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## ARTICULO ORIGINAL

# Formacion del inmunocomplejo radiactivo en un ria de doble anticuerpo

# Radioactive immunocomplex formation in a double antibody ria

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**RESUMEN**

Se ha elaborado un modelo para el estudio de la cinética y equilibrio de las reacciones antígeno-anticuerpo implicadas en el radioinmunoanálisis (RIA) de doble anticuerpo correspondiente al Péptido C. Se pretende caracterizar la influencia de las concentraciones iniciales de antígeno marcado (M) y no marcado (Q) sobre la formación del inmunocomplejo (PM) al segundo anticuerpo (J) inmovilizado sobre una bola. Se realizan 30 experiencias para el estudio del efecto de las variables antes mencionadas. Los resultados obtenidos son concordantes con el modelo propuesto.

***PALABRAS CLAVE***: Cinética, RIA, Doble Anticuerpo, Péptido C

**ABSTRACT**

A model has been produced for the kinetic and equilibrium study of antigen-antibody reactions in the radioimmunoassay (RIA) of C-Peptide double antibody. The aim is (1) to characterise the influence of initial concentrations of labelled (M) and unlabelled (Q) antigen and that of the initial concentration of the antibody in solution (P), and (2) to study the binding of the immunocomplex (PM) to the second antibody (J) immobilised on a bead, and the replacement of M by Q in the PMJ immunocomplex. In order to study the effect of such variables, 44 experiments were conducted. The results are in line with the model proposed.

***KEYWORDS***: Kinetics, RIA, Double Antibody, C Peptide

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**NOMENCLATURE**

P = antibody in solution, Q = unlabelled antigen, M = $^{125}$Iodine-labelled antigen, J = second antibody coated on plastic beads, $P_0$, $M_0$ , $Q_0$ = initial concentrations in arbitrary units, PQ, PQJ = non-radioactive immunocomplexes, PM, PMJ = radioactive immunocomplexes, [P], [Q], [M], [PQ], [PM],[PQJ], [PMJ] = concentrations in mol/L, [J] = concentration of vacant biding sites in antibody J, Z = cpm activity in each tube after reaction ($Z=Z_{sp}+Z_0$). The tables include a sub-index indicating the experiment number. $Z_{sp}$ = cpm activity from the radioactive immunocomplex corresponds to specific binding. $Z_0$ = value of Z at t=0, corresponds to non-specific binding. $Z_\infty$ = value of Z obtained at t infinity. $Z_e$ = value of Z at equilibrium ($Z_e=Z_\infty-Z_0$), t = time in minutes**,** k = rate constant, K = equilibrium constant, r = correlation coefficient.

## 1. INTRODUCTION

C-peptide is a polypeptide (31 amino acid residues) with a relative molecular mass (RMM) of 3018 Dalton. It is part of the proinsulin molecule and has the following structure: B chain – Arg – Arg -C-peptide – Lys – Arg – A chain.

In the pancreatic -cells, proinsulin is enzymatically cleaved into insulin (A chain and B chain) and the C-peptide molecule. Both are simultaneously secreted in equimolar concentrations into blood. Insulin has a rather short half-life -5 minutes- while the half-life of C-peptide is 30 minutes. Therefore, the molar ratio between C-peptide and insulin in peripheral blood ranges between 3:1 and 5:1. The main degradation site for C-peptide is the kidney. Consequently, patients with renal dysfunction have a longer half-life and higher basal values. Among other reasons, its determination is indicated in the study of pancreatic reserves in individuals with diabetes and pancreatectomy patients, and in insulinoma diagnosis.

Radioimmunoassay (RIA) is used in C Peptide assessment. It is a competitive technique in which the antigen molecule to be determined (Ag) competes with a radioactive tracer (labelled antigen: $Ag^*$) in order to bind to a specific antibody (Ab) that binds to both antigens until equilibrium is reached, in which circumstance both immunocomplexes -the radioactive one and the non-radioactive or “cold” one- can coexist[1]:

$$Ag + Ab + Ag^* \Leftrightarrow (Ag — Ab) + (Ag — Ab)^*$$

By keeping tracer ($Ag^*$) and antibody (Ab) quantities constant, the higher or lower proportion in the immunocomplexes formed will solely depend on the amount of cold antigen (Ag) in the sample to be analysed.

If the tracer behaves similarly when bound or in solution, then the separation of the bound and free fractions is essential. In our case, separation is accomplished by fixation on a second antibody coated on a plastic bead.

