QUANTITATIVE ASPECTS OF ANTIGEN-ANTIBODY REACTIONS

II. SOME COMPARISONS BETWEEN THE THEORY AND THE EXPERIMENTAL RESULTS

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(With 5 Figures in the Text)

In previous papers (Teorell, 1943, 1946) a quantitative theory was developed for the interaction between an antigen and the corresponding antibody. The theory made it possible to predict the whole course of precipitin reactions as regards the amount of precipitate formed and the composition of the precipitate in terms of added quantities of antigen and antibody and 'dissociation' constants, 'valencies', etc. The object of the present communication is a comparison between a number of available experimental results and the predictions according to the proposed theory. Some statements with regard to the flocculation velocity will also be discussed in the light of this theory.

A full discussion of all the various observations reported in the immunological literature cannot of course be given in this communication; we must limit the comparisons with the experimental findings to a few typical instances. For reviews of the available experimental evidence the reader is referred to Marrack's excellent works (1938, 1942). It should be emphasized that our theory has postulated a single, homogeneous antibody. In actual practice, however, one often seems to deal with mixtures of antibodies with different 'avidities' ('Multiplizität der Antikörper', according to Haurowitz (1939, 1943)). These circumstances may naturally complicate a comparison, but they may also partly explain divergences which may be found.

In the theoretical part it was attempted to make a classification of the different reaction types. In the first place the generally adopted division into an α *procedure* (constant amount of antibody, variable amounts of antigen) and a β *procedure* (constant antigen, variable antibody) was maintained. In each procedure a subdivision was then performed with regard to the 'inhibition' resulting from the solubility of certain antigen-antibody components (in general denoted by A_nG); thus complete insolubility of all AG components, i.e. no inhibition phenomenon, was described as an X *case*. The Y *case* refers to solubility of the compound AG and exhibits one inhibition zone (in excess of antigen), whereas the Z *case* shows two such zones (both in excess antigen and excess antibody) owing to solubility of AG and A_NG (A is 1-valent and G is N-valent). The six resulting types of reaction curves were shown graphically in the previous paper; they will here be referred to as αX, βX, αY cases etc. (cf. Table 1).

Table 1. *Scheme of classification (with typical examples)*

	No inhibition zones	One inhibition zone	Two inhibition zones
Constant antibody + variable antigen	αX (pneumococcus VIII)	αY (egg albumin (rabbit))	αZ (diphtheria toxin (horse))
Constant antigen + variable antibody	βX (—)	βY (egg albumin (rabbit))	βZ (—)

In the first section below, the following types of reaction and examples of corresponding experimental cases will be considered:

αX ... Type VIII pneumococcus polysaccharide-homologous antibody (horse).

αY ... Egg albumin-anti-egg albumin (rabbit).

βY ... Egg albumin-anti-egg albumin (rabbit).

αZ ... Diphtheria toxin-antitoxin (horse).

As regards the remaining types (βX and βZ) there do not yet seem to exist any experimental results suitable for a quantitative comparison.

In the last section a hypothesis concerning the flocculation velocity will be analysed in relation to available experimental findings.

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1. THE GENERAL COURSE OF THE PRECIPITATION CURVES

In the following account the considerations will be restricted to a *qualitative* comparison of the *general courses* between some selected experimental curves and the theoretical *type* curves, the latter being presented as 'insets' in the diagrams of the former. A comparison with regard to the *heights* of the curves must accordingly not be made. It seemed of little use to attempt a more quantitative correlation between experiment and theory by empirically determining those parameters of the theoretical equations, such as valencies, molecular weights, equilibrium constants, etc., which would result in more closely fitting curves. For simplicity, therefore, the purely arbitrary cases worked out in our previous paper have been chosen as theoretical

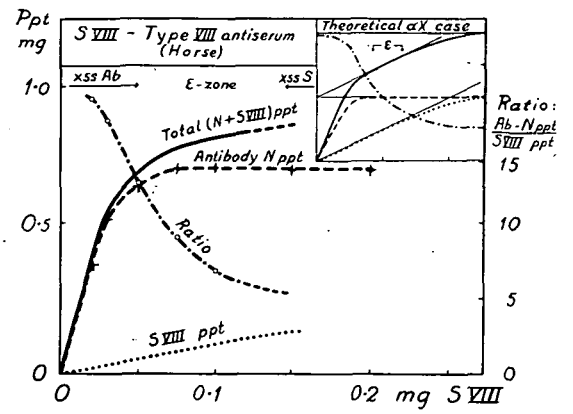

Fig. 1. Precipitate analysis on the *Pneumococcus type VIII polysaccharide-homologous antiserum* (*horse*) system (constant amount of antibody) (from Heidelberger *et al.* (1937), Table II, p. 490). The inset diagram refers to a theoretical calculation based on the theory and data presented in the previous paper.

