

*Citation:*

Arrhenius, S. & Hamburger, H.J., On the nature of precipitin-reaction, in:  
KNAW, Proceedings, 9 I, 1906, Amsterdam, 1906, pp. 33-45

I have tried to find a meltingpoint-line of the already described type in the monoclinic derivatives in which some degree of form-analogy is noticeable. However, in none of the three binary mixtures this was the case; the lower melting point was *lowered* on addition of the component melting at the higher temperature, without formation of mixed crystals. For instance:

A mixture of 82,3% 1-2-3- and 17,7% 1-3-5-*Nitrodibromo-benzene* melted at  $48\frac{1}{2}^{\circ}$  C.

A mixture of 76,5% 1-2-6- and 23,5% 1-3-5-*Nitrodibromo-benzene* at  $68\frac{1}{2}^{\circ}$  C.

A mixture of 90,5% 1-3-4- and 9,5% 1-2-6-*Nitrodibromo-benzene* at  $54^{\circ}$  C.

Moreover, no mixed crystals could be obtained from mixed solutions.

The slight form-analogy with the *Nitro-dichloro-benzenes*<sup>1)</sup> investigated by me some time ago is rather remarkable.

*Nitro-2-3-Dichloro-Benzene* ( $62^{\circ}$  C. rhombic) and *Nitro-2-6-Dichloro-Benzene* ( $71^{\circ}$  C. monoclinic) exhibit practically no form-analogy with the two *Dibromo*-compounds. There is also nothing in the *Dichloro*-derivatives corresponding with the isomorphotropic mixture of the 2-3- and 2-5-*Dibromo*-product. The sole derivatives of both series which might lead to the idea of a direct isomorphous substitution of two *Cl*- by two *Br*-atoms are the *Nitro-3-5-Dihalogen-Benzenes* ( $65^{\circ}$  C. and  $104^{\circ},5$  C.); the melting point of the *Dichloro*-derivative is indeed *elevated* by an addition of the *Dibromo*-derivative.

As a rule, the differences in the crystal-forms of the compounds of the brominated series are much less than those between the forms of the chlorinated derivatives — a fact closely connected with the much greater value which the molecular weight possesses in the *Nitro-Dibromo-Benzenes* than in the corresponding *Chloro*-derivatives.

Zaandam, April 1906.

**Physiology.** — “*On the nature of precipitin-reaction.*” By Prof. H. J. HAMBURGER and Prof. SVANTE ARRHENIUS (Stockholm).

(Communicated in the meeting of April 27, 1906).

One of the most remarkable facts discovered during the last years in the biological department, is most certainly the phenomenon that when alien substance is brought into the bloodvessels the individual reacts upon it with the forming of an antibody. By injecting a

<sup>1)</sup> These Proc. VII, p. 668.

toxin into the bloodvessels, the result is, that this is bound and free antitoxin proceeds. EHRLICH explains this as follows. When a toxin is injected, there are most probably cells which contain a group of atoms able to bind that alien substance. Now WEIGERT has stated the biological law, that when anywhere in the body tissue is destroyed, the gap usually is filled up with overcompensation. So, it may be assumed, that when the cell loses free groups of atoms, so many of these new ones are formed, that they can have no more place on this cell and now come in free state in circulation. This group of atoms is the antitoxin corresponding to the toxin.

As a special case of this general phenomenon the forming of precipitin is to be considered.

When a calf is repeatedly injected with horseserum, which can be regarded as a toxic liquid for the calf, then after some time it appears that in the bloodserum of that calf an antitoxin is present. In taking some bloodserum from this calf and by adding this to the horseserum a sediment proceeds. This sediment is nothing else than the compound of the toxin of the horseserum with the anti-toxin that had its origin in the body of the calf. We are accustomed to call this antitoxin *precipitin*, and the toxin here present in the horseserum, and which gave cause to the proceeding of precipitin, *precipitinogen* substance. The compound of both is called *precipitum*.

It is very remarkable that such a precipitate proceeds only, when the precipitin is brought in contact with its *own* precipitinogen substance. Indeed by adding the designed calfserum containing precipitin, not to the horseserum but to the serum of another animal, no precipitate proceeds. In this we have also an expedient to state if in a liquid (e.g. an extract of blood stain) horseserum is present or not (UHLÉNTHUTH, WASSERMANN inter alia). Meanwhile such a calfserum gives notwithstanding also a precipitum with serum of the ass related to the horse.

