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CONTRIBUTIONS TO THE DISCUSSION OF THE AGGLUTINATION-INHIBITION METHOD

BY

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INTRODUCTION

1. The Agglutination-Inhibition Method.

As is well known, the Agglutination-Inhibition Method for the measurement of the strength of a group-antigen is based on the following facts:

1° that the antibody contained in the serum from a person of the blood group *A* will cause blood corpuscles from a person of the blood group *B* to agglutinate and

2° that antigen from a person of the blood group *A* or any antigen of the same type will "fix" the serum antibody from a person of blood group *B*, thereby inhibiting more or less the agglutination of blood corpuscles of group *A* brought into touch with the serum antibody.

If in 1° and 2° *A* is replaced by *B* and *B* by *A* the statements thus formulated are likewise true. It may be noted that the serum from a person of blood group *B* is called anti-*A* because it reacts with the antigen from a person of blood group *A*. Similarly the serum antibody from a person of blood group *A* is called anti-*B*.

Now let the problem be to measure the strength of a group-antigen of a known blood group, say *A*. The antigen may be contained in an aqueous extraction from an organ of a person of this blood group or in a solution of a secretion from such a person. The agglutination inhibition method of measurement then generally takes the following shape. A series of small test tubes arranged in a stand is used. Into the first tube of the series 0.1 cm^3 of the original antigen extraction or solution is introduced; its concentration may be denoted by 1. Into the second tube 0.1 cm^3 of a solution of the concentration $^1/_2$ is inserted, into the third tube 0.1 cm^3 of a solution of the con-

centration $^1/_4 = {}^1/_2{}^2$ etc. Hence into the tube no. n 0.1 cm^3 of a solution of the concentration $^1/_2{}^{n-1}$ is introduced. Then 0.1 cm^3 of a serum anti-A is added to the contents of alle the tubes. Thus after this operation the antigen concentrations in the series of tubes are as $^1/_2$, $^1/_2{}^2$, $\cdots$ $^1/_2{}^n$. After the introduction of the serum the test tubes are kept at about 20° C for an hour in order that there may be time for the fixation of the serum antibody to take place. Then one drop of a 5 p.c. suspension in saline of washed blood corpuscles of the group A (A_1) is added to all the tubes. The tubes are again left to themselves for about 2 hours, when they are shaken and the effect of this shaking observed. It will generally be found that in all the tubes up to and including a certain number, no. n, there is no agglutination, while in tube no. $n+1$ and in all the following tubes agglutination has taken place. The explanation of this observation is fairly evident. In all the tubes up to tube no. n the concentrations of the antigen have been high enough to fix the serum antibody to such an extent that the remainder is unable to produce any appreciable agglutination. From tube no. $n+1$ and above, the fixation is no longer complete and so free antibody is present, causing agglutination of the blood corpuscles. Obviously the number n of the last tube in which there is still no agglutination will be the higher, the higher the concentration of the antigen in the original solution or extraction. So n may appropriately be taken as a measure for the strength or concentration of the antigen in question.

The series of tubes with their contents of antigen solution or extraction, serum and blood corpuscles may obviously be vizualised as a scale, the titer scale, on which the strength of the antigen extraction or solution in question is characterized by the number n of the last tube in which no agglutination is perceptible. Let $C_{A\cdot 0}$ be the concentration of the original extraction or solution of antigen, then in tube no. n the antigen concentration will be $\frac{C_{A\cdot 0}}{2^n}$. Let C_s be the concentration of the serum in the tubes and let $C_{s\cdot m}$ be the highest value of the serum concentration without any appreciable effect of agglutination, then the relation between the titer reading n and the antigen concentration $C_{A\cdot 0}$ in question may be written

(1) $$\frac{C_{A\cdot 0}}{2^n} = k\,(C_s - C_{s\cdot m})$$

expressing that in tube no. n all the serum short of the amount $C_{s\cdot m}$ has been fixed by the antigen. The factor k need not be a real constant but may depend on the concentrations of the antigen or of the serum antibody or of both. From (1) it follows that if two antigens of the same type give the readings n_1 and n_2 on two scales with the same serum then the concentration $C_{A\cdot 1.0}$ and $C_{A\cdot 2.0}$ of the antigens must satisfy the relation

(2) $$\frac{C_{A\cdot 1.0}}{C_{A\cdot 2.0}} = 2^{n_1 - n_2}$$

for in the two tubes no. n_1 and n_2 of the two titer scales the antigen concentrations as well as the serum concentrations are in this case the same, and so the factor k must have the same value.

2. The experimental Basis of the present Discussion.

The discussion which will be reported below is based on certain experiments to which the use of the agglutination inhibition method gave rise. When suitably treated these experiments

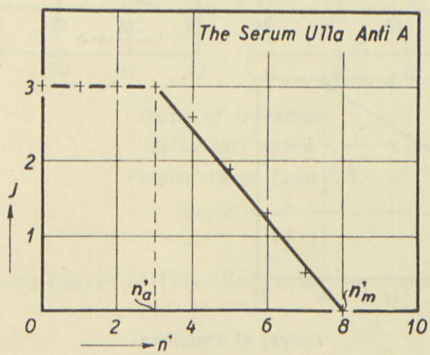

Fig. 1. Serum Titration Curve for Serum Ulla Anti *A*.

turn out to yield results of such a simple and characteristic nature that it occurred to the author that valuable information as to the process of fixation between antigen and serum antibody

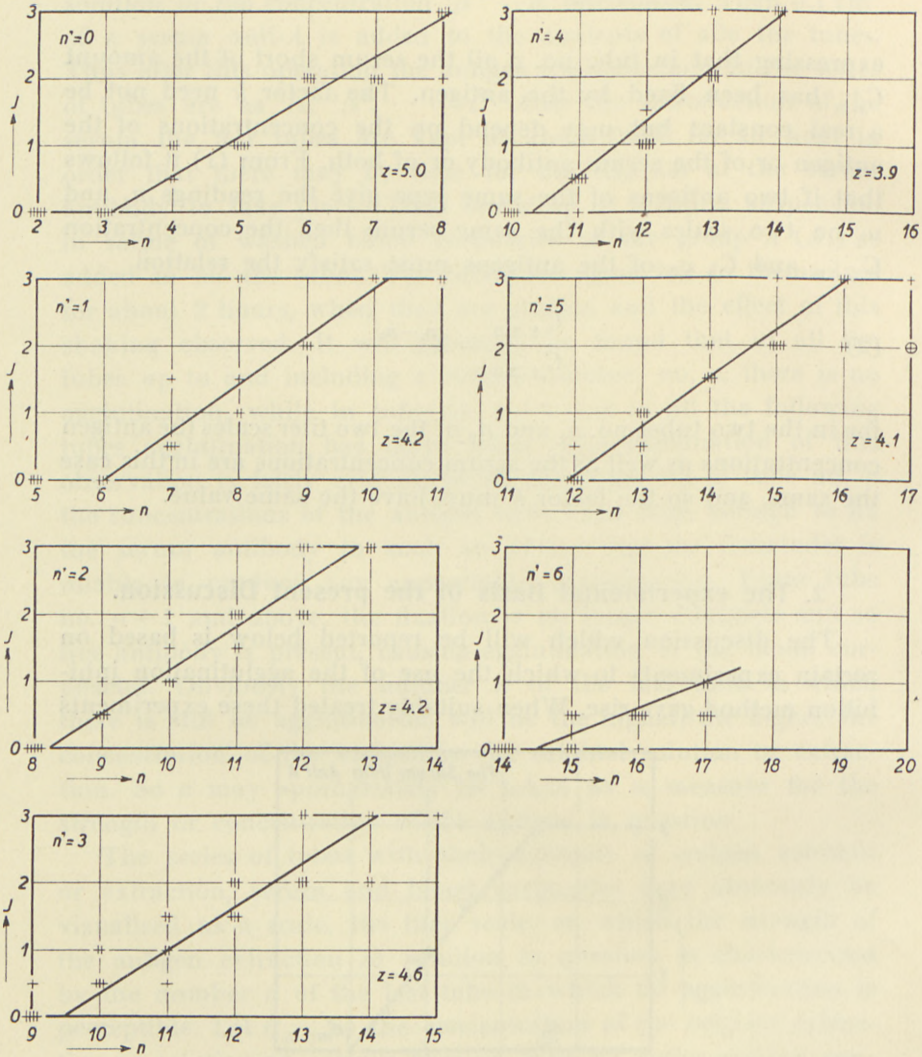

Fig. 2. Smoothed-out Curves of Transition from the Kaas-Ulla Experiment.

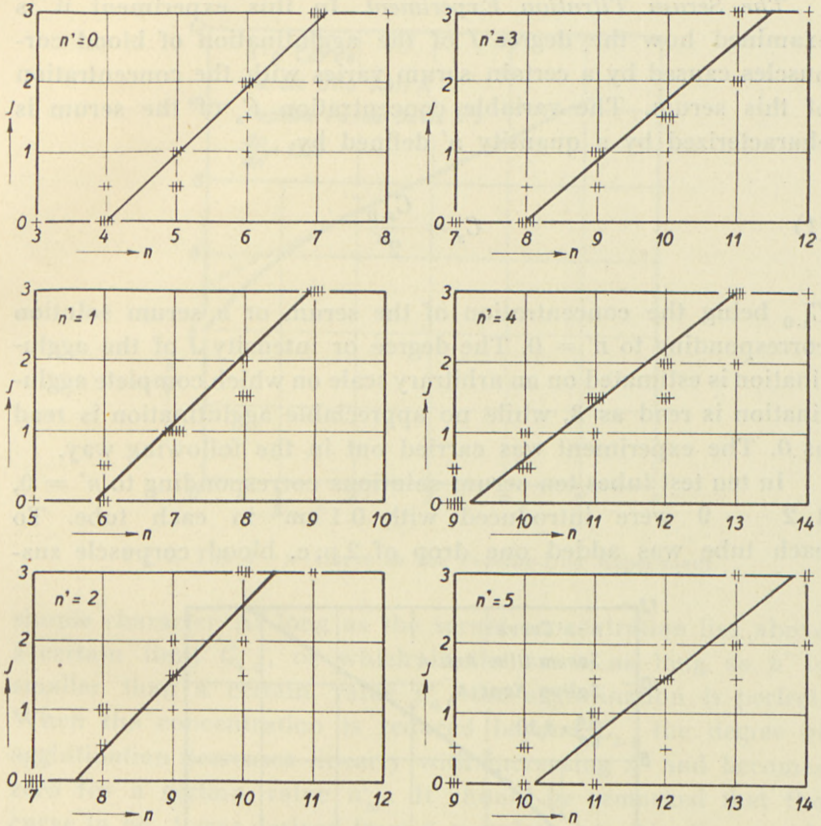

Fig. 3. Smoothed-out Curves of Transition from the Pepsine-Ulla Experiment.

might probably be derived from these results by mathematical analysis. The experiments referred to will now be considered.

