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EUCLID AVENUE AND KINGSHIGHWAY

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Dear Michael:

I am pleased to know from your letter of October 29 that you have been interested in our work. I am sorry if we gave the impression that your experiment with Ea was an isolated result; to be sure we realize that both your result and the one or two others cited are amply confirmed.

Before taking up the interesting calculations you have made, let me first say something about the general question of heterogeneity of antibody. I do not think that your results and ours are in fundamental disagreement. We do not suppose that the antibody in any one serum is completely homogeneous. The statement on p. 288, "Except for the contrary finding. . ." means merely that if there were no other source of information, we would have concluded from our experiments that A is homogeneous. Since there are your experiments showing very clearly that it is not, the statement is perhaps superfluous. Certainly we should not have implied that the evidence of heterogeneity was based on one experiment. I think the third paragraph of the introduction of the paper (p.281) precludes this implication, and indicates that we have not drawn conclusions opposed to yours, except perhaps in emphasis. That paragraph also states the issue of importance to us. This is not whether heterogeneity exists, which we feel has been answered by your work, but whether the degree of heterogeneity is sufficient to nullify theories of reaction based on the assumption of homogeneity. Regarding the usefulness of rate-measurements based on this assumption, which I understand from Hershey that you have questioned, I think our data show clearly that heterogeneity is not an important factor, even when different sera are compared. The one serious discrepancy, between the two samples of serum 3, table 3, is clearly the effect of some change in the antibody occurring during storage. The data of table 4, and the more careful experiment with serum 29, also establish that at least in a crude way, the rate of reaction is a measure of antibody-content of fractionated sera. Frankly, when we began this work, we wouldn't have been surprised to find, say, a 10-fold decrease in rate of reaction with no detectable change in other measures of A-content, following fractional absorption. Fortunately, such discrepancies were not encountered. Undoubtedly, more elaborate experiments would have revealed small discrepancies, just as you have found by more exact methods.

Our statement (p. 281) that the "diversity is slight, and could not account for various combining proportions" might be misunderstood. It occurs to us now that we may also have misunderstood Northrop, whose citation was intended to narrow the issue. As we interpreted his remarks, he felt that if you had homogeneous antibody it should combine in only one ratio with antigen, independently of the proportions in which they were mixed, which is of course

contrary to all current thinking about immune reactions. Whatever Northrop meant, we do not believe this, and I am sure you do not either. Our statement was not intended to imply that different fractions of antibody could not combine in different proportions with antigen. We hope it has not been so interpreted. The statements immediately preceding, to the effect that diversity of this kind has been observed by you, and "it is not unlikely that this could be confirmed by more extensive application of the methods used below", should prevent this misunderstanding.

Coming now to your calculations, I think you have overestimated the accuracy of our data, especially for the region of A-excess. It must be remembered that the combining ratio is estimated by difference after analysis of supernates. I think you will picture the difficulty of you contemplate attempting to decide between, say, GA_3 and GA_4 in a precipitate formed in the presence of a 5-fold excess of A by analyzing the supernate. This would be almost as difficult by N-determinations.

In the region of only 20 per cent removal of A, which is where you find the difference, the combining ratios might easily vary in repeated tests between 15 and 45 x 10^{-14} ml per lytic unit for serum 29 (see variations in k-determination in table 3, first paper). We have looked up the original data, and find that 3 analyses were made of the supernate in tube #2, serum 29A (table 2). The "per cent absorbed" varied between 14 and 39 per cent, with a mean of 31. Only one analysis was made of the corresponding supernate of serum 29 (21%). Therefore, the difference you point out, while it may be real, certainly is not established by the data. As stated in the paper, repeated analyses were usually made only of the tubes corresponding to 50 per cent absorption, nos. 3 and 4 in this case. In this region the error is much less.

Another illustration of this effect is seen in table 6, where a single serum is tested against two preparations of phage. The same sort of discrepancy appears in the region of 20 per cent absorption of antibody. This means merely that the experimental error becomes very large. It will be noticed that combining ratios may either rise or fall in this region, for the same reason. It happens that serum 29A supernates, the last series to be tested, were titrated with special care. This was to answer another question, whether there really is a significant trend in combining ratio depending on relative excess of A. We had been unable to decide this definitely after perhaps 30 different titrations of this kind. The test with serum 29A convinced us that the trend is real, as might be expected. The fact that the combining ratio drops in tube #2, serum 29, shows that this result is in error. These errors are unavoidable, and it is for this reason that attention was centered on the region of 50 per cent absorption.

I think, however, that an experiment could be done carefully enough to find out whether any difference exists. It would be necessary to set up whole and fractionated serum side by side, using the same preparation of phage, and making titrations of supernates simultaneously. Serums 29 and 29A were not done in this way, as shown in the table by the different amounts of phage in correspondingly numbered tubes. Following your suggestion, we may try this.

Our approach was different. The purpose of the titrations in table 2 was merely to estimate the relative amounts of A in the various sera. the 50 percent endpoint is best for this. Evidence of heterogeneity was sought by comparing these results with those of rate-measurements. This was done in table 3.

It was argued that any marked heterogeneity ought to show up very noticeably in rate-measurements. The comparisons between different sera in table 3 confirm this. No difference was found with the fractionated serum 29. Of course, it would be nice to have more data.

Similar considerations apply to table 4. Two of the 3 sera show a difference. However, as stated, these are very crude tests. The more careful one with serum 29 again showed no difference, so the result is 50-50. The purpose of the experiment of table 4 was primarily to decide whether precipitating and neutralizing A is the same thing. The data are probably sufficient for this. Again it is possible that more careful experiments would reveal significant differences. The one experiment directed to this end (serum 29) failed.

In paper 1, our table 3 is poorly constructed. All measurements of k, except the two tests with serum 1, which are not properly k-measurements at all, were done with P_o about 10^6. The values for serum 29 and 29A are therefore comparable, and stand in the same relation to each other as the other measures of antibody-content (table 3, second paper).

As to combining ratio inabsolute units, 5000 is of course reasonable enough if the MW is 10^8 or so. We have tried to reconcile our data with the much lower molecular size indicated by diffusion measurements.

Regarding Dr. Meyer's query about parallelism of curves of fig. 1, I think if you picture the way the experiments were done, you will agree with our statement. Suppose we have two sera, one containing twice as much A as the other. To a series of tubes containing equal amounts of P, we add various amounts of each serum, and make plaque counts after the reaction is completed. In one of the tubes of each series there will be, for instance, 50 per cent neutralization. The respective amounts of serum in these two tubes will necessarily differ by a factor or two, if the sera themselves differ only in A-content. Since volumes are kept constant (even this is not necessary), the contents of the two tubes are identical. The ratio ml serum/phage differs by a factor of two, and since the abcissa of fig. 1 is on a log scale, this factor appears as a linear distance which is the log of the ratio between antibody-contents of the two sera. Similarly, every point on the one curve would be duplicated by a corresponding point moved to right or left by this constant difference. The resulting curves are superimposable. I do not think this requires any proof, as it is simply a question of arithmetic, which would be the same for any possible relation between amount of A and neutralization. The form of the curves indicates the nature of this relation.

Sincerely yours,

JB/McK

Above all, no matter what we may say in our ignorance, do not take it ever as a "challenge" as you seem to have this time