Kinetics and equilibrium in antigen-antibody reactions are determining factors of the rapidity, analytical range, and reliability[2] of immunoanalytical techniques. Likewise, the search for more reliable faster immunoassays is one of the main development areas in this field. This has caused the overall process to be progressively automated, from sample handling to statistical assessment of results (3-6). Yet, despite the large number of immunoanalytical systems developed in recent years, very few of them include kinetic analysis.

Several models have been proposed for the adjustment of the kinetic data on association and dissociation reactions. In our previous research[3,4,5,6,7,8], different characteristics related to the kinetics of antigen-antibody reactions used in analytical techniques were studied, incorporating radioactivity as a measurable magnitude.

Equilibrium data analysis is broadly used in the determination of the ability of a substance to bind to one or several receptor populations. However, as pointed out by Weber[9], the detection of two binding sites by such assays requires the ligand to have very different affinity for both binding sites.

Zúber et al.[10] analysed the kinetics of a new homogeneous immunoassay based on fluorescent energy detection, fitting the obtained values to three models: monomolecular, hyperbolic, and Gompertz; they drew the conclusion that the best correlation is reached in the first model. Later on, the same authors[11] realized that experimental data fit in with the monomolecular or descriptive model already used in the previous research, good overall correlation being found -not so good in the first portion of the curve possibly due to an inflection point unforeseen by the equation used.

Through continuous flow immunoassays, Rabbany et al.[13] analysed the dissociation kinetics of the complex labelled antigen – immobilised antibody in the absence of unlabelled antigen. In another paper, *Rabbany et al.* (13) studied displacement kinetics for an immunoassay in trinitrotoluene (TNT) continuous flow against its antigen immobilised on a porous membrane. The latter assay type suggests that lower flow rates produce a longer interaction between the injected unlabelled antigen and the labelled antigen - antibody complex, and consequently a greater labelled antigen displacement, more intense signals being recorded.

## 2. GENERAL MODEL

### 2.1. Influence of the initial labelled antigen concentration on reaction kinetics

Radioactive immunocomplex formation:

$$P + M + J \underset{k_I}{\overset{k'_D}{\Leftrightarrow}} PMJ$$

its equilibrium constant is: $K'_M = \frac{[PMJ]}{[P]\cdot[M]\cdot[J]} = \frac{k'_D}{k_I}$

The above reaction can be assigned the following mechanism:

$k_1$

$P + M \Leftrightarrow P\cdots M$

$k_{-1}$

$k_2$

$P\cdots\cdot M \Leftrightarrow PM$

$k_{-2}$

$k_3$

$PM + J \Leftrightarrow PMJ$

$k_{-3}$

where the first stage consists of the diffusion approximation of the reacting molecules until the encounter complex (P····M) is formed. It is considered reversible, since the encounter complex can be dissociated, but this is not very likely due to the cage effect (22). At the second stage, the intermediate immunocomplex (PM) is formed, and in the third stage the binding of the immunocomplex to the second antibody immobilised on a bead takes place.

Matter conservation requires:

$$[P_0] = [P] + [P\cdots M] + [PM] + [PMJ]$$

$$[M_0] = [M] + [P\cdots M] + [PM] + [PMJ]$$

By derivation of the first of the previous equations, we obtain:

$$0 = \frac{d[P]}{dt} + \frac{d[P\cdots M]}{dt} + \frac{d[PM]}{dt} + \frac{d[PMJ]}{dt}$$

Let us assume stage 1 to be the slowest one -hence a rate limiting stage-, and let us assume that P····M and PM are stationary, which means their concentrations are very small, then the following can be

written:

$$0 = \frac{d[P]}{dt} + \frac{d[PMJ]}{dt}$$

The overall reaction rate is given by:

$$\frac{d[PMJ]}{dt} = -\frac{d[P]}{dt} = k_1 \cdot [P] \cdot [M] - k_{-1} \cdot [P \cdots M]$$

$$\frac{d[P \cdots M]}{dt} = 0 = k_1 \cdot [P] \cdot [M] - k_{-1} \cdot [P \cdots M] - k_2 \cdot [P \cdots M] + k_{-2} \cdot [PM]$$

$$[P \cdots M] = \frac{k_1 \cdot [P] \cdot [M] + k_{-2} \cdot [PM]}{k_{-1} + k_2}$$