The Serum Titration Experiment. In this experiment it is examined how the degree J of the agglutination of blood corpuscles caused by a certain serum varies with the concentration of this serum. The variable concentration C_s of the serum is characterized by a quantity n' defined by

$$C_s = \frac{C_{s.0}}{2^{n'}} \tag{1}$$

$C_{s.0}$ being the concentration of the serum or a serum solution corresponding to $n' = 0$. The degree or intensity J of the agglutination is estimated on an arbitrary scale on which complete agglutination is read as 3, while no appreciable agglutination is read as 0. The experiment was carried out in the following way.

In ten test tubes ten serum solutions corresponding to $n' = 0, 1, 2 \cdots 9$ were introduced with 0.1 cm^3 in each tube. To each tube was added one drop of 2 p. c. blood corpuscle sus-

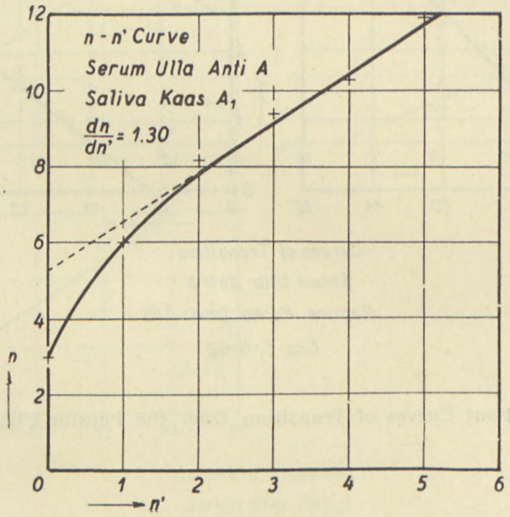

Fig. 4. The $n - n'$-Curve in the Kaas-Ulla Experiment.

pension of the type able to agglutinate under the influence of the serum. After two hours the intensity of the agglutination

was read. Fig. 1 shows the result of an experiment with a serum "Ulla Anti-*A*". It will be noted that the $J-n'$-curve is of a very

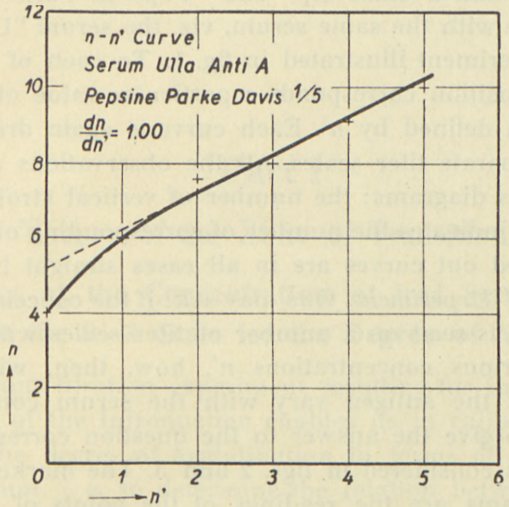

Fig. 5. The $n-n'$-Curve in the Pepsine-Ulla Experiment.

simple character. As long as the serum concentration lies above a certain limit $C_{s.a}$, or which is the same, as long as n' is smaller than a certain value n'_a, the agglutination is perfect. When the concentration is reduced beyond $C_{s.a}$ the degree of agglutination decreases linearly with increasing n' and becomes zero for a certain value n'_m. It should be remarked that the curve in fig. 1 was derived from 6 complete determinations, each point being thus the average of 6 independent observations.

The Zone of Transition. In the reading of the titer scale in the agglutination inhibition measurement one does not, as a rule, confine oneself to simply finding the last tube n in which no trace of agglutination is perceptible. The degree of agglutination in the following tubes $n+1, n+2, \cdots n+\Delta \cdots$ is made the subject of observation, the same scale for the degree of agglutination being used as in the case of the serum titration just described. In tube no. n the degree of agglutination is zero; in the following tubes the degree gradually rises, generally to complete agglutination, read as 3. The zone within which this rise takes place we shall call the zone of transition. Figs. 2 and 3

show a number of curves of transition originating from two comprehensive investigations carried out with two different antigens, viz. "Saliva Kaas A_1" and "Pepsine Parke Davis $^1/_5$", but otherwise with the same serum, viz. the serum "Ulla Anti-A" from the experiment illustrated in fig. 1. To each of the several curves of transition corresponds a particular value of the serum concentration defined by n'. Each curve is again drawn on the basis of 6 separate titer scales. All the observations are marked in the various diagrams; the number of vertical strokes in each of the points indicates the number of corresponding observations. The smoothed out curves are in all cases straight lines.

The $n - n'$-Experiment. One may ask: if the concentration of a given antigen is read on a number of titer scales with the same serum in various concentrations n', how, then, will the titer reading n of the antigen vary with the serum concentration? Figs. 4 and 5 give the answer to the question corresponding to the two cases considered in figs. 2 and 3. The marked ordinates of the diagrams are the readings of the points of intersection between the various curves of transition and the axes of abscissa in the diagrams of figs. 2 and 3. It will again be noted that the observations determine rather smooth and regular curves.

The experimental material recorded in figs. 1—5 forms the direct basis of the discussion that follows.

Part I

Theory of the Zone of Transition.

1. Variation of the Concentration of free Serum with the Reading on the 0—3 Scale for the Degree of Agglutination.

The serum titration experiment resulting for instance in the curve fig. 1 of the Introduction enables us to calibrate our 0—3 scale for the degree of agglutination in terms of concentration of free serum, i. e. to determine the relation between this concentration C_s and the reading J on the scale. Let fig. 6 represent the titration curve. Then the relation between J and the figure n' characterizing the concentration of the serum in the titration experiment (where the concentration of serum is identical with the concentration of free serum) is obviously

$$J = J_a \frac{n'_m - n'}{n'_m - n'_a}. \tag{1}$$

We introduce instead of the qualities n' the corresponding serum concentrations determined by

$$C_{s.a} = \frac{C_{s.0}}{2^{n'_a}}, \quad C_s = \frac{C_{s.0}}{2^{n'}}, \quad C_{s.m} = \frac{C_{s.0}}{2^{n'_m}} \tag{2—4}$$

from which

$$n'_a = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_{s.a}}\right), \; n' = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_s}\right), \; n'_m = \frac{1}{l_e 2} \cdot l_e\left(\frac{C_{s.0}}{C_{s.m}}\right). \tag{2a—4a}$$

If the latter expressions are introduced into (1) we get

$$J = J_a \frac{l_e\left(\frac{C_s}{C_{s.m}}\right)}{l_e\left(\frac{C_{s.a}}{C_{s.m}}\right)} \tag{5}$$

from which it follows that

$$\frac{C_s}{C_{s.m}} = \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{J}{J_a}}. \tag{6}$$

In the case of serum Ulla it appears from fig. 1 that $n'_a = 3$, $n'_m = 8$. Seeing that $\frac{C_{s.a}}{C_{s.m}} = 2^{n'_m - n'_a}$ and noting that J_a stands for the reading 3 on the 0—3 scale it follows that for this serum

$$\frac{C_s}{C_{s.m}} = 32^{\frac{J}{3}} = 2^{\frac{5}{3}J}. \tag{7}$$

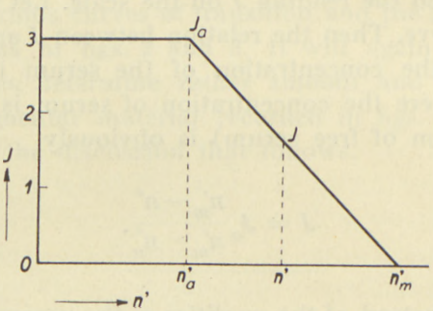

Fig. 6. The Serum Titration Curve (Diagram).

2. Variation of free and fixed Serum within the Zone of Transition.

Employing now our calibrated scale of agglutination we may be able to tell how the concentration of free serum varies within the zone of transition, seeing that we know how the degree of agglutination varies.

It appears from figs. 2 and 3 that the curves of transition may generally be considered as straight lines. If $n+\Delta$ is the

number of a tube within the zone we may appropriately take Δ as abscissa in the representation of the curve of transition when we may write the relation between J and Δ as

$$J = J_a \cdot \frac{\Delta}{\Delta_m}, \tag{1}$$

Δ_m being the width of the zone. It follows from (1) and from (6) paragraph 1 that the concentration of free serum C_s varies with Δ according to the expression

$$\frac{C_s}{C_{s \cdot m}} = \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}}. \tag{2}$$

In the case of the Kaas-Ulla experiment, fig. 2, Δ_m is on an average found to be 4.6 titers and so $\frac{C_s}{C_{s \cdot m}} = 2^{\frac{5}{4.6}\Delta} = 2^{1.09\Delta}$.

The concentration of fixed serum $C_{s \cdot f}$ is hereafter readily derived from the concentration of free serum C_s and from the (total) concentration of serum used in the setting up of the titer scale. It has been suggested that for the latter concentration the value $C_{s \cdot a} = \frac{C_{s \cdot 0}}{2^{n'_a}}$, comp. fig. 1[1], should be adopted. Assuming this choice the concentration of fixed serum is determined by

$$C_{s \cdot f} = C_{s \cdot a} - C_s \tag{3}$$

or by

$$\frac{C_{s \cdot f}}{C_{s \cdot m}} = \frac{C_{s \cdot a}}{C_{s \cdot m}} - \frac{C_s}{C_{s \cdot m}} \tag{3a}$$

and so by virtue of (2)

$$\frac{C_{s \cdot f}}{C_{s \cdot m}} = \frac{C_{s \cdot a}}{C_{s \cdot m}}\left[1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]. \tag{4}$$

[1] It should here be noted that if the concentration $C_{s \cdot a}$ is aimed at in the production of the titer scale, a serum of concentration $n'_a - 1$ should be used, seeing that the concentration of serum is reduced to half its former value by being added to the antigen solution in the titer scale.