To the same phenomenon the fact is to be brought, that when a rabbit has been injected with oxenserum, the serum taken from the rabbit does not only give a precipitate with oxenserum but also with that of the sheep and the goat, which are both related to the ox.

Some time ago an expedient was given to distinguish also <sup>1)</sup> serum proteid from *related* species of animals by a quantitative way, and in connection with this a method <sup>2)</sup> was proposed to determine accu-

<sup>1)</sup> H. J. HAMBURGER, Eine Methode zur Differenzirung von Eiweiss biologisch verwandter Thierspecies. Deutsche Med. Wochenschr. 1905, S. 212.

<sup>2)</sup> H. J. HAMBURGER, Zur Untersuchung der quantitativen Verhältnisse bei der Präcipitinreaction. Folia haematologica. II Jahrg. No. 8.

rately the quantity of precipitate which is formed by the precipitin reaction. This method also permitted to investigate quite generally the conditions which rule the formation of precipitate from the two components.

Immediately two facts had pushed themselves forward by a preliminary study which were also stated in another way by EISENBERG <sup>1)</sup> and ASCOLI <sup>2)</sup>.

1. That when to a fixed quantity of calfserum <sup>3)</sup> (precipitin = antitoxin) increasing quantities of diluted horseserum (precipitinogen substance = toxin) were added, the quantity of precipitate increased, in order to decrease by further admixture of diluted horse serum.

2. that whatever may have been the proportion in which the two components were added to each other, the clear liquid delivered from precipitate always give a new precipitate with each of the components separately. This leads to the conclusion that here is question of an equilibrium reaction in the sense as it has been stated and explained for the first time by ARRHENIUS and MADSEN <sup>4)</sup>.

This conclusion has become also the starting point of the now following researches of *which the purpose was to investigate by quantitative way the principal conditions by which precipitin reaction is ruled.*

#### *Methods of investigation.*

To a fixed quantity of calfserum <sup>5)</sup> (precipitin = antitoxin) increasing quantities of diluted horse-serum (precipitinogen substance =

<sup>1)</sup> EISENBERG. Beiträge zur Kenntniss der specifischen Präcipitationsvorgänge Bulletin de l'Acad. d. Sciences de Cracovie. Class. d. Sciences Mathem. et nat. p. 289.

<sup>2)</sup> ASCOLI. Zur Kenntnis der Präcipitinwirkung. Münchener Med. Wochenschr. XLIX Jahrg. S. 398.

<sup>3)</sup> They used sera of other animals.

<sup>4)</sup> ARRHENIUS und MADSEN. Physical chemistry to toxins and antitoxins. Festschrift ved indvielsen of Statens Serum Institut. Kjobenhavn 1902; Zeitschr. f. physik. Chemie 44, 1903, S. 7.

In many treatises the authors have continued these investigations; compare e.g. still:

ARRHENIUS. Die Anwendung der physikalischen Chemie auf die Serumtherapie. Vortrag gehalten im Kaiserl. Gesundheitsamt zu Berlin am 22 Sept. 1903. Arbeiten aus dem Kaiserl. Gesundheitsamt 20, 1903.

ARRHENIUS. Die Anwend. der physik. Chemie auf die Serumtherapeutischen Fragen. Festschrift f. BOLTZMANN 1904. Leipzig, J. A. BARTH.

<sup>5)</sup> To make it easy for the reader, we speak here only of calfserum and horse-serum. Compare the third note on this page.

toxin) are added. There upon the mixtures are heated for one hour at 37° and then centrifugated in funnelshaped tubes of which the capillary neck was fused at the bottom. The in 100 equal volumes calibrated capillary portion contains 0.02 or 0.04 c.c. The centrifugating is continued till the volume of the precipitate has become constant <sup>1)</sup>).

*Experiment with calf-horse serum.*

As it was of importance, at all events for the first series of proofs, to dispose of a great quantity of serum containing precipitin, a large animal was taken to be injected. Dr. M. H. J. P. THOMASSEN at Utrecht was so kind to inject at the Gouvernement Veterinarschool there, a large calf several times with fresh horse serum and to prepare the serum out of the blood drawn under aseptic precautions.

