St. Louis
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Dr. Michael Heidelberger
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Dear Dr. Heidelberger,

I am extremely happy to get your letter. Naturally enough, no one else has been sufficiently interested to come forward with any intelligent objections. So far the reaction has been "anything can be got into an equation", which may be true but is a particularly irritating form of criticism. I will do my best with the points you raise.

p. 457. The general theory requires no assumption concerning a. Beginning on p. 461, however, multivalence is introduced, to see in what way specific aggregation will affect the postulated equilibria. The conclusion is reached that no great disturbance results so long as selective precipitation is ignored. Therefore, equation [4] is approximately valid, within the framework of the general theory, independently of a.

Your objection to my choice of initial reactions is disturbing, because this is indeed the fundamental and fatal point. I shall try, therefore, to clarify my argument, which is not always perfectly clear in my own mind. The range of precipitation, as in your experiments with albumin, covers A_o/G_o ratios from about 2 to 10, i. e. antibody is in molecular excess throughout. For explicitness, consider the mixture of 3 mols A to 1 mol G. Ignoring 3rd order combination, only 1 reaction is possible momentarily: G + A = GA. But there are now the following possibilities:

$G + A = GA$ (a)
$GA + A = GA_2$ (b)
$GA + G = G_2A$ (c)
$GA + GA = G_2A_2$ (d) etc.

While all these are possible, they are by no means equally probable. Thus the concentration of A is 3x that of G at the outset, and the latter rapidly disappears as the reaction proceeds. The concentration of G would be virtually nil by the time $x = 2$, at which time the concentration of A is still $= G_o$. Accordingly, the reactions of type (b) are overwhelmingly more frequent than those of type (c). As for reaction (d), let us imagine that all G has combined to yield GA. We have then 2 mols of A to one of GA. Hence reaction (b) is roughly twice as probable as reaction (d), and the disproportion would be greater in the actual disordered system. In general, aggregations are slower than the initial reactions.

However, aggregations (d) do occur simultaneously with the initial reactions, of course, and the separation is purely conceptual. The advantage is simplicity: compare [4] and [7]. Equation 4 deals wholly with the hypothetical initial reactions. It can be applied to actual precipitation only as an approximation. It is necessary to evaluate the degree of approximation.

Beginning on p. 461, a general reaction is set up leading to eq. [7]. Here no separation into initial reactions has been made, and no possible reaction excluded. It is true that multivalence has been assumed, but <u>some</u> assumption is necessary for explicitness. The result is perfectly general and valid (except that selective precipitation is ignored), but is not very useful. The remainder of the discussion aims to show by a series of approximations that [7] is very similar to the simple equation for the imaginary initial equilibrium [4], in other words, the real equilibrium is not very different from the initial equilibrium.

The equilibrium is not strictly speaking influenced more by G surfaces than by those of A. However, the <u>initial</u> equilibrium is between uncombined antibody, and the residual antigenic surfaces, i. e. antibody is univalent by definition in the initial reactions. It is true that the introduction of these reactions is somewhat artificial, but as I mention above, the result is not contingent on this assumption. The initial reactions are not wholly hypothetical, however, for experimental conditions can be so

chosen that these alone occur.

p. 460. This objection arises from my mistake in defining an equivalence point different from your equivalence zone. I should have used a new term. Definitions are on pages 457 and 500 respectively. According to my definition, the equivalence point is at x = 1 when g = a, and usually would be slightly greater than one. The absolute limits are 0 and 1.3 (p. 474). However, as I define it, the equivalence point is of little experimental interest.

p. 461. I agree that your reagglutination experiments should be interpreted in the light of your theory, but a possible objection could be made that the effect is due to removal of antibody, or of something else (Jones and Orcutt?). I seem to remember that Hooker made this objection. Duncan's experiments seem quite unequivocal to me, though this may be my prejudice.