Now, this expression is derived on the assumption that the concentration of the serum in the titer scale (before fixation) is just $C_{s.a}$ (corresponding to n'_a in fig. 1). If the concentration is $C_{s.n'}$ corresponding to n', where $n' < n'_a$, the formula is slightly modified. In that case

$$\frac{C_{s.f}}{C_{s.m}} = \frac{C_{s.n'}}{C_{s.m}} - \frac{C_s}{C_{s.m}} \tag{3b}$$

leading to

$$\frac{C_{s.f}}{C_{s.m}} = \frac{C_{s.n'}}{C_{s.m}} \left[1 - \frac{C_{s.a}}{C_{s.n'}} \cdot \left(\frac{C_{s.a}}{C_{s.m}} \right)^{\frac{\Delta}{\Delta_m} - 1} \right] \tag{4a}$$

where it is assumed that the curve of transition is still a straight line and where Δ_m is the width of the zone, which may or may not be equal to that of the zone with the serum concentration $C_{s.a}$.

3. Relative Variation of fixed Antigen within the Zone of Transition.

We will now derive a formula for the relative variation of the fixed antigen within the zone of transition. We shall write the concentration of fixed antigen in tube no. $n + \Delta$ under the form

$$C_{A.f} = \frac{1}{k_\Delta} \cdot \frac{C_{A.0}}{2^{n+\Delta}} \tag{1}$$

thus a certain fraction $\frac{1}{k_\Delta}$ of the antigen present in the said tube. Most likely $\frac{1}{k_\Delta}$ is a function of Δ, i. e. of the concentration of the antigen. In deriving the function we shall make the sole assumption that the antigen will fix serum in a definite ratio, i. e. that the amount of antigen required to fix a certain amount of serum is proportional to the latter amount. This assumption is expressed in the equation

$$C_{A.f} = c \cdot C_{s.f} \tag{2}$$

where c is a constant. Introducing the value for $C_{s\cdot f}$ derived from (4) in the preceding paragraph we find

$$(3) \qquad C_{A\cdot f} = cC_{s\cdot a}\left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]$$

and finally from (3) and (1)

$$(4) \qquad \frac{1}{k_\Delta} = \frac{cC_{s\cdot a}}{C_{A\cdot 0}}\cdot 2^n\left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

The factor preceding the brackets is with a given titer scale a constant, and a formula for the relative variation of $\frac{1}{k_\Delta}$ with Δ may thus be written

$$(5) \qquad \frac{1}{k_\Delta} = \left[1-\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

Here it is supposed that the concentration of the serum in the titer scale is $C_{s\cdot a}$ corresponding to n'_a in fig. 1. If the concentration is $C_{s\cdot n'}$ corresponding to $n' < n'_a$, then the expression (4a) of paragraph 2 for $C_{s\cdot f}$ should be introduced into (2), when we get instead of (5)

$$(5\,a) \qquad \frac{1}{k_\Delta} = \left[1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\cdot\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}\right]\cdot 2^{\Delta}.$$

4. The Question of a Law of Mass-Action for the Fixation within the Zone of Transition.

Let us assume that one molecule of antigen unites with one molecule of the antibody to form one single molecule of the combination product. Then we might presumably expect a law of mass-action of the form

$$(1) \qquad \frac{C_s C_A}{C_{A\cdot f}} = K \quad \text{(constant)}$$

C_s being the concentration of free serum, C_A the concentration of free antigen, and $C_{A \cdot f}$ the concentration of fixed antigen (equal or proportional to the concentration of fixed serum and to the concentration of the combination product). Introducing for the concentrations the following expression

$$C_s = \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s \cdot m} \tag{2}$$

$$C_{A \cdot f} = \frac{1}{k_\Delta} \frac{C_{A \cdot 0}}{2^{n+\Delta}} \tag{3}$$

$$C_A = \frac{C_{A \cdot 0}}{2^{n+\Delta}} \left(1 - \frac{1}{k_\Delta}\right) \tag{4}$$

we may rewrite (1) in the shape

$$K = \frac{C_s C_A}{C_{A \cdot f}} = \frac{1 - \frac{1}{k_\Delta}}{\frac{1}{k_\Delta}} \cdot \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s \cdot m}. \tag{5}$$

Now, obviously, $\frac{1}{k_\Delta}$ here stands for the absolute value of the fraction of antigen fixed by the serum. Our theory, as expressed by the formulae (5) and (5a) of paragraph 3, yields relative values only. If the figures derived from the formulae are all divided by the value of $\frac{1}{k_\Delta}$ for $\Delta = 0$, i. e. if the formulae (5) and (5a) of the said paragraph are transcribed to

$$\frac{1}{k_{\Delta \cdot 1}} = \frac{1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{\frac{\Delta}{\Delta_m} - 1}}{1 - \left(\frac{C_{s \cdot a}}{C_{s \cdot m}}\right)^{-1}} \cdot 2^\Delta \tag{6}$$

and

$$\frac{1}{k_{\Delta\cdot 1}} = \frac{1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{\Delta}{\Delta_m}-1}}{1-\frac{C_{s\cdot a}}{C_{s\cdot n'}}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{-1}}\cdot 2^{\Delta} \tag{6a}$$

we arrive at relative values of $\frac{1}{k_\Delta}$ defined by $\frac{1}{k_\Delta}$ being 1 for $\Delta = 0$. We shall make these values our starting point in the following test and denote the absolute value of $\frac{1}{k_\Delta}$ to be introduced into (5) by $a \cdot \frac{1}{k_{\Delta\cdot 1}}$, where a is a constant so far unknown. What we can now do is to try whether the quantity $K = \frac{C_s C_A}{C_{A\cdot f}}$ may assume a constant value with a suitably chosen value of a.

If there be such a value it should satisfy the equation

$$\frac{dK}{d\Delta} = 0 \quad \text{for} \quad \Delta = 0. \tag{7}$$

Conversely we may try whether the value of a derived from this relation will make K a constant within the zone of transition or a greater or smaller part of that zone.

Confining ourselves to the case $C_{s\cdot n'} = C_{s\cdot a}$ we may for the sake of simplicity write (6) as

$$\frac{1}{k_{\Delta\cdot 1}} = \frac{1-z^{\frac{\Delta}{\Delta_m}-1}}{1-\frac{1}{z}}\cdot 2^{\Delta} \tag{8}$$

where $z = \frac{C_{s\cdot a}}{C_{s\cdot m}}$. Introducing $a \cdot \frac{1}{k_{\Delta\cdot 1}}$ for $\frac{1}{k_\Delta}$ in (5) we get

$$K = \frac{z-1-a\left(z-z^{\frac{\Delta}{\Delta_m}}\right)\cdot 2^{\Delta}}{a\left(z-z^{\frac{\Delta}{\Delta_m}}\right)\cdot 2^{\Delta}}\cdot z^{\frac{\Delta}{\Delta_m}}. \tag{9}$$

Differentiating K with regard to Δ and putting $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ we find, after a series of rather tedious transcriptions, the following expression for a

$$a = \frac{z}{z-1} - \frac{l_e 2}{l_e z} \cdot \Delta_m. \tag{10}$$

Generally z is large compared to 1 and thus, approximately, we may write

$$a = 1 - \frac{l_e 2}{l_e z} \cdot \Delta_m. \tag{10a}$$

The latter formula is also found if an approximate expression is derived for K on the assumption of small values of Δ. On this assumption and assuming that z is large compared to 1 (9) may be replaced by

$$K = \frac{1 - a \cdot 2^{\Delta}}{a \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}. \tag{9a}$$

If K is here differentiated with regard to Δ and if $\frac{dk}{d\Delta}$ is equalised with zero for $\Delta = 0$ we can immediately write down the expression for a in (10a). Again it may be noted that the value of K corresponding to $\Delta = 0$ is found to be

$$K = \frac{1-a}{a} \tag{11}$$

both from (10) and from the approximate expression (10a). Now our deduction is based on the formula (6) for $\frac{1}{k_{\Delta \cdot 1}}$, i. e. a formula applying only to the case where the serum concentration in the tubes of the titer scale is just identical with $C_{s \cdot a}$. If the said concentration is larger, viz. $C_{s \cdot n'}$ $(n' < n'_a)$ the formula (6a) should be used. Using the abbreviations $\frac{C_{s \cdot a}}{C_{s \cdot m}} = z$ and $\frac{C_{s \cdot a}}{C_{s \cdot n'}} = u$ (6a) may be rewritten as

$$\frac{1}{k_{\Delta \cdot 1}} = \frac{1 - uz^{\frac{\Delta}{\Delta_m} - 1}}{1 - u \cdot \frac{1}{z}} \cdot 2^{\Delta}. \tag{12}$$

If this expression is introduced into the formula (5) we get

$$K = \frac{z - u - a\left(z - uz^{\frac{\Delta}{\Delta_m}}\right) \cdot 2^{\Delta}}{a\left(z - uz^{\frac{\Delta}{\Delta_m}}\right) \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}. \tag{13}$$

Now, it should here be noted that u is a fraction such as $\frac{1}{2}$ or $\frac{1}{4}$ or $\frac{1}{5}$ etc. thus smaller than unity. Hence for suitably small values of Δ we may use the approximate formula

$$K = \frac{1 - a \cdot 2^{\Delta}}{a \cdot 2^{\Delta}} \cdot z^{\frac{\Delta}{\Delta_m}}, \tag{14}$$

i. e. exactly the same formula as in the particular case of $C_{s \cdot n'} = C_{s \cdot a}$. Hence, in the more general case also, we may calculate an approximate value a corresponding to $\frac{dK}{d\Delta} = 0$, $\Delta = 0$ from (10a), and the value of K for $\Delta = 0$ from the formula (11).

5. Application of the Formulae to the Kaas-Ulla- and the Pepsine-Ulla Experiment.

We will apply our formulae to the experiments described in paragraph 2 of the Introduction. We shall illustrate the mode of calculation by the Kaas-Ulla experiment. The various steps are given in Table I. We consider the case $n' = n'_a = 3$ (comp. fig. 1 Introduction). With serum Ulla $n'_m = 8$ and so $C_{s \cdot a}/C_{s \cdot m} = 2^{8-3} = 2^5 = 32$. Again in the Kaas-Ulla experiment $\Delta_m = 4.6$ (comp. fig. 2 Introduction). With these values the formula (5) of paragraph 3 becomes

$$\frac{1}{k_{\Delta}} = \left[1 - 32^{\frac{\Delta}{\Delta_m}-1}\right] \cdot 2^{\Delta}.$$

The first 6 columns of Table I show the calculation of $\frac{1}{k_{\Delta}}$ according to this formula. In the following column that of $\frac{1}{k_{\Delta \cdot 1}}$ is given. Now from the formula (10) of paragraph 4 we find $a = \frac{32}{31} - 0.2 \cdot \Delta_m = 0.111$. In column 8 the values of $a \cdot \frac{1}{k_{\Delta \cdot 1}}$ are stated, in column 9 then the values of $\frac{1 - a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$ and finally in column 10 the figures found for the quantity

$$K = \frac{1 - a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}} \cdot 32^{\frac{\Delta}{\Delta_m}}$$

derived from (5) paragraph 4 where we have neglected the constant quantity $C_{s \cdot m}$. Table I further comprises a calculation of K based on another value of a; the meaning of this calculation will be explained below.