The serum used for the following series of experiments was collected Nov. 28, 1905, sent to Groningen and there preserved in ice. On the day of the following experiment January 25, 1906, the liquid was still completely clear and free from lower organisms; there was only on the bottom a thin layer of sediment, which naturally was carefully left behind at the removing of the liquid. <sup>2)</sup>)

The horseserum used for the proof in question was fresh and 50 times diluted with a sterile NaCl-solution of 1%.

Each time two parallel proofs were taken as a control. The capillary portion of the funnel shaped tubes used for this experiment had a calibrated content of 0.04 cc. Each division of the tubes thus corresponded to 0.0004 cc.

To this series of experiments another was connected in which the quantity of diluted horseserum was constant, but increasing quantities of calfserum were used.

From the first table it appears, that when to 1 cc. calfserum increasing quantities of diluted horseserum are added, the quantity of precipitate rises. When more horseserum is added as is the case in the second table, the quantity of precipitate descends. This appears from the following.

<sup>1)</sup> Compare Folia haematologica l. c. for further particulars of the method.

<sup>2)</sup> Fuller details of other proofs taken on other days with calf-horseserum, also of experiments with serum obtained by injecting rabbits with pig-, oxen-, sheep- and goat-serum will be communicated elsewhere.

TABLE I.

1 cc of the mixture of 1 cc. cal serum (precipitin or serum containing anti- toxin) + ... cc. horse- serum $\frac{1}{50}$ (precipitino- gen or toxin containing serum.	Volume of the precipitate, after centri- fugating for :  $\frac{1}{2}$ h. - $\frac{1}{2}$ h. - $\frac{1}{2}$ h. - $\frac{1}{2}$ h. - $\frac{1}{2}$ h. - 20m. - 15m.	The quantity of precipitate found in 1cc. of the mixtures calculated for the total quan- tity of the mixed compo- nents according to the last observation.
$\frac{0.04}{3}$ cc. horseserum $\frac{1}{50}$	1 — $\frac{1}{2}$ — not to be measured accurately	
$\frac{0.04}{3}$ » » »	1 — $\frac{1}{2}$ — » » » » »	
$\frac{0.08}{3}$ » » »	3 — 3 — 3 — 3 — 3 — 3 — 3	3.08
$\frac{0.08}{3}$ » » »	3 — 3 — 3 — 3 — 3 — 3 — 3	3.08
$\frac{0.1}{2}$ » » »	12 — 11 — 10 — 10 — 10 — 10 — 10	10.5
$\frac{0.1}{2}$ » » »	12 — 11 — 10 — 10 — 10 — 10 — 10	10.5
$\frac{0.16}{2}$ » » »	26 — 23 — 20 — 18 — 17 — 17 — 17	18.4
$\frac{0.16}{2}$ » » »	26 — 23 — 20 — 18 — 17 — 17 — 17	18.4
$\frac{0.2}{2}$ » » »	32 — 26 — 24 — 22 — 21 — 21 — 21	23.1
$\frac{0.2}{2}$ » » »	33 — 26 — 24 — 22 — 21 — 21 — 21	23.1
0.13 » » »	48 — 43 — 39 — 34 — 32 — 32 — 32	36.2
0.13 » » »	48 — 43 — 39 — 34 — 32 — 32 — 32	36.2
0.15 » » »	52 — 45 — 40 — 36 — 34 — 34 — 34	39.1
0.15 » » »	50 — 45 — 40 — 36 — 34 — 34 — 34	39.1
0.18 » » »	65 — 61 — 54 — 48 — 42 — 43 — 43	50.7
0.18 » » »	65 — 61 — 54 — 48 — 42 — 43 — 43	50.7
0.2 » » »	65 — 62 — 55 — 49 — 45 — 45 — 45	54
0.2 » » »	65 — 62 — 55 — 49 — 45 — 45 — 45	54
0.25 » » »	78 — 73 — 65 — 58 — 55 — 53 — 53	66.3
0.25 » » »	78 — 73 — 65 — 58 — 54 — 53 — 53	66.3
0.3 » » »	85 — 80 — 70 — 62 — 58 — 57 — 57	74.1
0.3 » » »	84 — 80 — 70 — 62 — 58 — 57 — 57	74.1

So e. g. the quantity of precipitate when 0.3 cc. horse serum is added to 1 cc. calfserum, is 74.1 (table I). But when, as may be read in the second table 0.5 cc. horse serum is added to 0.9 cc. calfserum the precipitate has a volume

TABLE II.

