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 MACHEBOEUF'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 lipoid 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 $p_H = \pm 9$, 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 p_H was 8.6. On acidifying with acetic acid to $p_H = 5$, 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 B_1, B_2 etc. The solutions of B_4, B_5 and B_6 were analysed, and gave the following results for the substance precipitated:

substance	B_4	B_5	B_6
% oleic acid	57.9	60.0	59.2
% protein	42.1	40.0	40.8

The composition of the precipitate obviously is sufficiently constant to treat the substance as a definite compound. The solution of B_6 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-p_H 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 $p_H = 6$ 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 $p_H > 7$, 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 [2]). 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 $p_H = 9$. 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 C_1, C_2, etc. The products C_4 and C_5 were analysed, giving:

substance	C_4	C_5
% oleic acid	40.6	41.3
% protein	59.4	58.7

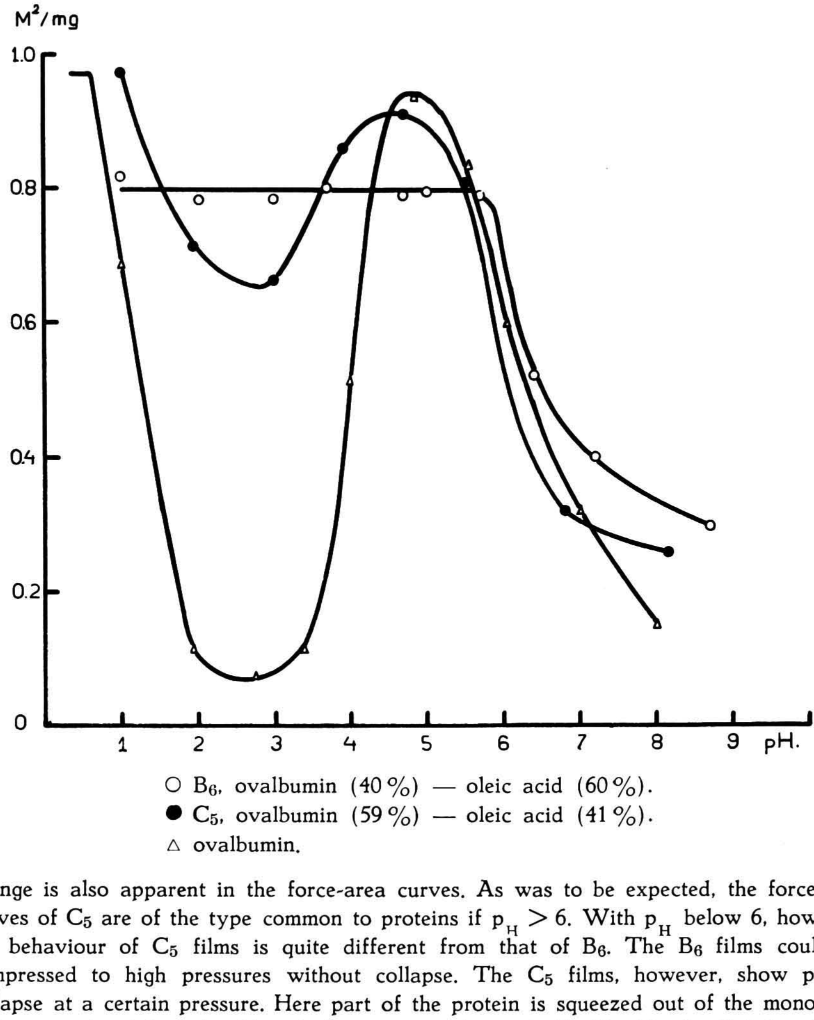
[1]) E. GORTER and J. J. HERMANS, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 804 (1942).

[2]) ST. J. V. PRZYLECKI and E. HOFER, Biochem. Z. **288**, 303 (1936); Acta Biol. Exp. **12**, 70 (1938).

[3]) Compare: E. GORTER, J. V. ORMONDT and F. J. P. DOM, Proc. Kon. Akad. v. Wetensch., Amsterdam, **35**, 838 (1932).

[4]) E. GORTER and collaborators, Proc. Kon. Akad. v. Wetensch., Amsterdam, **37**, 788 (1934); **29**, 371 (1926).

The spreading areas of C_5 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



○ B_6, ovalbumin (40 %) — oleic acid (60 %).
● C_5, ovalbumin (59 %) — oleic acid (41 %).
△ ovalbumin.

change is also apparent in the force-area curves. As was to be expected, the force-area curves of C_5 are of the type common to proteins if $p_H > 6$. With p_H below 6, however, the behaviour of C_5 films is quite different from that of B_6. The B_6 films could be compressed to high pressures without collapse. The C_5 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 B_6 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 B_6 and, in fact, the force-area curves of C_5 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.

[5]) J. H. SCHULMAN, Proc. Roy. Soc. London, A **155**, 701 (1936).
F. COCKBAIN and J. H. SCHULMAN, Trans. Farady Soc. **35**, 716 (1939).
A. HUGHES, Biochem. J. **29**, 430 (1935).
J. H. SCHULMAN and A. HUGHES, Biochem. J. **29**, 1243 (1935).
J. H. SCHULMAN and E. K. RIDEAL, Proc. Roy. Soc. London, B **122**, 29 (1937); **126**, 356 (1939).