A similar set of calculations was carried out from the data of the Pepsine-Ulla experiment. Here the width of the zone of transition Δ_m was 3.3 titers, from which the value of a defined by (7) or (10), paragraph 4, is found to be 0.371.

Again for both of the two experiments the variation of the quantity K with Δ was calculated corresponding to other values of a. The results of all the calculations here referred to will now be considered.

In fig. 7 the values of $\frac{1}{k_{\Delta \cdot 1}}$ from Table I have been plotted against Δ, curve a. The curve corresponds to a serum concentration n' (in the tubes of the titer scale) equal to $n'_a = 3$ (comp. fig. 1). It is seen that $\frac{1}{k_{\Delta \cdot 1}}$ rises to a maximum and then drops rapidly to zero at $\Delta = \Delta_m$. In the same figure curve b

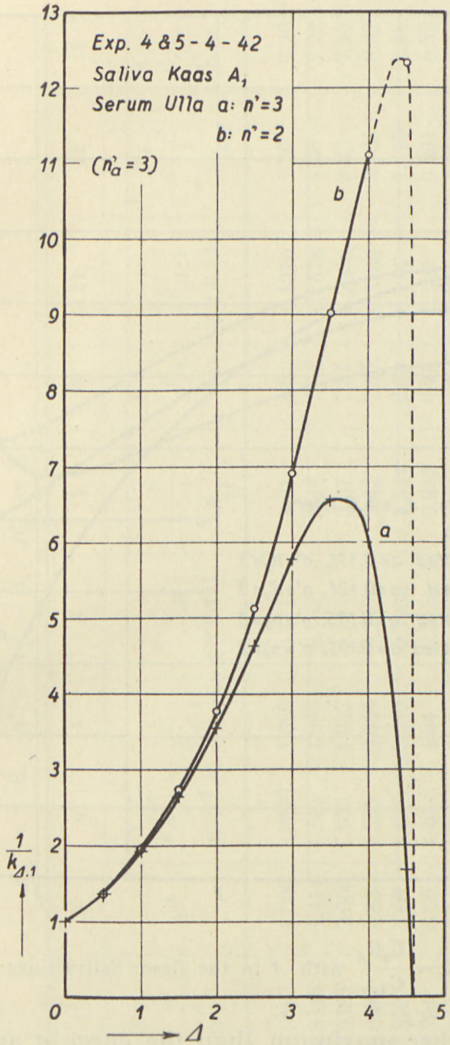

Fig. 7. Variation of $\frac{1}{k_{\Delta \cdot 1}}$ with Δ in the Case: Saliva Kaas A_1, Serum Ulla, $n' = n'_a = 3$, Curve a and $n' = 2$, Curve b.

represents the variation of $\frac{1}{k_{\Delta \cdot 1}}$ with Δ, corresponding to a titer scale with a serum concentration $n' = 2$. The curve has been calculated from formula (6a), paragraph 4, where $\frac{C_{s \cdot a}}{C_{s \cdot n'}} = \frac{1}{2}$.

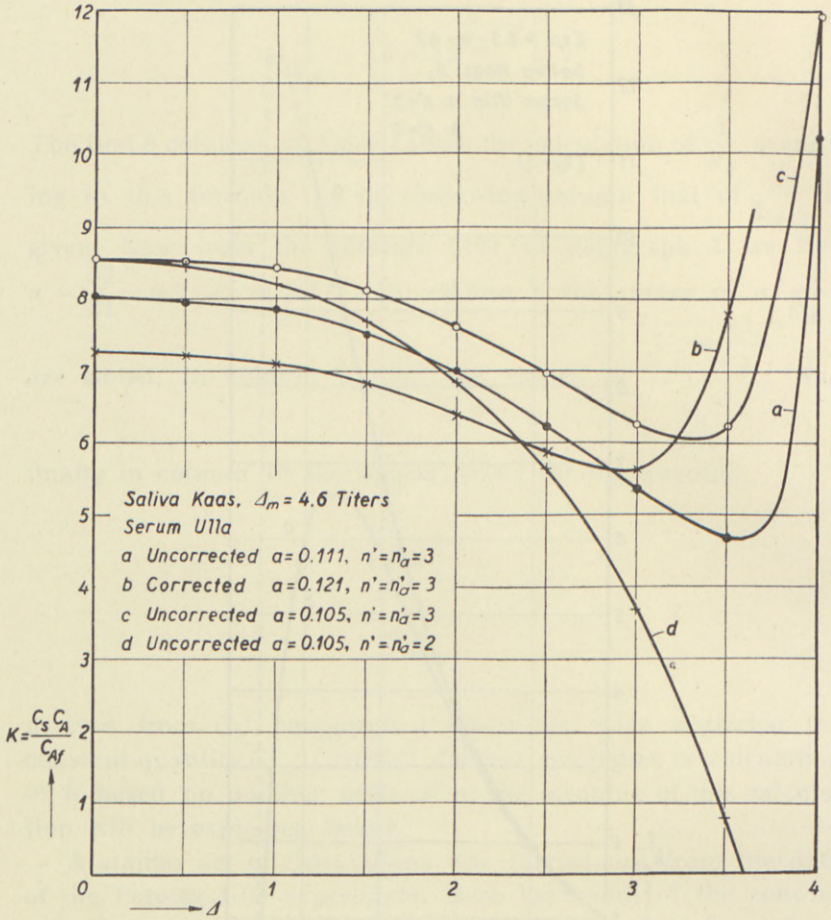

Fig. 8. Variation of $K = \frac{C_s C_A}{C_{A \cdot f}}$ with Δ in the Case: Saliva Kaas A_1, Serum Ulla.

It rises to a higher maximum than the curve *a* and the maximum is located closer to Δ_m than in the latter case. In fig. 8 the variations with Δ of K from Table I have been represented in the curves *a* and *b*. It will be noted that the quantity K is approximately constant within part of the zone of transition. It gradually decreases to a minimum and then, towards the end of the zone, rises rapidly. Fig. 8 further comprises two other curves *c* and *d* both calculated for a value of *a* slightly different from that of curve *a*, viz. the value 0.105. The curves

Table I.

Saliva Kaas A_1, Serum Ulla Anti-A

$\Delta_m = 4 \cdot 6, \quad n' = n'_a = 3, \quad n'_m = 8$

1	2	3	4	5	6	7	8	9	10	11	12	13
Δ	$\frac{\Delta}{4 \cdot 6}$	$\frac{\Delta}{32^{\frac{\Delta}{4 \cdot 6}-1}}$	$M = \frac{\Delta}{1-32^{\frac{\Delta}{\Delta_m}-1}}$	2^{Δ}	$\frac{1}{k_{\Delta}} = \frac{}{M \cdot 2^{\Delta}}$	$\frac{1}{k_{\Delta \cdot 1}}$	$a \cdot \frac{1}{k_{\Delta \cdot 1}}$ $a = 0.111$	$\frac{1-a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$	$K = \frac{C_s C_A}{C_{A \cdot f}}$	$a\frac{1}{k_{\Delta \cdot 1}}$ $a = 0.121$	$\frac{1-a\frac{1}{k_{\Delta \cdot 1}}}{a\frac{1}{k_{\Delta \cdot 1}}}$	$K = \frac{C_s C_A}{C_{A \cdot f}}$
0	0	0.0313	0.9687	1.000	0.9687	1.000	0.111	8.01	8.01	0.1210	7.26	7.26
0.5	0.1088	0.0455	0.9545	1.414	1.347	1.391	0.156	5.46	7.94	0.1678	4.96	7.22
1.0	0.2175	0.0667	0.9333	2.000	1.865	1.927	0.2140	3.67	7.84	0.2308	3.33	7.10
1.5	0.3260	0.0970	0.9030	2.83	2.555	2.635	0.2925	2.42	7.50	0.3130	2.195	6.82
2.0	0.4350	0.141	0.859	4.00	3.435	3.545	0.3935	1.544	6.98	0.414	1.415	6.40
2.5	0.5440	0.206	0.794	5.66	4.49	4.64	0.515	0.942	6.21	0.529	0.891	5.88
3.0	0.6525	0.300	0.700	8.00	5.60	5.78	0.642	0.558	5.35	0.630	0.588	5.64
3.5	0.7610	0.437	0.563	11.31	6.36	6.58	0.730	0.370	5.19	0.643	0.556	7.77
4.0	0.8700	0.637	0.363	16.00	5.81	6.00	0.666	0.502	10.22	0.409	1.445	42.9
4.5	0.9780	0.927	0.073	22.60	1.64	1.69	..	..	..	..	..	..

correspond to the two values of the serum concentration $n' = 3$ and 2 respectively. It will be noted that the minimum is more pronounced in the case of the higher serum concentration than in that of the lower.

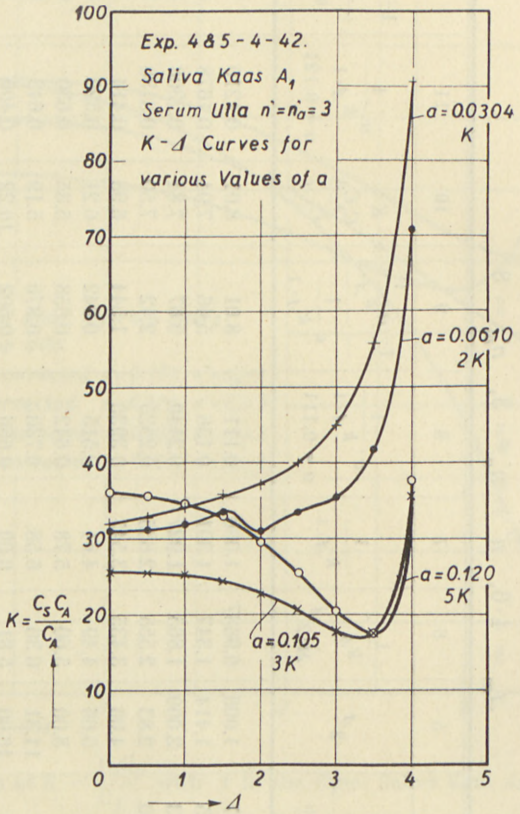

Fig. 9. Comparison of $K-\Delta$-Curves corresponding to various Values of the Factor a: Saliva Kaas A_1, Serum Ulla $n' = n'_a = 3$.

The curves in fig. 8 have been calculated for values of a making $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ or approximately so. It would seem of interest to compute K-curves for other values of a. Results of such a calculation have been presented in fig. 9. In order to render possible a more direct comparison various multiples of

K have been plotted. It will be noted that all the curves are approximately horizontal within a certain initial part of the zone of transition.

