out of it, which takes more time.

The method used by Rideal and Hughes (7) who study protein crystals, which fall on the surface of the tray from a modified Nernst-balance gives results which are similar to ours.

Their numbers for gliadin at different pressure give a spreading of about one square meter per milligram, if the results are extrapolated to zero-pressure. This is the number we found for most proteins (8).
We are indebted to the ROCKEFELLER foundation for a grant by means of which these investigations have been made possible.

LITERATURE:

Physics. — On the electronic states of the \( N_2^+ \)-molecule and their energies of dissociation. By H. H. BRONS. (Communicated by Prof. D. COSTER.)

(Communicated at the meeting of November 24, 1934).

Introduction. Of the \( N_2^+ \)-molecule four electronic states have been found. These are three \( ^2\Sigma \)-states and one \( ^2\Pi \)-state. The three \( ^2\Sigma \)-states (see fig. 4) are known from transitions \( b^2\Sigma \rightarrow a^2\Sigma \) and \( c^2\Sigma \rightarrow a^2\Sigma \), the last transition recently having been found by WATSON and KOONTZ 1).

The fourth electronic state \( ^2\Pi \) is only known from the perturbations it causes in the \( b^2\Sigma \)-state. Selection rules forbid transitions \( c^2\Sigma \rightarrow ^2\Pi \) and also \( b^2\Sigma \rightarrow ^2\Pi \). Transitions between the \( ^2\Pi \)-state and the \( a^2\Sigma \)-state have not been found in the region of 2000 Å — 10000 Å, which might be understood if we assume these molecular states to lie at a very small distance from each other. Therefore all the knowledge about the \( ^2\Pi \)-state has to be obtained from the perturbations it causes in the \( b^2\Sigma \)-state. Several of these perturbations have already been studied 2). From these perturbations the values \( B_{II} \) of the vibrational levels \( v = X \), \( v = X + 4 \) and \( v = X + 7 \) (see fig. 2) are calculated in the following way 3):

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2) D. COSTER and H. H. BRONS, ZS. f. Phys. 73, 747, 1932.
   ALLAN E. PARKER, Phys. Rev. 44, 90, 1933 and 44, 914, 1933.
   H. H. BRONS, Physica 1, 739, 1934.