$$\frac{d[PM]}{dt} = 0 = k_2 \cdot [P \cdots M] - k_{-2} \cdot [PM] - k_3 \cdot [PM] \cdot [J] + k_{-3} \cdot [PMJ]$$

$$[PM] = \frac{k_2 \cdot k_1 \cdot [P] \cdot [M] + k_2 \cdot k_{-3} \cdot [PMJ]}{k_{-2} \cdot k_{-1} + (k_{-1} \cdot k_3 + k_2 \cdot k_3) \cdot [J]}$$

$$\frac{d[PMJ]}{dt} = \left( \frac{k_2 \cdot k_1}{k_{-1} + k_2} \right) \cdot [P] \cdot [M] - \left( \frac{k_{-1} \cdot k_{-2}}{k_{-1} + k_2} \right) \cdot \frac{k_2 \cdot k_1 \cdot [P] \cdot [M] + k_2 \cdot k_{-3} \cdot [PMJ]}{k_{-2} \cdot k_{-1} + (k_{-1} \cdot k_3 + k_2 \cdot k_3) \cdot [J]}$$

The value of [J] corresponds to the concentration of vacant binding sites on the antibody coating the bead. If we assume it to be in excess, [J] can be considered constant; by simplifying and calling $k'_D$ and $k_I$ the constants groups ruling the overall direct and reverse processes, then a differential rate equation is obtained in the form

$$\frac{d[PMJ]}{dt} = k'_D \cdot [P] \cdot [M] - k_I \cdot [PMJ]$$

The terms in square brackets represent $mol \cdot L^{-1}$ concentrations. They must be multiplied by the adequate factor to convert them to the practical units used since the experimental data are radioactivity measurement. Then, the equation is transformed into:

$$\frac{dZsp}{dt} = k_D \cdot (P_0 - Zsp) \cdot (M_0 - Zsp) - k_I \cdot Zsp$$

By integration, it takes the following form:

$$Z = Z_e\left[\frac{\left(1-\exp\left(-t\cdot k_D\cdot\left(\frac{P_0\cdot M_0}{Z_e}-Z_e\right)\right)\right)}{1-\frac{{Z_e}^2}{P_0\cdot M_0}\cdot\exp\left(-t\cdot k_D\cdot\left(\frac{P_0\cdot M_0}{Z_e}-Z_e\right)\right)}\right]+Z_0 \quad \textbf{(Eq.1)}$$

By assuming $\frac{{Z_e}^2}{P_0\cdot M_0} << 1$ and therefore $Z_e << \frac{P_0\cdot M_0}{Z_e}$ (which applies if the labelled reagent is in excess in relation with the antibody), then the process behaves as an apparently irreversible process, and **Eq.1** for two binding site types transforms into:

$$Z = Z_{e1}\cdot\left[1-\exp\left[-t\cdot k_{D1}\cdot\left(\frac{P_{01}\cdot M_0}{Z_{e1}}\right)\right]\right]+Z_{e2}\cdot\left[1-\exp\left[-t\cdot k_{D2}\cdot\left(\frac{P_{02}\cdot M_0}{Z_{e2}}\right)\right]\right]+Z_0$$

(Eq.2)

**2.2. Influence of initial labelled and unlabelled antigen concentrations on reaction kinetics**

If unlabelled peptide Q competes with M in the binding to the antibody, the following process will take place simultaneously:

$$P + Q + J \underset{k'_I}{\overset{k''_D}{\Leftrightarrow}} PQJ$$

its equilibrium constant being $K'_Q = \frac{[PQJ]}{[P]\cdot[Q]\cdot[J]} = \frac{k''_D}{k'_I}$

In line with the previous descriptions, and by calling $K_M$ and $K_Q$ the products of $K'_M \cdot [J]$ and $K'_Q \cdot [J]$, the conservation conditions are now:

$$Z = \frac{P_{01}\cdot M_0}{M_0 + b\cdot Q_0 + c}\left[1-\exp\left(-t\cdot k_{D1}\cdot\left(M_0 + b\cdot Q_0 + c\right)\right)\right] +$$
$$+\frac{P_{02}\cdot M_0}{M_0 + i\cdot Q_0 + j}\left[1-\exp\left(-t\cdot k_{D2}\cdot\left(M_0 + i\cdot Q_0 + j\right)\right)\right] + Z_0$$

$$[P]_0 = [P] + [PMJ] + [PQJ] = [P] + K_M \cdot [P] \cdot [M] + K_Q \cdot [P] \cdot [Q] =$$