A similar set of curves corresponding to the Pepsine-Ulla Experiment are reproduced in figs. 10, 11 and 12. The $\frac{1}{k_{\Delta \cdot 1}}$—$\Delta$-curves are of the same character as those of fig. 7. The K—Δ-curves

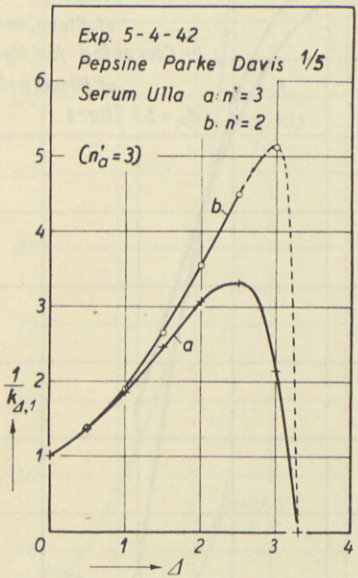

Fig. 10. Variation $\frac{1}{k_{\Delta \cdot 1}}$ with Δ in the Case: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla, $n = n'_a = 3$, Curve a and $n' = 2$, Curve b.

have also much the same character as the corresponding curves in fig. 8 apart from the minima being much more pronounced in fig. 11 than in fig. 8. They are, in fact, located below $K = 0$. Finally fig. 13, curve c, and fig. 14 correspond to an antigen, Stomach Pt. I. 140, giving with serum Ulla a very wide zone of transition, viz. $\Delta_m = 6$. No complete investigation with the combination of this antigen and serum Ulla was carried out but the existence of a zone of the width $\Delta_m = 6$ was established for the combination. With this width there is no (positive) value of a which will make $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$ (comp. formula

(10) paragraph 4). So the $K-\Delta$-curves in fig. 14 were calculated for a number of small positive values of a.

From the discussion now recorded it would seem that no very simple law of mass-action dominates the fixation between

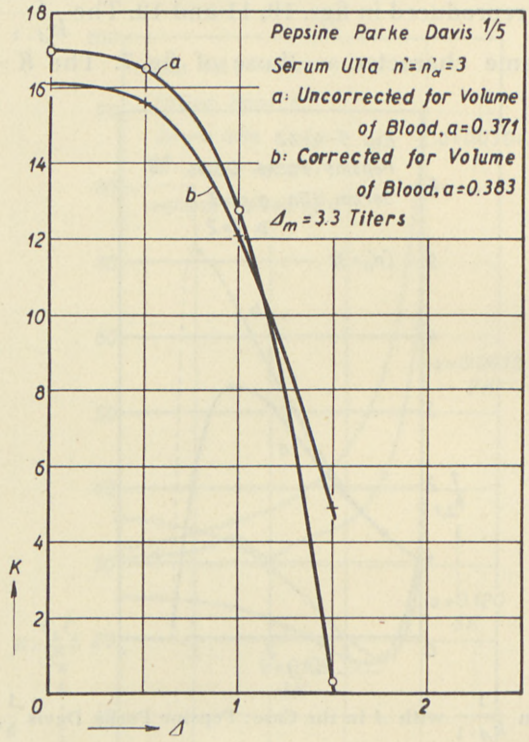

Fig. 11. Variation of $K = \frac{C_s C_A}{C_{A \cdot f}}$ with Δ in the Case: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla $n' = n'_a = 3$.

the antigen and the serum antibody in the agglutination inhibition test—though there may be an approximation to such a law within certain ranges of concentration of the two components.

6. Corrections for the Volume of the Blood Drop.

In the titer curve fig. 1 for the serum the abscissa characterizes the concentration of the serum—by the quantity n'—as it was before the addition of a drop of blood. Now the volume

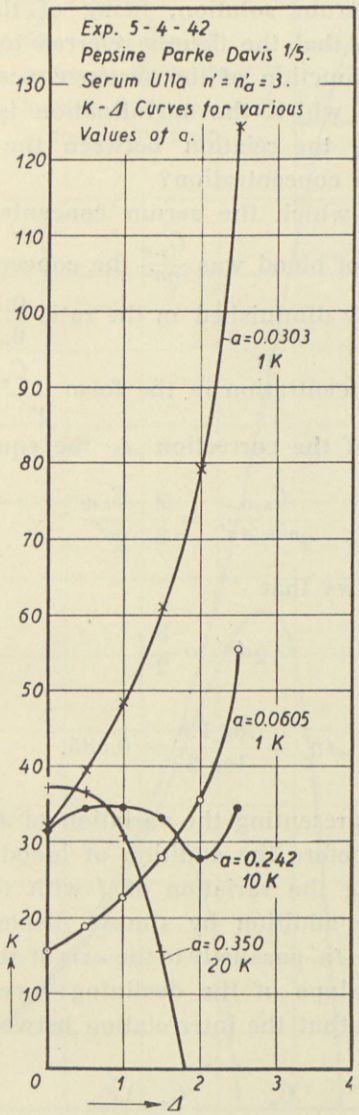

Fig. 12. Comparison of K — Δ-Curves corresponding to various Values of the Factor a: Pepsine Parke Davis $\frac{1}{5}$, Serum Ulla $n' = n'_a = 3$.

of a "drop" is not at all inappreciable compared to the volume 0.1 cm^3, of the serum solution, being of the order of size 0.05 cm^3. It follows that the figures referred to do not give the agglutination as a function of the serum concentration prevailing in the tube in which the agglutination is read. We may ask: what will be the relation between the agglutination J and the true serum concentration?

In the tube in which the serum concentration before the addition of a drop of blood was $\frac{C_{s.0}}{2^{n'}}$ the concentration after the addition is obviously diminished in the ratio $\frac{0.10}{0.15} = \frac{2}{3}$. We may write the actual concentration in the form $\frac{C_{s.0}}{2^{n'+\Delta n'}}$ and have for the determination of the correction $\Delta n'$ the equation

$$\frac{C_{s.0}}{2^{n'+\Delta n'}} = \frac{2}{3} \cdot \frac{C_{s.0}}{2^{n'}} \tag{1}$$

from which it follows that

$$2^{\Delta n'} = \frac{3}{2} \tag{2}$$

or

$$\Delta n' = \frac{\log 1.5}{\log 2} = 0.585. \tag{2a}$$

Hence the curve representing the variation of J with the serum concentration (n') before the addition of blood is changed into a curve representing the variation of J with the concentration $(n'+\Delta n')$ after the addition by simply displacing the former curve by the amount $\Delta n'$ parallelly to the axis of abscissae. Through this operation the slope of the declining curve branch is not altered. This means that the interrelation between C_s and J may still be written

$$\frac{C_s}{C_{s.m}} = \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{J}{J_a}} \tag{3}$$

where $C_{s.a}$ and $C_{s.m}$ on the right-hand side may have the values read on the uncorrected curve or, as well, the corrected values read on the displaced curve, while $C_{s.m}$ on the left-hand side

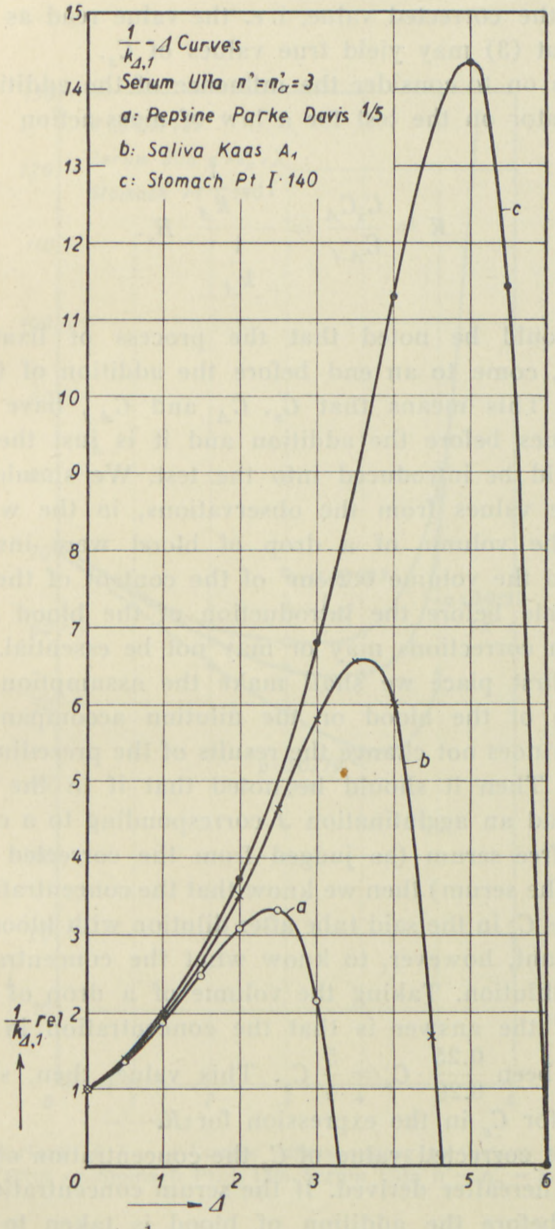

Fig. 13 Comparison of $\frac{1}{k_{\Delta \cdot 1}} - \Delta$-Curves corresponding to various Antigens: *a* Pepsine Parke Davis $\frac{1}{5}$, *b* Saliva Kaas A_1, *c* Stomach Pt. I. 140. Serum Ulla $n' = n'_a = 3$.

should be the corrected value, i. e. the value read as $n'_m + \Delta n'$ in order that (3) may yield true values of C_s.

We pass on to consider the influence of the addition of the blood indicator on the test for a law of mass-action

$$K = \frac{C_s C_A}{C_{A \cdot f}} = \frac{1 - \frac{1}{k_\Delta}}{\frac{1}{k_\Delta}} \cdot B_s .$$

Here it should be noted that the process of fixation has, presumably, come to an end before the addition of the blood suspension. This means that C_s, C_A and $C_{A \cdot f}$ have assumed definite values before the addition and it is just these values which should be introduced into the test. We should directly derive these values from the observations, in the way stated above, if the volume of a drop of blood were insignificant compared to the volume 0.2 cm^3 of the content of the tubes of the titer scale before the introduction of the blood indicator. Now certain corrections may or may not be essential.