'type patterns' (an exception is Fig. 4). Nevertheless, the agreement between experiment and theory is, on the whole, surprisingly good.

In the diagrams, curves from the experiments and from the theory which correspond to each other have been plotted with identical signs (points, dashes, etc.).

(*a*) *The αX case* of the theory seems to be well represented by some experiments published by Heidelberger, Kabat & Shrivastava (1937) on the *Pneumococcus VIII polysaccharide* (*S* VIII) and its *homologous antibody* (compare Fig. 1, plotted from their Table II, loc. cit. p. 490). Though these authors determined only the nitrogen contents of the precipitates obtained on mixing increasing amounts of *S* VIII with the antiserum, it is permissible to draw the curves for precipitated *S* VIII ('*S* VIII ppt.') and for total precipitate (represented by 'Total (*N* + *S* VIII) ppt.')* up to the beginning of the excess antigen zone ('xss. *S*'). The precipitate curves, and also the curve of the ratio (antibody *N* ppt.) : (antigen ppt.), have a course closely resembling the corresponding theoretical type case. The divergency in the magnitudes of *A* (antibody) ppt. and *G* (antigen) ppt. and their ratio might be ascribed to the fact that the 'molecular weight' ('minimum reactive chain weight') of *S* VIII is considerably lower than the weight of the antibody globulin; the valency may also be higher than 4 (cf. Heidelberger, 1938). (In the theoretical case in the 'inset' figure the molecular weights were taken as equal, and the valency as 4.)

The presented experimental data, as well as data for other polysaccharide types obtained by Heidelberger & Kendall (*1935a, b, c*), also indicate that *the equivalence zone* ('*ε-zone*') *appeared before the maximal precipitation* was approached, exactly as demanded by the theory.

From Heidelberger & Kendall's work (1935*a*) one can infer that they suppose the 'equivalence point ratio' to be approximately half the maximum value attained in the region of extreme antibody excess, and they seem inclined to interpret this fact as due to the formation of a definite, *single* compound. In certain cases our theory also yields *roughly* 'half the maximum ratio' at the ϵ point, but fails to ascribe this circumstance to the presence of a single compound. On the contrary, the ϵ point precipitate is always considered as a *mixture* of *AG* compounds. Furthermore, Heidelberger & Kendall found that the ratio in the excess antibody zone can be represented by a straight line. This fact cannot be verified by the presented theory. (A closer study of the data of Heidelberger & Kendall seems to disclose that the straight line relationship is a rough approximation, a fact that, in some other cases, has forced these authors (1935*c*) to use a purely empirical relationship for the ratio curve.)

(*b*) *αY and βY cases. The egg albumin-rabbit anti-egg albumin* systems studied by Taylor, Adair & Adair (1934) (cf. also Culbertson (1932), Heidelberger & Kendall (1935*b*), Pappenheimer (1940)) are typical inhibition cases, where excess of the antigen diminishes or dissolves the precipitate formed. The diagrams for precipitate nitrogen published by Taylor *et al.* include α and β procedures. In regard to the general course, the location of the ϵ point, the maximum precipitation point (and perhaps also the ratio) the agreement of the diagrams based on their data with the theoretical αY and βY cases is remarkably good (Figs. 2, 3).

Taylor *et al.* have also investigated a *horse serum albumin* system and their results show, on the whole, that it was similar to the egg albumin case, but differed

* Strictly speaking *N* should be multiplied by a factor to give the amount of antibody *protein*.