As to why specific and non-specific forces necessarily oppose, the sentence on p. 461 does not seem to make much sense, and does not I think refer to this. The footnote on page 491, however, makes this statement. The argument is this. The effect of selective precipitation (which I think must be taken into account if A is multivalent) is to cause more antibody to be left over than [4] predicts in the region above $g/2 = r$, while the effect of non-specific precipitation, which ultimately means a reaction of A with A, is to reduce the dissociation of A from the precipitate.

p. 469. Should read "For the case $x > (g-1)...$"

p. 474. Again the definition of equivalence point.

p. 475. Maximal precipitation of A should occur, as you have found, with largest amount of G compatible with reasonably complete precipitation of initial compounds. However, since (my) equivalence point is in the inhibition zone, the region of complete precipitation necessarily lies above this, i. e. with residual A in excess. See also p. 500. I am afraid I haven't done the best with terminology.

p. 479. The T-A system certainly has something peculiar, see p. 504, footnote. Do you have any explanation for the peculiarities of horse antiprotein systems? I am not entirely satisfied with Pappenheimer's dissymmetry, tho it does explain a lot.

p. 480-2. I agree that there is no difference other than effects of concentration as between constant-G and constant-A titrations. However, the optimal ratios have considerable significance in connection with the restricted theory. Personally, I question whether my treatment of the kinetics of precipitation will ever be of any use, but so far as the theory goes I believe it hangs together. Please notice that no difference in composition of precipitates for the two titrations is predicted, except for the small volume-effect. But the optima necessarily lie at different points. These ratios provide the only independent estimate of g, and the lack of data on this point is a handicap in testing the theory.

Paper II. So far as any practical use is concerned, I am sure your linear equation is irreplaceable. My equation is not linear, and I felt that it was more instructive to plot it as a deviation from the straight lines of my fig. 2.

As for the values of k, g, and a, these are evaluated in a strictly unarbitrary way, as will perhaps be made clearer in my 4th paper, which you have probably seen in MS. As for k itself, something must vary from serum to serum. It seems to me a dissociation constant is a more plausible variable than a valence, but this is a matter

of taste. I have made quite a point of the constancy of g, but I must admit the data available are not very encouraging. This of course hangs on optimal ratios.(table 4)

p . 499. I am sorry about the misquote. I recall at the time I couldn't seem to find exactly what I thought I had read in your papers. I should have got in touch with you.

p. 502. The nature of the "valence" is very much up in the air, as you will doubtless agree. I have recently got in trouble with one of the referees (Boyd?) over this. I don't see how the p icture can be simplified very much in the light of results like those of Landsteiner and VanderScheer, J.I.M. 1940, 71, 445.

p. 503. There is indeed evidence for heterogeneity of A, and I have not meant to deny it. My argument is, that with the exception of certain horse sera, there is not much indication that the diversity is sufficient to be a strong factor in determining the behavior of the system. Apart from this, I have suggested the variable is k, rather than g. In any case, I do not see any evidence that there are valences directed toward different determinant structures ("immunological dissimilarity" p. 502), on the contrary Landsteiner cited above.

Paper III. I have tried not to ignore unfavorable results.

pp. 516-517. I have tried to substitute a single variable, k, for your two variables g and a. k would of course affect the entire range of reaction, for that matter so do g and a (equation [4]).

p. 518. I agree that recovery of undifferentiated A by dissociation evidences uniformity. However, the removal by absorption of a small fraction of A with unusually small k, and therefore p ossessing cross-reactivity, might not be detectable in the homologous reaction, but would of course in the heterologous.

If the change in flocculation rate is due to removal of a fraction with small k, it ought to occur on sorp tion with either homol. or heterol. G, but perhaps more sharply in the latter case.

p. 522. I missed the point about dialysis. Is it possible that the precipitate once formed in the presence of salt is sufficiently saturated by virtue of its structure so that the subsequent addition of A would be extremely slow? This result also seems to contradict your explanation for the effect of salt.

~~Does salt affect the composition of the precipitate at optimal ratios determined with and without salt respectively?~~ Are the compositions at optimal ratios determined with high and low salt respectively, different?

I notice you have not questioned the assumption of reversibility of the initial reactions, also fundamental. Some experiments with phage have disappointingly failed to demonstrate reversibility. I have been struggling for a couple of years with a theory developed along the lines of yours,assuming irreversibility. Of two results, one is non-integrable. In general, I would guess that the eventual conclusion will be that the reactions cannot be treated as entirely irreversible. Probably you have reached this conclusion long ago. If I thought you had the time, I would like you to look at the thing as far as I have got. Possibly you could make something of it; I doubt if I can.

I have covered a lot of paper. I hope that doesn't mean it is still confused. I am satisfied that what I cant say is not understood. So I would like to try again on the worst points, if you will p oint them out.

Sincerely

A O Heidelberger.