In the first place we shall make the assumption that the introduction of the blood or the dilution accompanying this introduction does not change the results of the preceding process of fixation. Then it should be noted that if in the tube no. $n + \Delta$ we read an agglutination J corresponding to a concentration C_s of free serum (as judged from the corrected curve of titration of the serum) then we know that the concentration of the free serum is C_s in the said tube after dilution with blood suspension. We want, however, to know what the concentration was before the dilution. Taking the volume of a drop of blood to be 0.05 cm^3 the answer is that the concentration in question must have been $\frac{0.25}{0.20} \cdot C_s = \frac{5}{4} C_s$. This value, then, should be introduced for C_s in the expression for K.

From the corrected value of C_s the concentration of fixed serum $C_{s \cdot f}$ is hereafter derived. If the serum concentration in the titer scale before the addition of blood is taken to be $C_{s \cdot a}$, compare fig. 6, we have

$$C_{s \cdot f} = C_{s \cdot a} - \frac{5}{4} C_s . \tag{4}$$

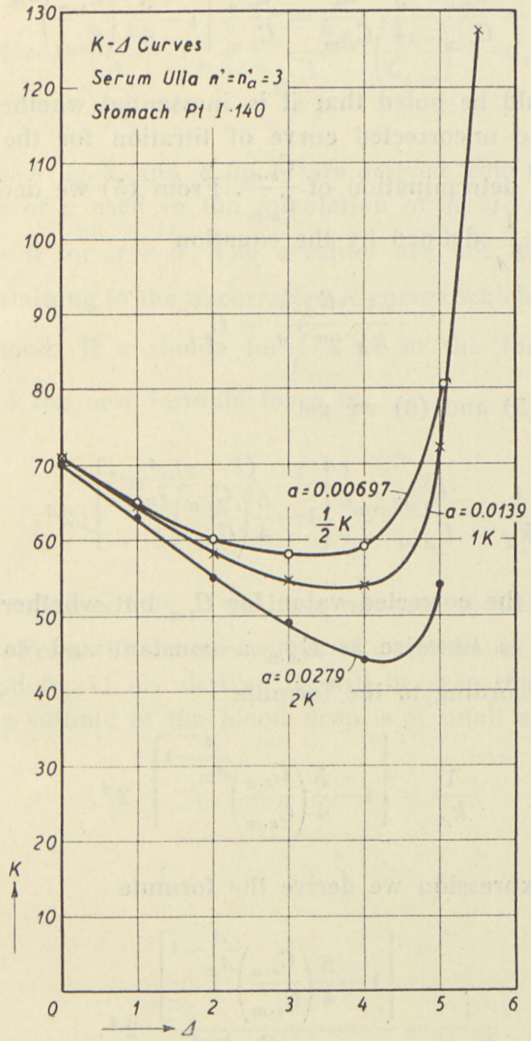

Fig. 14. Comparison of K — Δ-Curves corresponding to various Values of the Factor a: Stomach Pt. I. 140. Serum Ulla $n' = n'_a = 3$.

From (4) we get

$$(5) \qquad \frac{C_{s\cdot f}}{C_{s\cdot m}} = \frac{C_{s\cdot a}}{C_{s\cdot m}} - \frac{5}{4}\cdot\frac{C_s}{C_{s\cdot m}} = \frac{C_{s\cdot a}}{C_{s\cdot m}}\left[1 - \frac{5}{4}\cdot\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right].$$

Here it should be noted that it is inessential whether the corrected or the uncorrected curve of titration for the serum is used for the determination of $\frac{C_{s\cdot a}}{C_{s\cdot m}}$. From (5) we derive an expression for $\frac{1}{k_A}$ defined by the equation

$$(6) \qquad \frac{1}{k_A}\,\frac{C_{A\cdot 0}}{2^{n+A}} = C_{s\cdot f}.$$

Combining (5) and (6) we get

$$(7) \qquad \frac{1}{k_A} = \frac{C_{s\cdot a}}{C_{A\cdot 0}}\cdot 2^n\cdot\left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]\cdot 2^A.$$

Here $C_{s\cdot a}$ is the corrected value for $C_{s\cdot a}$ but whether corrected or not, $C_{s\cdot a}$ is likewise as $C_{A\cdot 0}$ a constant and so $\frac{1}{k_A}$ varies relatively, according to the formula

$$(7\,a) \qquad \frac{1}{k_A} = \left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]\cdot 2^A.$$

From this expression we derive the formula

$$(7\,b) \qquad \frac{1}{k_{A\cdot 1}} = \frac{\left[1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{\frac{A}{A_m}-1}\right]}{1 - \frac{5}{4}\left(\frac{C_{s\cdot a}}{C_{s\cdot m}}\right)^{-1}}\cdot 2^A.$$

This formula should be compared to the uncorrected formula (6), paragraph 4 for $\frac{1}{k_{A\cdot 1}}$. The values for $\frac{1}{k_{A\cdot 1}}$ derived from

(7b) are those which should be introduced into the formula for K, viz.

$$(8) \qquad K = \frac{1 - a\frac{1}{k_{\Delta}}}{a\frac{1}{k_{\Delta}}} \cdot C_s = \frac{1 - a\frac{1}{k_{\Delta}}}{a\frac{1}{k_{\Delta}}} \cdot \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{\Delta}{\Delta_m}} \cdot C_{s.m}.$$

The curves b fig. 8 and b fig. 11 are derived from this formula. The values of a used in the calculation of K are such as will make $\frac{dK}{d\Delta} = 0$ for $\Delta = 0$. The a-values are not identical with those appertaining to the uncorrected K-curves which will readily be understood. If z stands for $\frac{C_{s.a}}{C_{s.m}}$ as in the formula 10 of paragraph 4 the new formula for a is

$$(9) \qquad a = \frac{z(z-1)}{\left(z - \frac{5}{4}\right)^2} - \frac{z-1}{z-\frac{5}{4}} \cdot \frac{l_e 2}{l_e z} \Delta_m.$$

If $\frac{5}{4}$ is here replaced by 1 we have returned to the formula 10 referred to. From (9) the two values of a written on the b-curves of fig. 8 and fig. 11 are derived. It will be seen that the correction for the volume of the blood drop is of small moment only.

Part II.

Theory of the $n-n'$-Curve.

1. Shape of the $n-n'$-Curve. The $\frac{1}{k}-n$-Curve.

In fig. 15 the observed $n-n'$-curves from figs. 4 and 5 of the Introduction have been redrawn with the change that n' now corresponds to the concentration of the serum in the titer-scale proper; n' fig. 15 is, thus, greater by 1 than the n' in figs. 4 and and 5 of the Introduction. The observed values of n are marked by crosses. The curves shown have been drawn as smoothed out curves between the crosses. Then it was tried whether the curves might be represented by an expression of the form

$$n^2 = \beta (n' - n'_0) \tag{1}$$

thus by the analytical expression for a parabola with its axis coinciding with the n'-axis in fig. 15 and with its vertex in the point $n' = n'_0$. The values of β and n'_0 may appropriately be determined by means of two sets of points situated on the smoothed out curve. In the case of Saliva Kaas the points with the abscissae $n' = 1$ and $n' = 6$ were used, the corresponding ordinates being read as $n = 3.0$ and $n = 11.7$ respectively. If the two sets of coordinates are introduced for n' and n in (1) two equations are obtained yielding the values $\beta = 25.6$ and $n'_0 = 0.649$, thus the expression

$$n^2 = 25.6\,(n' - 0.649) \qquad \text{(Saliva Kaas).}$$

In a similar manner the formula

$$n^2 = 17.2\,(n' - 0.070) \qquad \text{(Pepsine)}$$

was derived for Pepsine Parke Davis $\frac{1}{5}$. The points marked by

circles in fig. 15 were calculated from these formulae. It appears that the formulae cover the experimental curves with almost astonishing exactness.

It would thus seem that we may with good approximation represent the interrelation between n and n' by the formula (1).

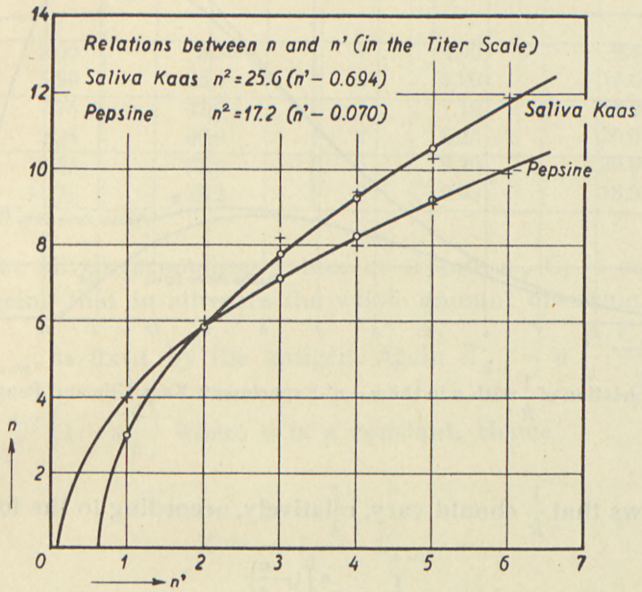

Fig. 15. $n-n'$-Curves from the Kaas-Ulla- and the Pepsine-Ulla Experiments.

Now the fraction $\frac{1}{k}$ of antigen fixed by the serum is defined by

(2) $$\frac{1}{k} \cdot \frac{C_{A\cdot 0}}{2^n} = c \cdot \frac{C_{s\cdot 0}}{2^{n'}}$$

or by

(3) $$\frac{1}{k} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{n-n'}$$

where c is so far assumed to be a constant.

Eliminating n' from (3) by means of (1) we get

(5) $$\frac{1}{k} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} 2^{n - \frac{1}{\beta} n^2 - n'_0} = c \cdot \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{-n'_0} \cdot 2^{n\left(1 - \frac{n}{\beta}\right)}.$$

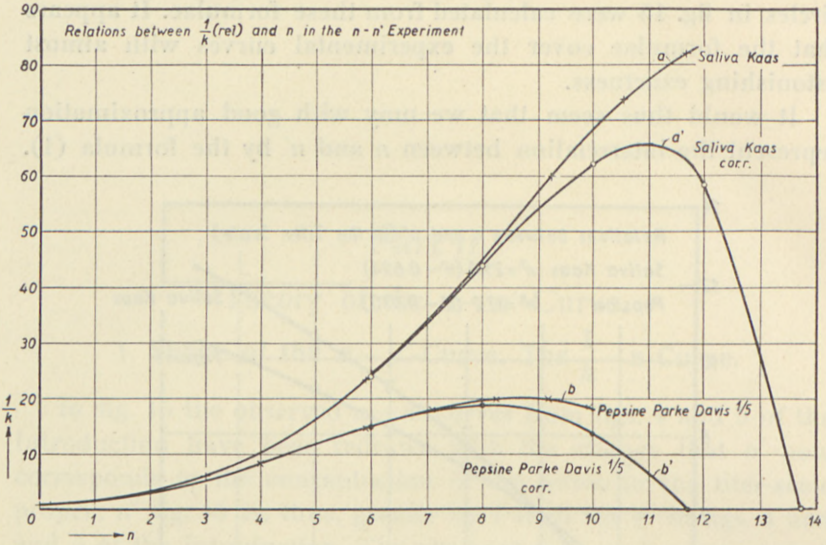

Fig. 16. Variation of $\frac{1}{k}$ with n in the $n-n'$-Experiments Kaas-Ulla and Pepsine-Ulla.