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in so far as it was possible to demonstrate a considerable excess of both the antigen and its antibody at the ϵ point. This is, however, a consequence that the presented theory can anticipate in the case, where the values of the equilibrium (dissociation) constants approach numerically the total concentration of the constant antibody (or antigen), or, in other words, in

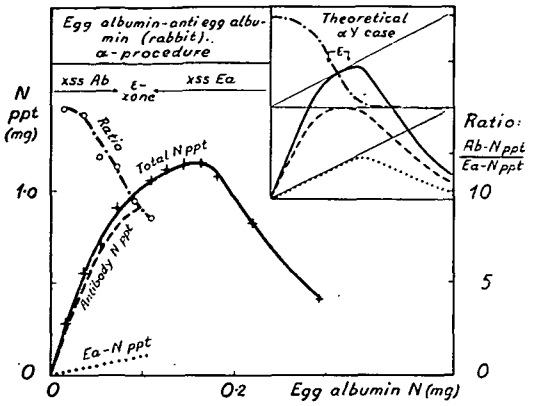

Fig. 2. Precipitate analysis on an *egg albumin-anti-egg albumin (rabbit)* system (constant amount of antibody) (from Taylor *et al.* (1934), Exp. 4, p. 122). Inset: a theoretically calculated αY case.

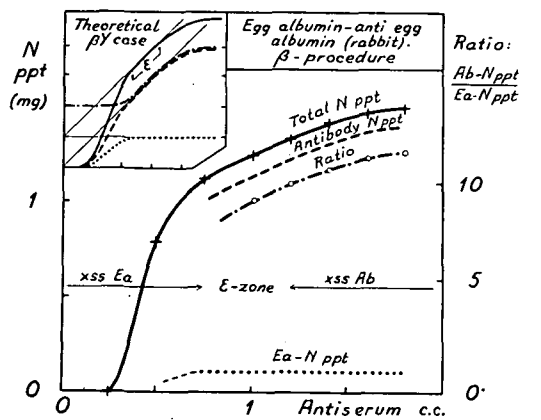

Fig. 3. Precipitate analysis on an *egg albumin-anti-egg albumin (rabbit)* system (constant amount of antigen) (from Taylor *et al.* (1934), Exp. 7, p. 124). Inset: a theoretically calculated βY case.

the case where the dissociation tendency of the AG compounds is appreciable (cf. discussion of 'the completeness of the ϵ point precipitation' in the preceding paper, p. 234). The explanation suggested by Taylor *et al.* that the serum albumin contains more than one antigen is therefore superfluous.

(c) *αZ and βZ cases.* An experimental correspondence of the theory to the Z case should exhibit two inhibition zones: not only in the presence of excess of antigen but also in excess of *antibody*. So far very few such cases have been investigated quantitatively. A case important in practice is the *diphtheria toxin-horse antitoxin* system, which is described by Marrack (1938, p. 160) as 'abnormal' since precipitation occurs only within a narrow range around the equivalence point (cf. Healey & Pinfield (1935) and Pappenheimer *et al.* (1940)). Pappenheimer & Robinson (1937) have published some data on such a case. A diagram based on their Table I (loc. cit. p. 294) is given in Fig. 4. A comparison with the theoretical curves in the inset shows a reasonable agreement between experiment and the predictions for an αZ case; hence there is no reason to call this reaction 'abnormal'.

The egg albumin-*horse* anti-egg albumin systems recently analysed by Pappenheimer (1940) and by

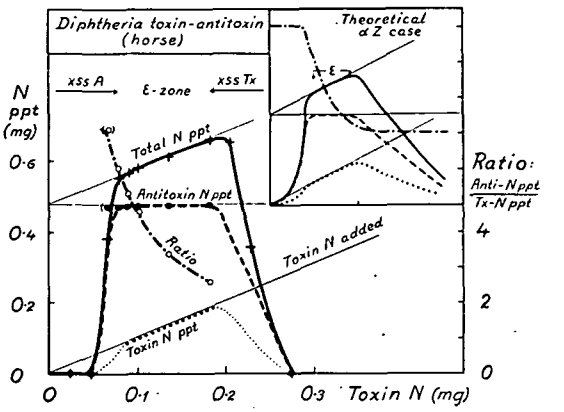

Fig. 4. Precipitate analysis on a *diphtheria toxin-antitoxin (horse)* system (constant amount of antitoxin) (from Pappenheimer & Robinson (1937), Table I, p. 294). Inset: a theoretically calculated αZ case. Here $N = 6$ and $k_1 \ldots k_6$ equal to $10^{-3}, \frac{1}{2}10^{-5}, 10^{-7}, 10^{-9}, 10^{-11}, 10^{-13}$.

Heidelberger *et al.* (1940) also fit the αZ case very well.

The well-known Ramon 'titration' of diphtheria antitoxins is a β procedure. Unfortunately, no quantitative data seem to be available. In their general behaviour, however, the results of the Ramon method seem to show great similarity with the βZ case of the theory (cf. Fig. 8 of the preceding paper).