1 cc of the mixture of 0.5 cc horseserum $\frac{1}{50} + \dots$ cc calfserum.	Volume of the precipitate, after centrifuging for							The quantity of precipitate found in 1cc of the mixtures calculated for the total quantity of the mixed components, according to the last observation.
	$\frac{1}{2}$ h.	- $\frac{1}{2}$ h.	- $\frac{1}{2}$ h.	- $\frac{1}{2}$ h.	- $\frac{1}{2}$ h.	- 20m.	- 15m.	
0.1 cc calfserum,	1 — $\frac{1}{2}$ — not to be measured accurately							
0.1 » »	1 — $\frac{1}{2}$ — » » » » »							
0.3 » »	2	— 2	— 2	— 2	— 2	— 2	— 2	16
0.3 » »	2	— 2	— 2	— 2	— 2	— 2	— 2	1.6
0.5 » »	6	— 5	— 5	— 5	— 5	— 5	— 5	5
0.5 » »	7	— 5	— 5	— 5	— 5	— 5	— 5	5
0.7 » »	48	— 36	— 32	— 28	— 25	— 25	— 25	30
0.7 » »	50	— 38	— 33	— 29	— 25	— 25	— 25	30
0.9 » »	84	— 65	— 57	— 50	— 43	— 43	— 43	51.6
0.9 » »	81	— 63	— 55	— 49	— 43	— 43	— 43	51.6
1.1 » »	95	— 81	— 67	— 58	— 52	— 50	— 50	80
1.1 » »	94	— 81	— 68	— 56	— 52	— 50	— 50	80
1.3 » »	92	— 79	— 66	— 59	— 59	— 55	— 55	99
1.3 » »	97	— 80	— 69	— 60	— 59	— 55	— 55	99
1.5 » »	96	— 84	— 74	— 65	— 62	— 59	— 59	118
1.5 » »	95	— 84	— 73	— 64	— 62	— 59	— 59	118
1.9 » »	90	— 75	— 65	— 55	— 53	— 51	— 51	122.4
1.9 » »	89	— 75	— 65	— 55	— 53	— 51	— 51	122.4

of 51.6. If instead of 0.9 calfserum 1 cc. was used the quantity of horseserum would necessarily have amounted to  $0,5 \times \frac{1}{0,9} = 0,55$  cc. So it appears that by the addition of 0.3 cc. horseserum to 1 cc. calfserum the precipitate amounts to 74.1 and by the addition of 0.55 cc. horseserum but to 37.3 <sup>1)</sup>.

This decrease must be attributed partly to the solubility of the precipitum in NaCl-solution, a solubility which is felt the more strongly as a greater quantity of diluted horseserum is added. (Compare also Fol. Haematol l. c.).

So we see that the clear liquid above the precipitate contains, besides free precipitin and free precipitinogen substance, as has already been stated, also dissolved precipitate.

These three substances must form a variable equilibrium, which according to the rule of GULDBERG and WAAGE is to be expressed by the following relation.

**Concentration of the free precipitinogen subst.  $\times$  Concentr. of the precipitin  $= k_1 \times$  Concentr. of the dissolved precipitate . . . . (I)**

in this  $k_1$  is the constant of reaction.

Meanwhile it appears from the experiment, that a greater quantity of precipitate must be dissolved than corresponds with this equation, or to express it more clearly, than corresponds with the conception that the solubility of the precipitate in NaCl solution is the only fact by which the quantity of sediment decreases.

To take away the difficulty, the hypothesis was made *that still another portion of the precipitate forms a dissolvable compound with free precipitinogen substance* (of horseserum) and that we have here a case analogical to the reaction of  $\text{CaH}_2\text{O}_2$  with  $\text{CO}_2$ . As is known  $\text{CaH}_2\text{O}_2$  is precipitated by  $\text{CO}_2$ , but by addition of more  $\text{CO}_2$  the sediment of  $\text{CaCO}_3$  decreases again, while  $\text{CO}_2$  with  $\text{CaCO}_3$  forms a dissolvable substance.

As will soon be seen, a very satisfactory conformity between calculated and observed quantity of precipitate is obtained through this hypothesis, which could afterwards be experimentally affirmed.

Let us now try, reckoning both with the solubility of the precipitate in NaCl-solution and with the forming of a dissolvable mixture of precipitate with precipitinogen substance, to precise more closely equation I.