It follows that $\frac{1}{k}$ should vary, relatively, according to the formulae

$$\frac{1}{k} = 2^{n\left(1-\frac{n}{\beta}\right)}. \tag{5}$$

Representations of the variation of $\frac{1}{k}$ with n for the two antigens: Saliva Kaas and Pepsine Parke Davis $\frac{1}{5}$ are given in Table II and in graphical form in fig. 16, curves a and b. It follows from (5) that the curves representing the interrelation between $\frac{1}{k}$ and n are symmetrical with regard to $n = \frac{\beta}{2}$ for which abscissa $\frac{1}{k}$ exhibits a maximum.

One might ask whether free and fixed antigen and serum distribute themselves according to a simple law of mass-action in the $n-n'$-experiment. We may again write down an expression for the quantity

$$K = \frac{C_A \cdot C_s}{C_{A \cdot f}}.$$

Table II.

Saliva Kaas			Pepsine Parke Davis $\frac{1}{5}$		
n'	n	$\frac{1}{k} = 2^{n\left(1-\frac{n}{25.6}\right)}$	n'	n	$\frac{1}{k} = 2^{n\left(1-\frac{n}{17.2}\right)}$
1	3.00	6.30	1	4.00	8.45
2	5.90	23.4	2	5.90	14.6
3	7.75	42.5	3	7.10	18.0
4	9.25	60.0	4	8.25	20.0
5	10.55	73.6	5	9.20	20.0
6	11.70	82.2	6	9.95	18.5

Here, for all corresponding values of n and n', C_s is equal to $C_{s.m}$, seeing that in all cases the whole amount of serum, apart from $C_{s.m}$, is fixed by the antigen. Again $C_{A.f} = a\,\frac{1}{k} \cdot \frac{C_{A.0}}{2^n}$ and $C_A = \frac{C_{A.0}}{2^n}\left(1 - a\frac{1}{k}\right)$ where a is a constant. Hence

$$K = \frac{1 - a\frac{1}{k}}{a\frac{1}{k}} \cdot C_{s.m}.$$

Quite obviously this function cannot assume a constant value if $\frac{1}{k}$ varies with n. In fig. 17 the variation of K with n is represented for the case of Saliva Kaas and for a value of $a = 0.00758$ determined so as to make $a\,\frac{1}{k_{\max}} = 0.5$, where $\frac{1}{k_{\max}}$ is the maximum value of $\frac{1}{k}$ read on the curve a' fig. 16. The meaning of this latter curve will now be explained.

Our formula (5) for $\frac{1}{k}$ is approximate only, seeing that we have in (2) neglected the quantity $C_{s.m}$. Actually (2) should be replaced by the equation

$$\frac{1}{k}\frac{C_{A.0}}{2^n} = \frac{C_{s.0}}{2^{n'}} - C_{s.m} \tag{7}$$

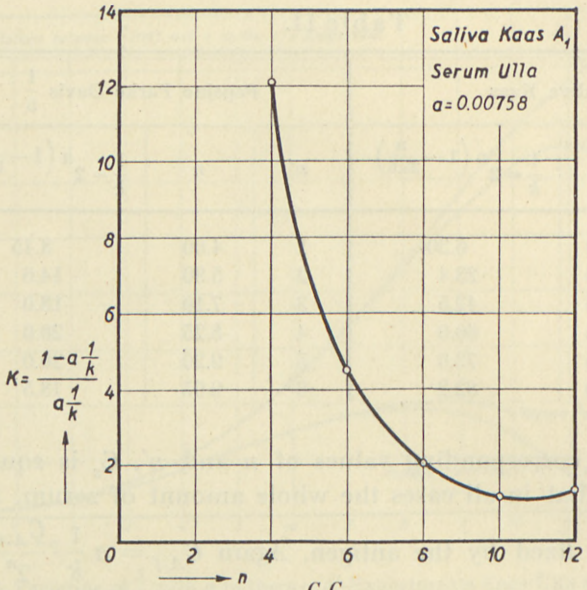

Fig. 17. Relative Variation of $K = \frac{C_s C_A}{C_{A\cdot f}}$ in the $n-n'$- Experiment Kaas-Ulla.

if for the sake of convenience we put $c = 1$. From (7) and (1) we readily derive

$$(8)\quad \left\{\begin{aligned} \frac{1}{k} &= \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{n-\frac{n^2}{\beta}-n'_0} - \frac{C_{s\cdot m}}{C_{A\cdot 0}} \cdot 2^n \\ &= \frac{C_{s\cdot 0}}{C_{A\cdot 0}} \cdot 2^{-n'_0} \cdot 2^{n\left(1-\frac{n}{\beta}\right)} \left[1 - \frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}}\right], \end{aligned}\right.$$

i. e. relative values of $\frac{1}{k}$ are given by the formula

$$(9)\quad \frac{1}{k} = 2^{n\left(1-\frac{n}{\beta}\right)} \left[1 - \frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}}\right].$$

From this formula it appears that the values derived from (5) are too large by an amount of $\frac{C_{s\cdot m}}{C_{s\cdot 0}} \cdot \frac{2^{n'_0}}{2^{-\frac{n^2}{\beta}}} \cdot 100$ p. c. In the case of Saliva Kaas and Serum Ulla we have:

$\frac{C_{s\cdot m}}{C_{s\cdot 0}} = 2^{-8}$ (comp. fig. 1 Introd.), $\beta = 25.6$, $n'_0 = 0.649$.

From these data we derive $\frac{C_{s \cdot m}}{C_{s \cdot 0}} \cdot 2^{n'_0} = 0.00618$. Introducing the value into (9) and calculating the percentage correction corresponding to a number of values of n we arrive at the curve a', the corrected $\frac{1}{k} - n$-curve, in fig. 16, and in the same way at the curve b' for Pepsine Parke Davis $\frac{1}{5}$. It will be noted that the corrections are pronounced for larger values of n only.

2. Comparison of the $\frac{1}{k}$-Curves derived for the Zone of Transition, $\frac{1}{k_\Delta}$, and from the $n - n'$-Experiment.

It would now seem of interest to compare the two curves: the $\frac{1}{k_\Delta} - \Delta$-curve derived for the zone of transition and the $\frac{1}{k} - n$-curve derived from the $n - n'$-experiment. This comparison has been made in fig. 18. The $\frac{1}{k_\Delta} - \Delta$-curves are those already represented in figs. 7 and 10 of Part I, while the $\frac{1}{k} - n$-curves are reproductions of the curves a and b in fig. 16 or rather of the lower parts of these curves. Again curves representing 2^n are drawn. It will be noted that the $\frac{1}{k}$-curves would seem to coincide, at any rate within a rather wide interval, with the $\frac{1}{k_\Delta}$-curve corresponding to a serum concentration (in the titer scale) between $n' = 2$ and $n' = 3$, say $n' = 2.5$. This is not far from being an average of the values of n' covered by the $n - n'$-experiment. It would thus seem, at any rate at first sight, that a certain relationship has been established between the two quantities $\frac{1}{k_\Delta}$ and $\frac{1}{k}$, derived from the two sources, viz. the zone of transition- and the $n - n'$-experiment.

Before this relationship is further discussed attention should, however, be drawn to the following experience. The parabolic $n - n'$-curves may within limited intervals be regarded practically as straight lines. In a preliminary investigation the $n - n'$-curves were actually interpreted as straight lines. Now the

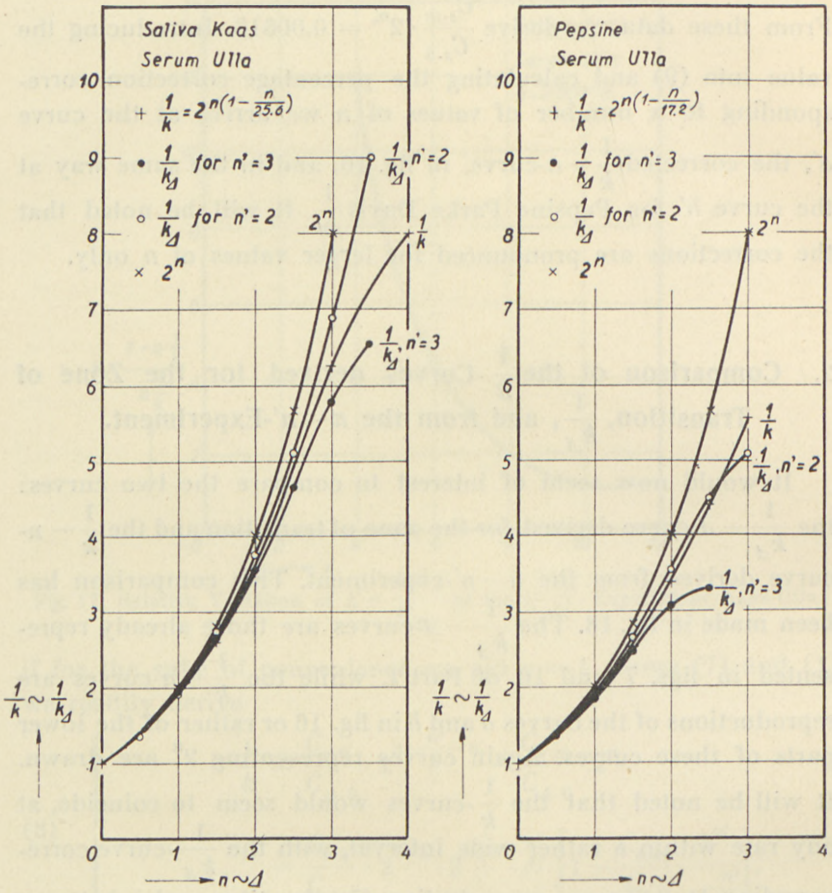

Fig. 18. Comparison of the $\frac{1}{k_\Delta}-\Delta$-Curve with the $\frac{1}{k}-n$-Curve.

experience referred to is as follows. It would seem that $n-n'$-curves of greater steepness A, i. e. with a greater (average) value of $\frac{dn}{dn'}$, also mean a greater width Δ_m of the zone of transition. The correlation is illustrated in Table III. Apparently Δ_m is approximately proportional to $\frac{dn}{dn'}$. True, the value of $\Delta_m \Big/ \frac{dn}{dn'}$ is, in the case of saliva AL, comparatively low. This, however, most likely finds its explanation in the circumstance that $\frac{dn}{dn'}$ is estimated too high. The experiment with saliva AL is just

Table III.