According to Pappenheimer *et al.* (1940) the maximum number of antitoxin molecules which can combine with one diphtheria toxin molecule is eight ($N = 8$). It is conceivable that not only the compound A_8G but also A_7G (perhaps even A_6G) enter as soluble compounds besides AG (possibly also A_2G (A_3G?)) in this Z case. The authors also point out that the *antibody* excess inhibition is due to a property of the *horse* antibody. The corresponding rabbit antisera do not show this 'prozone', and behave, it appears, as Y cases (cf. Boyd's 'R' and 'H' type sera referred to in the following section).

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2. A HYPOTHESIS ON THE VELOCITY OF THE FLOCCULATION

The velocity of flocculation has been the subject of much discussion in the literature, in particular as regards the relation between the 'equivalence' or 'neutralization point', the 'maximum precipitation point' and the 'optimum proportion point' (cf., in particular, Marrack, 1938, Chapter v; Taylor *et al.* 1934; and recently Boyd, 1941 and Hershey, 1941).

The 'optimum proportion' is a practically useful concept introduced by Dean & Webb (1926) for the ratio (dilution of antigen) : (dilution of antibody) that gives rise to the most rapid flocculation. Within the limits of error, it is regarded as depending on the relative, and not the absolute, proportions of the reagents. Hooker & Boyd (1935) found the velocity of flocculation approximately proportional to the total antigen quantity present (in the excess antibody zone). Boyd (1941) suggests that most antisera can be classified into two main types: the 'R type' which gives an optimum only in the α procedure, and the 'H type' giving an optimum in both α and β procedures. The two optima in the latter case may be more or less different as regards the ratio mixed antigen/mixed antibody. In many systems the optimum point is reported to coincide with the neutral point; in other systems it is said to be located in either of the excess zones.

No extensive attempts will be made here to analyse the 'optimum point' in terms of the proposed theory. Some remarks may, however, be put forward as a contribution to the discussion on the significance of this concept. Under certain provisions, including the absence of substances exhibiting colloidal protective action, the velocity of flocculation of hydrophobic particles depends on the initial number of centres of aggregation (cf. von Smoluchowski's (1918) theory, and also Boyd (1941)). In terms of the presented theory, this amounts to saying that *the velocity of flocculation is proportional to the amount of antigen precipitated* (*G ppt.*) because each molecule of any insoluble AG compound formed contains one molecule of G. In a region of antibody excess this hypothesis includes the above rule of Hooker & Boyd (1935) since there is G ppt. equal to the total antigen quantity. In this connexion it may be instructive to study the G ppt. curves of the preceding paper (Figs. 2–4, 6–8). 'True' optimum points corresponding to well-defined maxima of G ppt. ought to be anticipated only in the αY, αZ and βZ cases (loc. cit. Figs. 3, 4, 8). In the cases presented these hypothetical optimum points coincide approximately with the maximum precipitations. A closer investigation reveals, however, that the 'maximum G ppt. point', in systems with other values of the dissociation constants, can occur at a ratio (antigen dilution) : (antibody dilution) other than that characterizing the maximum precipitation point or the equivalence point. *Thus the optimum point, the maximum precipitation point and the equivalence (neutral) point are theoretically independent, although they may sometimes more or less coincide.*

A rough check on the applicability of the above hypothesis to the velocity of flocculation can be made, using the extensive data recently published by Boyd (1941). He has expressed his results in a very compact way as 'isochrone' diagrams (isochrone = contour line of equal time of flocculation in mixtures of antigens and antibodies). Incidentally, Boyd's isochrone maps of the 'R type' (rabbit) antisera have a course greatly resembling the graph of the total amount of precipitate which illustrated the preliminary communication of our theory (Teorell, 1943). That graph represented a case with an inhibition zone due to antigen excess (Y case, according to the proposed terminology). For the sake of easier comparison the graph in question has been rearranged and is plotted here in Fig. 5 (full lines). This diagram, too, shows a remarkably good agreement with some of Boyd's isochrone maps, in particular his 'H type' (horse) cases.

It should be emphasized that the contour line diagrams presented here refer to amounts of *total* precipitate (Tot. ppt.). They may also, however, as regards *shape*, approximately refer to the amounts of *precipitated antigen* (G ppt.), since it can be shown that the Tot. ppt. curves and the G ppt. curves, although not proportional to each other, run quite parallel (cf. several figures of the preceding paper). Now, according to the hypothesis made here, it is the G ppt. that should determine the time relations of the flocculation. Hence, it seems permissible, as is done here, to compare Boyd's flocculation-time curves with the constructed Tot. ppt. contour lines.