<sup>1)</sup> The hyperbolic form of the precipitate curve with ncreasing quantity of horseserum may still appear from the following series of experiments taken on another day (Table III). This series has not been used for the following calculation.



TABLE III.

1 cc of the mixture of 1 cc calf serum + . cc horse- serum $\frac{1}{50}$ .	Volume of the precipitate after centrifuging for $\frac{1}{2}$ h. — $\frac{1}{2}$ h. — $\frac{1}{2}$ h. — $\frac{1}{2}$ h. — 20 m. — 15 m. — 10 m.	The quantity of precipitate found in 1 cc. of the mixtures calculated for the total quantity of the mixed components according to the last observation.
0 1 cc horseser. $\frac{1}{50}$	38 — 38 — 28 — 24 — 23 — 23 — 23	25 3
0 1 » » »	40 — 22 — 20 — 24 — 23 — 23 — 23	25 3
0 2 » » »	66 — 54 — 48 — 44 — 42 — 42 — 42	50 4
0.2 » » »	59 — 50 — 45 — 43 — 41 — 41 — 41	50.4
0 3 » » »	88 — 69 — 65 — 56 — 55 — 55 — 55	71.5
0 3 » » »	87 — 68 — 65 — 56 — 55 — 55 — 55	71 5
0 4 » » »	98 — 76 — 70 — 62 — 58 — 57 — 57	79 8
0.4 » » »	89 — 73 — 68 — 62 — 58 — 57 — 57	79.8
0 6 » » »	84 — 62 — 57 — 49 — 44 — 44 — 44	70 4
0 6 » » »	71 — 57 — 53 — 47 — 43 — 43 — 43	68 8
0.7 » » »	65 — 49 — 45 — 39 — 37 — 37 — 37	62.9
0 7 » » »	66 — 49 — 45 — 39 — 37 — 37 — 37	62 9
0.8 » » »	61 — 45 — 40 — 38 — 33 — 31 — 31	55.8
0 8 » » »	62 — 45 — 40 — 38 — 33 — 31 — 31	55.8
0 9 » » »	41 — 30 — 26 — 25 — 22 — 21 — 21	39 9
0 9 » » »	41 — 30 — 26 — 25 — 22 — 21 — 21	39 9
1 » » »	24 — 17 — 15 — 15 — 13 — 13 — 13	26
1 » » »	25 — 10 — 15 — 15 — 13 — 13 — 13	26
1 2 » » »	2 — 2 — 2 — 2 — 2 — 2 — 2	4
1 2 » » »	2 — 2 — 2 — 2 — 2 — 2 — 2	4
1 4 » » »	not to be measured	
1.4 » » »	» » » »	

Firstly we shall try to find an expression for the three substances occurring in the clear liquid which stands above the precipitate: for the free precipitinogen substance, for the free precipitin which it contains and for the quantity of dissolved precipitate.

Firstly the quantity of *free precipitinogen substance*. Let  $A$  be the *total* quantity of that substance used for an experiment. To determine how much of this is still present in the liquid in free state, it is to be determined how much is bound. Bound is:

1. a certain quantum to form the precipitate which is present in solid condition. If we set down as a rule that 1 mol. precipitum proceeds from 1 mol. precipitinogen substance and 1 mol. precipitin, then the wanted precipitinogen substance will be expressed by  $P$ , if the molecular quantity precipitate also amounts to  $P$ .

2. a quantity  $pV$  when  $p$  represents the percentage of the quantity of dissolved precipitate and  $V$  the total volume of the liquid.

3. a quantity necessary to form the compound of precipitate and precipitinogen substance. Admitting that 1 mol. of this compound proceeds from 1 mol. precipitate and 1 mol. precipitinogen substance and then that  $y$  of this compound is present, then together  $2y$  must be charged, while in each of the two components  $y$  mol. precipitinogen substance is present, so that the quantity of precipitinogen substance, which is left in free state, amounts to

$$A - P - pV - 2y.$$

So when the volume of the liquid is  $V$ , the *concentration* of the free precipitinogen substance =

$$\frac{A - P - pV - 2y}{V} \quad \dots \quad (2)$$

It is possible to calculate in the same way the concentration of the free precipitin.

If  $B$  is the *total* quantity of precipitin, which is used for the experiment, then there is to be subtracted from this:

1<sup>st</sup>. a quantity  $P$  for the same reason as is given at the calculation of the free precipitinogen substance (see above).