Correlation between Δ_m and $\frac{dn}{dn'}$. Serum Ulla, Anti *A*.

Antigen	$\frac{dn}{dn'}$	Δ_m	$\Delta_m / \frac{dn}{dn'}$
Saliva Kaas A_1	1.30	4.6	3.54
Pepsine Parke Davis $\frac{1}{5}$	1.00	3.3	3.30
Saliva *AL*	2.2	6.0	2.7

one of those preliminary tests in which the $n - n'$-curve or rather the lowest part of it was interpreted as a straight line. This obviously means that the result of this experiment is bound to yield a relatively high value of $\frac{dn}{dn'}$ as compared to the two other experiments of Table III, seeing that the values of $\frac{dn}{dn'}$ in these latter cases were derived from the upper, less steep part of the parabola.

An attempt will now be made to show that the interrelation between Δ_m and $\frac{dn}{dn'}$ may actually be predicted from what has been stated above—or at any rate an interrelation approximately to that effect. The expressions for $\frac{1}{k_\Delta}$ and $\frac{1}{k}$ may be written in the shapes.

$$(1) \qquad \frac{1}{k_\Delta} = \frac{1 - \left(\frac{C_{s.a}}{C_{s.m}}\right)^{\frac{\Delta}{\Delta_m} - 1}}{1 - \frac{C_{s.m}}{C_{s.a}}} \cdot 2^{\Delta}$$

$$(2) \qquad \frac{1}{k} = 2^{n} \cdot 2^{-\frac{n^2}{\beta}}$$

it being assumed in the expression (1) that the serum concentration in the tubes of the titer scale is $C_{s.a}$ or that $n' = n'_a$. In

the case of serum Ulla $\frac{C_{s\cdot a}}{C_{s\cdot m}} = 2^5$, and so the equation (1) may be rewritten as

$$\frac{1}{k_\Delta} = \frac{1-2^{5\left(\frac{\Delta}{\Delta_m}-1\right)}}{1-2^{-5}} \cdot 2^\Delta. \tag{1a}$$

Now it should here be remarked that $2^x = e^{l_e 2 \cdot x}$ where $l_e 2$ is the natural logarithm of 2. Hence (1a) may further be changed into

$$\frac{1}{k_\Delta} = \frac{1-e^{5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}}\cdot 2^{-5}}{1-2^{-5}} \cdot 2^\Delta = \left(1-e^{5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}}\cdot 2^{-5}+2^{-5}\right)2^\Delta \tag{1b}$$

seeing that 2^{-5} is small compared to 1. Confining ourselves to small values of Δ we may replace $e^{5\cdot l_e 2\frac{\Delta}{\Delta_m}}$ by the three first terms of its series development. Hence

$$\left\{\begin{aligned} \frac{1}{k_\Delta} &= \left[1-\left(1+5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}+\frac{1}{2}\left(5\cdot l_e 2\cdot\frac{\Delta}{\Delta_m}\right)^2\right)2^{-5}+2^{-5}\right]\cdot 2^\Delta \\ &= \left[1-\frac{5\cdot l_e 2}{2^5}\cdot\frac{\Delta}{\Delta_m}-\frac{(5\cdot l_e 2)^2}{2^6}\cdot\left(\frac{\Delta}{\Delta_m}\right)^2\right]\cdot 2^\Delta \\ &= \left[1-0.108\,\frac{\Delta}{\Delta_m}-0.220\left(\frac{\Delta}{\Delta_m}\right)^2\right]\cdot 2^\Delta. \end{aligned}\right. \tag{3}$$

In much the same way we may rewrite (2) in the shape

$$\frac{1}{k} = 2^n \cdot e^{-l_e 2\cdot\frac{n^2}{\beta}}. \tag{4}$$

Developing in series we get

$$\frac{1}{k} = \left[1-l_e 2\cdot\frac{n^2}{\beta}+\frac{1}{2}(l_e 2)^2\cdot\frac{n^4}{\beta^2}\right]\cdot 2^n. \tag{5}$$

Now from the equation of the parabola, viz.

$$n^2 = \beta\,(n'-n'_0) \tag{6}$$

it follows that

$$\frac{dn}{dn'} = \frac{\beta}{2n} \tag{7}$$

thus proportional to the quantity $\frac{\beta}{n}$. Let A be an average value of $\frac{dn}{dn'}$ or of $\frac{\beta}{2n}$ within a certain minor interval of the $n-n'$-curve covering small values of n only, then we may with a certain degree of approximation rewrite (5) as

$$\frac{1}{k} = \left[1 - \frac{l_e 2}{2} \cdot \frac{n}{A} + \frac{(l_e 2)^2}{8} \cdot \frac{n^2}{A^2}\right] \cdot 2^n. \tag{8}$$

A comparison of (3) and (8) now shows that, for such small values of n and Δ that the terms of the second degree in n and Δ may be neglected, the two expressions will yield identically the same values for $\frac{1}{k_\Delta}$ and $\frac{1}{k}$, with $n = \Delta$, if

$$\frac{l_e 2}{2} \cdot \frac{1}{A} = \frac{5 \cdot l_e 2}{2^5 \cdot \Delta_m},$$

i. e. if

$$\Delta_m = \frac{5}{2^4} \cdot A.$$

Hence the condition for coincidence of the two characteristics, viz. the $\frac{1}{k_\Delta} - \Delta$-and the $\frac{1}{k} - n$-characteristics, would, with small values of Δ and n, seem to be that of $A = \frac{dn}{dn'}$ being proportional to Δ_m or conversely. Seeing that the two characteristics actually coincide the conclusion may presumably be drawn that they do so because the interrelation referred to is substantially a reality, though it is hardly one of exact proportionality.

3. The Power of Fixation of the Antigen.

Above we have defined the quantities $\frac{1}{k}$ and $\frac{1}{k_\Delta}$ as the fractions of the antigen uniting with the serum antibody. Another way of visualizing the said quantities is to consider them as

measures of the ability of the antigen to fix the serum antibody. For this reason $\frac{1}{k_A}$ or $\frac{1}{k}$ may appropriately be termed the power of fixation of the antigen. A most conspicuous result of the preceding discussion is that $\frac{1}{k}$ in the $n-n'$-experiment is approximately the same function of n as $\frac{1}{k_A}$ is of A in the zone of transition experiment. (Comp. figs. 18). It is rather obscure why it should be so and the author is unable to offer any explanation. *A priori* one might guess at a constant value of the power of fixation of the antigen. It will, however, readily be seen from the experimental results that $\frac{1}{k_A}$ and $\frac{1}{k}$ cannot be constant but that they must increase with decreasing values of the antigen concentration. This follows immediately from the two experimental facts: 1° that the zone of transition has generally a greater width than should be expected if the power of fixation had a constant value independent of the antigen concentration, 2° that the (average) steepness of the $n-n'$-curve is greater than 1, i. e. the constant value it would exhibit in the case of a constant power of fixation. We shall terminate our discussion by showing this.

Let the titer reading for a given antigen on a given titer scale be n. Then, if the power of fixation were constant, there would in tube no. $n+1$ of the scale be an amount of free serum equal to $\frac{C_{s\cdot a}}{2}$, in tube no. $n+2$ the amount $\frac{3}{4}\,C_{s\cdot a}$ and in tube no. $n+3$ the amount $\frac{7}{8}\,C_{s\cdot a}$, $C_{s\cdot a}$ being the serum concentration in the tubes of the titer scale. For in tube no. $n+1$ the amount of antigen is half that of tube no. n and so half of the serum should be fixed if $\frac{1}{k_A}$ were constant, in tube no. $n+2$ the amount of antigen is $\frac{1}{4}$ of that of tube n and consequently with constant power of fixation $\frac{1}{4}$ of the serum should be fixed, leaving $\frac{3}{4}$ of the serum free etc.

It may be noted that we have here neglected the amount of serum $C_{s\cdot m}$, i. e. the amount of free serum corresponding to the

degree of agglutination 0 in the titer curve of the serum.—It is seen that in the case of a constant value of $\frac{1}{k_A}$ a practically complete agglutination should be anticipated within a zone of transition covering 3 titers only. Now actually the zone may have a width considerably in excess of this, say a width of up to 6 titers. This clearly suggests the idea of a power of fixation increasing with decreasing antigen concentration. For if in tube no. $n+1$ more than half of the serum is fixed, in tube no. $n+2$ more than $\frac{1}{4}$ is fixed and so on, then obviously the zone of transition must be wider and the more so the greater the increase of the power of fixation. Again, if the power of fixation of the antigen $\frac{1}{k}$ were constant, i. e. independent of the antigen concentration, the titer reading n should in the $n-n'$-experiment rise by one unit for each unit's increase of n' or, we should find $\frac{dn}{dn'} = 1$. Now, as a general rule $\frac{dn}{dn'}$ is found to assume higher (average) values. This experience too shows clearly that the power of fixation $\frac{1}{k}$ must increase with decreasing values of the antigen concentration. For it means that if the serum concentration is reduced to half its former value, i. e. n' increased by 1, then the antigen concentration must be diminished not to $\frac{1}{2}$ but to a value smaller than that, say to $\frac{1}{4}$ of its former value, in order to just fix the serum (apart from $C_{s.m}$). But this can only be so, if the power of the antigen to fix serum rises considerably when the antigen concentration is reduced.

On the other hand it is clearly seen that there must be a limit to the increase of the power of fixation with decreasing antigen concentration. Such a limit is obviously given by $\frac{1}{k_A} = 2^A$ $\left(\text{or } \frac{1}{k} = 2^n\right)$. The latter relation would mean that with half the concentration of antigen the power of fixation should be doubled, i. e. the amount of serum antibody which could be fixed would remain unaltered. The consequence hereof would be a zone of transition of infinite width and an $n-n'$-curve of infinite steep-

ness. This is directly seen but it follows also from the results of our discussion. For it may be noticed that the two expressions (3) and (8) of the preceding paragraph show that $\frac{1}{k_A}$ and $\frac{1}{k}$ vary approximately as 2^A and 2^n respectively and that a variation exactly as 2^A and 2^n must imply $A_m = \infty$ and $A = \infty$. So the deviations from the latter variations expressed by the factors in brackets just account for the finite values of the zone of transition and of the steepness of the $n - n'$-curve. Geometrically these deviations have been illustrated in figs. 18, where curves for 2^n (2^A) have been drawn.

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