In spite of the approximate method of comparison, one may perhaps take Boyd's results as arguments in favour of the hypothesis that the velocity of flocculation is determined by the amount of antigen precipitated. It might be expected, however, that colloidal protective action exerted by antigen (or antibody) excess, or by soluble AG compounds, would in actual practice cause the results to diverge greatly from the theory.

One might anticipate that such a protective action on the hydrophobic AG compounds would result in a displacement of the most rapid flocculation point towards the equivalence zone,[1] as has actually been reported in many experimental cases. For instance, in an X or βY case the rate of flocculation ought theoretically to

[1] The experimental location of the equivalence point by tests on the supernatants might be complicated, not only by protective effects, but also by inhibition due to formation of soluble products and increased dissociation and solubility resulting from dilution of the system.

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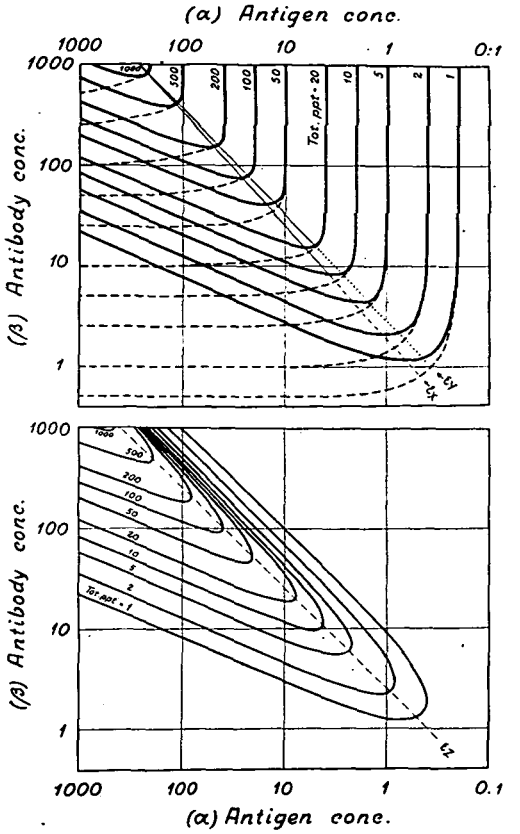

Fig. 5. Theoretical diagram of effects of varying antigen-antibody ratios on amount of precipitate (Tot. ppt.). Variation along horizontal rows shows α procedures, along vertical rows shows β procedures. *The upper part* refers to X (dashes) and Y cases (full lines), *the lower part* to Z cases. Lines marked ϵ_X, ϵ_Y and ϵ_Z refer to 'equivalent points', which approximately coincide with the Dean & Webb (1926) 'optimum proportion points'.

rise towards a maximum value and then remain constant (see the G ppt. curves of Figs. 2, 6 and 7 of the preceding paper). Protective action by excess A in the βY case, may, however, retard the aggregation, and there consequently may appear a 'false' optimum point close to the equivalence zone (such a result has been reported by Taylor, Adair & Adair, 1934, Exp. 7).

The factors determining the velocity of flocculation are probably many and presumably act in a rather complex manner, which may explain the divergent results reported in the literature. Further investigations, including determinations of the electro-kinetic potential, seem desirable before these problems can be finally settled.

SUMMARY

The quantitative theory for the interaction between antigen and antibody presented in the previous paper has been compared with some experimental precipitin reactions published in the literature. These reactions include Type VIII pneumococcus polysaccharide-homologous (horse) antibody, egg albumin-(rabbit) anti-egg albumin and diphtheria toxin-(horse) antitoxin.

1. The general course of the experimental precipitation curves (total amount of precipitate, amounts of precipitated antigen and antibody) corresponded well to the theoretical type curves. Hence it may be concluded that the precipitates may be composed of mixtures of compounds of the types AG, A_2G, A_3G, ..., A_NG in accordance with the law of mass action. In the cases with 'inhibition zones', however, AG, or A_NG, or both (and perhaps several more compounds) retain the same solubility as the free antigen (G) and free antibody (A).

2. With regard to the location of the 'equivalence zones', experiment and theory also showed a satisfactory agreement.

3. A hypothesis on the velocity of flocculation in the precipitin reaction is presented and compared with some recent results. The relation between the immunological concepts 'equivalence (neutral) point', 'optimum point' and 'maximum precipitation point' is also discussed.

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