Medicine. — The spreading of an ovalbumin-oleic acid complex. By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of September 26, 1942.)

In view of the results obtained with MACHEBOEF's lipoprotein from horse serum 1), it seemed interesting to examine a purely artificial lipoprotein of the type described by PRZYLECKI and HOFER 2). These authors prepared ovalbumin-oleic acid complexes of varying composition, the largest lipid content attainable being about 60%.

10 cc of a 10% solution of oleic acid in alcohol was added drop by drop from a burette into 120 cc of a 1% solution of ovalbumin in 0.04 molar borax buffer pH = ± 9, stirring all the while. The ovalbumin was prepared according to SÖRENSEN 3). The oleic acid was a KAHLBAUM product, redistilled by us at reduced pressure. The solution obtained was slightly opalescent, its pH was 8.6. On acidifying with acetic acid to pH = 5, a white precipitate was formed, which was centrifuged off and redissolved in borax. This procedure was repeated six times. For the sake of brevity we will denote the successive precipitates by B1, B2 etc. The solutions of B4, B5 and B6 were analysed, and gave the following results for the substance precipitated:

<table>
<thead>
<tr>
<th>Substance</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
</tr>
</thead>
<tbody>
<tr>
<td>% oleic acid</td>
<td>57.9</td>
<td>60.0</td>
<td>59.2</td>
</tr>
<tr>
<td>% protein</td>
<td>42.1</td>
<td>40.0</td>
<td>40.8</td>
</tr>
</tbody>
</table>

The composition of the precipitate obviously is sufficiently constant to treat the substance as a definite compound. The solution of B6 was spread in the usual way 4). The areas at zero pressure are given in the figure, where they have been compared with the corresponding ones for ovalbumin. It is seen that the minimum in the area-pH curve at the acid side has disappeared, showing that the oleic acid combines with the amino groups of the protein. It should further be mentioned that the pressure-area curves below pH = 6 are of the type common to lipids. Collapse of the film does not occur even at pressures which are higher than the collapse point of oleic acid itself. The fact that the ovalbumin thus strengthens the oleic acid film (and vice versa) is obviously of considerable interest to the physical chemistry of natural and artificial membranes.

At pH > 7, however, the films show protein-like behaviour, and pressures larger than about 25 dynes/cm cannot be maintained.

If ovalbumin is made to react with smaller quantities of oleic acid, compounds of varying composition can be obtained 5). It seemed interesting to study the effect of a larger protein content on the spreading properties of the lipoprotein. To that end only 2 cc of oleic acid, 10%, in alcohol was slowly added to 60 cc ovalbumin, 1% in borax of pH = 9. The resulting solution was acidified with acetic acid, the precipitate redissolved in borax, and so on. For convenience sake let us denote these precipitates by C1, C2, etc. The products C4 and C5 were analysed, giving:

<table>
<thead>
<tr>
<th>Substance</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>% oleic acid</td>
<td>40.6</td>
<td>41.3</td>
</tr>
<tr>
<td>% protein</td>
<td>59.4</td>
<td>58.7</td>
</tr>
</tbody>
</table>

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2) ST. J. V. PRZYLECKI and E. HOFER, Biochem. Z. 288, 303 (1936); Acta Biol. Exp. 12, 70 (1938).
The spreading areas of C5 are recorded in the graph. There clearly is a continuous change in the spreading properties of this lipoprotein if the protein content is altered. This change is also apparent in the force-area curves. As was to be expected, the force-area curves of C5 are of the type common to proteins if pH > 6. With pH below 6, however, the behaviour of C5 films is quite different from that of B6. The B6 films could be compressed to high pressures without collapse. The C5 films, however, show partial collapse at a certain pressure. Here part of the protein is squeezed out of the monolayer and we are then left with a film which is very similar to the B6 film. No detailed description of this behaviour will be given here, since pressure-area curves of a similar nature have been studied by SCHULMAN and others (5) who obtained complex films by injecting substances underneath monolayers.

In the present case it would seem that the protein is only partly squeezed out, since the resulting film is able to resist higher pressures than the oleic acid. This result is similar to that obtained with B6 and, in fact, the force-area curves of C5 in this later part are quite compatible with the assumption that no more protein is squeezed out than is needed to bring the lipid content up to about 60%, the largest figure obtained so far in the ovalbumin-oleic acid compounds prepared by the method described here.