it seems that we have to deal with a stable unmixing. In how far this is indeed stable and does not remain to exist only as a result of an insufficient formation of crystal-germs or (and) of a too small crystallization velocity can of course not be decided as long as the expected crystalline "colloid-colloid salts" (e.g. gelatin-arabinate) are not yet known.

Between the complex coacervation and the analogous unmixing in crystalloid salt solutions thus far the apparently fundamental difference existed that in the latter case the analogous inter-relation between the two oppositely charged ions only seemed to allow metastable unmixing, i.e. where the "coacervate" was only passed as a transition-stage to the stable ordered-crystalline condition.

In the examples described here the unmixing above certain temperatures is indeed stable and the fundamental difference which we considered still present between complex coacervation and its crystalloid analogue consequently does not exist.

Leiden, December 1936.

Chemistry. — *The spreading of Protamine Insulinate.* By E. GORTER and L. MAASKANT. (From the Laboratory of the Children's Hospital of the University of Leiden, Holland.) (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of December 19, 1936).

It is possible to combine the insulin with some basic group, so that the combination has its iso-electric point nearer to the p_H of the tissue fluid than insulin.

By combining the usual insulin hydrochloride (in solution p_H = about 2.5) with a protamine a compound is formed, which has its point of minimum solubility at about the p_H of the blood serum.

H. C. HAGEDORN, B. NORMAN JENSEN, N. B. KRARUP, J. WODSTRUP NIELSEN [1]) prepared a special insulin preparation which is absorbed more slowly and, therefore, has a more gradual effect than ordinary insulin. This "Leo Insulin Retard" is manufactured by the "Nordisk Insulin Laboratorium".

We have now studied the spreading of Leo Insulin Retard and an insulin-clupeïn complex, made by us.

GORTER and VAN ORMONDT [2]) showed that insulin is a very well-spreading substance. This protein shows a slightly diminished spreading at the acid side of the iso-electric point, which proves that the tendency to spread in a charged condition of the protein is high.

GORTER and his collaborators [3]) studied the spreading of artificially

[1]) Journ. Am. Med. Assn. Vol. **106** (1936).

[2]) Proc. Royal Acad. Amsterdam, **36**, 922 (1933).

[3]) E. GORTER, H. VAN ORMONDT, TH. M. MEYER, Biochem. J. **29**, 38 (1935).

prepared complex proteins of different types in order to prove the correctness of the supposition, that the type of the p_H-area curve depends on the ionizable groups.

We mentioned already, that insulin can be combined with a protamine. It is obvious that the latter, being a basic substance must combine with the free COOH groups of the insulin. When studying the spreading of this complex protein, we see no change at the acid side of the iso-electric point, whereas the minimum on the alkaline side disappears completely (see fig. 1). This figure shows, that the spreading measurements of these two complex proteins agree. The amount of the protamine in these complex proteins is small (1 : 10). Moreover the protamines themselves belong to that class of proteins, which do not spread owing to the too great solubility in the solutions used.

In order to study the effect of p_H on the amount of the spreading we used: dilute hydrochloric acid solutions between 1 and 3, a $^1/_{300}$ molar sodium acetate-acetic acid solution between p_H 3.6 and 5.5 and $^1/_{300}$ molar veronal-acetate buffer solutions according to MICHAELIS between p_H 5.5 and 7.5.

We have also made turbidity measurements in $^1/_{300}$ mol. phosphate

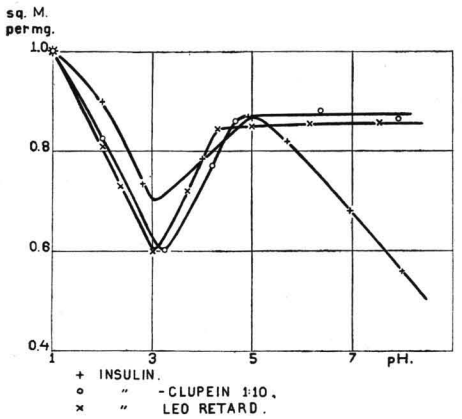

Fig. 1.

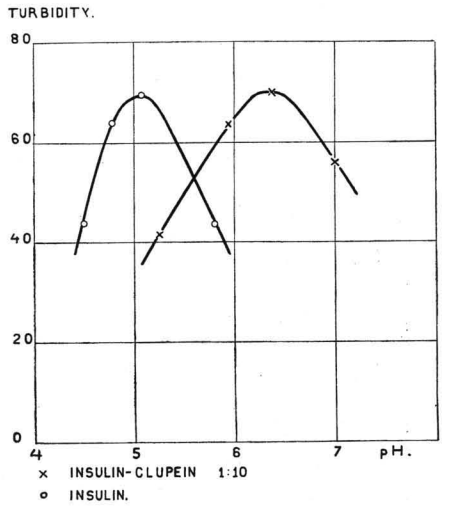

Fig. 2.

buffer solutions of the insulin-clupeïn complex, which show that the point of minimum solubility approach the p_H of the blood serum (see fig. 2).

These spreading experiments give also an explanation of the fact, that these insulin protamines have a more gradual effect, when injected in the body as the insulin.

We have made use of insulin of a pure preparation from „N.V. Organon" at Oss (Netherlands). To control the purity of the insulin this

substance was recrystallized after D. A. SCOTT [1]). There was no change in purity after the recrystallization.

The clupein was prepared according to KOSSEL [2]), from the sperm of the herring. The testicles are put through a mincing machine and the pulpy mass suspended in 4 or 5 volumes of water. The milky liquid is treated with dilute acetic acid until strongly acid to congo red. After filtering the precipitate is extracted with alcohol and ether. About 20 grams of the remaining white floury mass are shaken for half an hour with 100 cc 1 per cent sulphuric acid and filtered. The sulphuric acid extract is precipitated with three volumes of alcohol and the precipitate, consisting of the protamine sulphate, collected, dissolved in a little hot water and reprecipitated with alcohol. Further purification was effected by treating a warm aqueous solution of the protamine sulphate with sodium picrate. Clupein was obtained as the free base by treating the aqueous solution of the sulphate with baryta.

Insulin with clupein.

A solution of insulin in hydrochloric acid ($p_H = 2.5$), containing 5 mg. insulin per ml. was prepared and 2 cc. of this solution was mixed with 0.1 cc. of a clupein solution, containing 10 mg. clupein per ml.

Summary.

The spreading of Leo Insulin Retard and an insulin-clupein complex was studied in comparison with the results of the spreading of insulin. These experiments show, that the minimum on the alkaline side of the insulin p_H-area curve disappears completely, when this protein is combined with a protamine.

1) J. of Biol. Chem., **92**, 281 (1931).
2) A. KOSSEL, The protamines and histones, p. 19.

Chemistry. — *The spreading of urease and Bence-Jones protein.* By E. GORTER and L. MAASKANT. (From the Laboratory of the Children's Hospital of the University of Leiden, Holland.) (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of December 19, 1936).

We have now studied the spreading of two other proteins and examined the influence of the p_H of the solution in the tray on the spreading of urease and Bence-Jones protein.

The general rule is, that the spreading is maximal at the iso-electric