2<sup>nd</sup>. a quantity  $pV$ , likewise as explained there.

3<sup>d</sup>. a quantity necessary to form the compound precipitate-precipitinogen substance. While in this compound but 1 mol. precipitin is present, only  $1y$  is to be charged. So that the quantity of precipitin which remains in free state, amounts to  $B - P - pV - y$ .

While the volume of the liquid amounts to  $V$ , the concentration of the free precipitin is =

$$\frac{B-P-pV-y}{V} \dots \dots \dots (3)$$

As for the *concentration* of *dissolved precipitate* in the third place, this must be expressed by

$$\frac{pV}{V} \dots \dots \dots (4)$$

So the equation (1) becomes:

$$\frac{A-P-pV-2y}{V} \times \frac{B-P-pV-y}{V} = k_1 \frac{pV}{V}$$

or

$$(A-P-pV-2y)(B-P-pV-y) = k_1 pV^2 \dots \dots (5)$$

Now one more equation, expressing the reaction according to which precipitate combines with precipitinogen substance. This is to be written down as follows.

Concentration free precipitinogen substance  $\times$  concentr. dissolved precipitate  
 $= k_2$  concentr. compound precipitinogen subst. — precipitate.

$$\frac{A-P-pV-2y}{V} \times \frac{pV}{V} = k_2 \frac{y}{V}$$

or

$$(A-P-pV-2y)p = k_2 y \dots \dots \dots (6)$$

By putting shortly  $P+pV = P'$  and by substituting the value of  $y$  of equation (6) into equation (5) we obtain

$$\left( A-P'-2p \frac{A-P'}{k_2+2p} \right) \left( B-P'-p \frac{A-P'}{k_2+2p} \right) = k_1 pV^2 \dots \dots (7)$$

In this equation are known:

1<sup>st</sup>.  $A$ , the total quantity of precipitinogen substance (diluted horseserum added);

2<sup>nd</sup>.  $B$ , the total quantity of precipitin (calfserum) used;

3<sup>rd</sup>.  $V$ , the volume of the liquid resulting from the mixing of the two components;

4<sup>th</sup>.  $P$ , the quantity of solid precipitate directly observed.

Unknown are:

1<sup>st</sup>.  $p$ , the quantity in percentages of precipitate which is *dissolved* (so  $p$  represents the solubility of the precipitate);

2<sup>nd</sup>.  $k_1$ , the constant for reaction of the formation of precipitate;

3<sup>rd</sup>.  $k_2$ , the constant for reaction of the formation of the compound precipitate-precipitinogen substance;

4<sup>th</sup>.  $P'$ , this is however  $P + pV$  and therefore, known as soon as  $p$  has become known.

As equation (7) contains 3 unknown quantities three observations will be necessary to determine them.

When we introduce then the so found values in the other experiments and calculating the quantity of precipitate, it appears that the calculated quantities correspond in very satisfactory way with those which are observed.

Let us observe that to avoid superfluous zeros 1 cc calfserum ( $B$ ) is taken  $= 100$ .

While as appears from the experiments in the case in question 1 cc calfserum is equivalent to nearly  $\frac{1}{50}$  cc horseserum 1 : 50, 1 cc horseserum 1 : 50, that is  $A$ , obtains a value of 300.

So, where in the first experiment  $\frac{0,04}{3}$  cc. horse serum was used  $A$  obtains a value of  $\frac{0,04}{3} \times 300 = 4$ .

In the experiment, where on 1 cc. calfserum 0,3 cc. horseserum was used, with a value  $B = 100$ ,  $A$  becomes  $0,3 \times 300 = 90$ .

Let us now combine the two tables to one by calculating for the second table how much  $\frac{1}{50}$  horseserum is used on 1 calfserum..

*We see that the conformity between the determined and calculated precipitate (col. III and IV) is very satisfactory. The average of the discrepancy amounts to 1.3.*

This result deserves our attention not only in view of the knowledge of the precipitin reaction as such, but also from a more general point of view, this reaction belonging to the great group of the toxin-antitoxin reactions.

Till now, in studying the last, we were obliged to deduce the equilibrium conditions from the toxins, that is to say by determining the toxic-action which was left by the gradual saturation of the toxin by increasing quantities of antitoxin, but with the precipitin-reaction the equilibrium conditions may be deduced from the quantity of the formed toxin-antitoxin compound.

And not only that, but owing to the fact that the compound forms a precipitum, the quantity of this may be fixed in an accurate and direct way by simple measurement, thus without the aid of red blood corpuscles or of injecting-experiments in animals.

So there is good reason to expect that a further study of the precipitin-reaction will facilitate too the insight in other toxin-antitoxin reactions.

( 44 )

TABLE IV.

I	II	III	IV	V
<i>1 cc. calf serum, B = 100.</i>				
Used quantity of horseserum $\frac{1}{50}$ (on 1 cc. calf serum).	Used quantity of horseserum expressed in the just accepted units A.	Determined volumes of the precipitate in 1 cc of the mixtures.	Calculated volumes of the precipitate in 1 cc. of the mixtures.	Difference between III and IV.
0 013 cc.	4	not to be measu- red.	0 2	
0 027 »	8	3	3 9	+ 0.9
0.05 »	15	10	10.3	+ 0.3
0.08 »	24	17	17.8	+ 0 8
0.1 »	30	21	23 6	+ 2.6
0.13 »	39	32	29.7	- 2 3
0.15 »	45	34	34	0
0 18 »	54	43	40.1	- 2.9
0.2 »	60	45	43 9	- 1.1
0 25 »	75 $\left. \begin{smallmatrix} 53 \\ 51 \end{smallmatrix} \right\}$	52	52 1	- 0.1
0.266 »	79	51	53.6	+ 2.6
0.294 »	88.3	55	57.1	+ 2 1
0.3 »	90	57	57.5	+ 0 5
0.33 »	100	59	58.9	- 0.1
0.385 »	115.4	55	57.4	+ 2.4
0.457 »	137	50	51.3	+ 1.3
0 557 »	167	43	41.3	- 1.7
0 713 »	214	25	26.8	+ 1.8
1 »	300	5	5.5	+ 0.5
1.67 »	500	2	0	- 2

# R É S U M É.

We may resume our results as follows.

By mixing precipitin and precipitinogen substance (to compare resp. with antitoxin and toxin) an equilibrium reaction proceeds

obeying to the law of GULDBERG and WAAGE. By this equilibrium reaction part of the precipitin molecules combines with the corresponding quantity of molecules precipitinogen substance, while by the side of this compound a certain quantity of each of the two components remains in free state. The compound is partly precipitated and partly remains dissolved. How much remains dissolved depends for the greater part on the salt solution which is present, for the sediment is soluble in Na Cl-solution.

Besides this equilibrium reaction there is still another which consists in this, that part of the precipitate combines with free precipitinogen substance to a soluble compound. This reaction too obeys the law of GULDBERG and WAAGE. The case is to be compared with the precipitation of  $\text{Ca}(\text{OH})_2$  by  $\text{CO}_2$ . By excess of  $\text{CO}_2$  a part of the resulting  $\text{CaCO}_3$  is transformed in a soluble bicarbonate. So  $\text{CaH}_2\text{O}_2$  takes the function of the precipitin and  $\text{CO}_2$  that of the precipitinogen substance.

**Astronomy.** — "*Observations of the total solar eclipse of August 30, 1905 at Tortosa (Spain).*" By J. STEIN S.J. (Communicated by Prof. H. G. VAN DE SANDE BAKHUYZEN.)

At the invitation of Mr. R. CIRERA S. J., director of the new "Observatorio del Ebro" I went to Tortosa towards the end of June 1905 in order to take part in the observation of the total solar eclipse. I was charged with making the measurements of the common chords of the sun and moon at the beginning and at the end of the eclipse and had also to determine the moments of the four contacts. The results might perhaps contribute to the correction of the relative places of the sun and moon.

The determination of the co-ordinates was much facilitated by the circumstance that the signals of the three points Espina, Gordo and Montsià of the Spanish triangulation were visible at this place. The measurements of the angles with a theodolite yielded the following results:

$$\varphi = 40^\circ 49' 13''.43 \quad ; \quad \lambda = 1^m 58^s 18 \text{ east of Greenwich.}$$

In these results the spheroidal shape of the earth is accurately taken into account. Later measurements made by Mr. J. UBAGH gave the same results. Electric time-signals, directly telegraphed from the Madrid observatory, gave for the longitude:  $1^m 58^s.8$  east of Greenwich. As the most probable value we have adopted  $1^m 58^s.5$ ,