$$= [P] \cdot [1 + K_M \cdot [M] + K_Q \cdot [Q]]$$

$$[M]_0 = [M] + [PMJ] = [M] + K_M \cdot [P] \cdot [M] = [M] \cdot [1 + K_M \cdot [P]]$$

$$[Q]_0 = [Q] + [PQJ] = [Q] + K_Q \cdot [P] \cdot [Q] = [Q] \cdot [1 + K_Q \cdot [P]]$$

$$[PMJ] = K_M \cdot [P] \cdot [M] = \frac{K_M \cdot [P]_0 \cdot [M]}{[1 + K_M \cdot [M] + K_Q \cdot [Q]_e]} = \frac{K_M \cdot [P]_0 \cdot \frac{[M]_0}{1 + K_M \cdot [P]}}{1 + \frac{K_M \cdot [M]_0}{1 + K_M \cdot [P]} + \frac{K_Q \cdot [Q]_0}{1 + K_Q \cdot [P]}} =$$

$$= \frac{[P]_0 \cdot [M]_0}{\frac{1 + K_M \cdot [P]}{K_M} + [M]_0 + \frac{K_Q \cdot [1 + K_M \cdot [P]]}{[1 + K_Q \cdot [P]] \cdot K_M} \cdot [Q]_0} =$$

$$= \frac{[P]_0 \cdot [M]_0}{[M]_0 + \frac{K_Q \cdot [1 + K_M \cdot [P]]}{K_M \cdot [1 + K_Q \cdot [P]]} \cdot [Q]_0 + \frac{1 + K_M \cdot [P]}{K_M}}$$

By transforming the concentrations into activities and taking into account that Ze is the cpm value corresponding to [PMJ] and simplifying, then:

$$Z_e = \frac{P_0 \cdot M_0}{M_0 + b \cdot Q_0 + c} \qquad \textbf{(Eq.3)}$$

By substituting the value of Ze obtained by **Eq.3** in **Eq.2,** we have **(Eq.4)**

**2.3. Equilibrium equations**

These are obtained from rate equations by making time tend to infinity. By doing this, exponential terms containing such a variable disappear., Additionally the unspecific activity is subtracted.

**3. MATHERIAL AND METHODS**

*3.1. Reagents*

The reagents used belong to the RIA-coat® C-Peptid kit, manufactured by Byk-Sangtec Diagnostica GMBH & Co.KG. The kit includes:

- A polyclonal antiserum obtained by immunising goats with synthetic human C-peptide

- A second monoclonal antibody (mouse anti goat) coated on a plastic bead

- $^{125}$I-C-peptide: a vial with lyophilised labelled C peptide

-Unlabelled C peptide vials in different concentrations, with which different solutions were prepared, 0, 0.533 and 5.917 ng/mL being the final concentrations for the determination of the influence of initial labelled and unlabelled antigen concentrations, and 1, 3, 10 and 30 ng/mL for the study of the labelled antigen displacement resulting from the addition of the unlabelled antigen.

### 3.2. Instrumentation

LKB Gammamaster Automatic Gamma Counter, fitted with a computer with a Riacalc programme.

### 3.3. Computer programme

Statistica (Copyright© StatSoft, Inc.1993). It allows the fitting of experimental data using specific non-linear regression equations, and the production of the corresponding tables. As a statistical criterion for equation selection in the different models, AIC (Akaike's Information Criterion) was observed; it can be expressed as follows: $AIC = N \cdot \ln S + 2 \cdot P$, where N is the number of points, S the addition of the squares of the residuals, and P the number of parameters in the equation. The equation with the lowest AIC in the fitting must be chosen (See Results) No sé si este cambio sera posible.

### 3.4. Experimental procedure

30 experiments were performed in which were studied the influence of the influence of initial concentrations of labelled ($M_0$) and unlabelled ($Q_0$) C Peptide on reaction kinetics and equilibrium. Tube series were prepared with 100 μL of each of the different labelled and unlabelled antigen solutions, together with 100 μL of antibody solution and a bead in each tube. They were left to react in agitation for different time periods, after which they were washed, eliminating the liquid and leaving the bead in order to measure its radioactivity on the counter. One tube from each series was left to react for 24 hours, this being considered infinite time and therefore corresponding to the value at equilibrium.

## 4. RESULTS AND DISCUSSION

### 4.1. Previous Experiment

In this experiment tubes were added labelled C peptide (M) and an antibody-coated bead (J), the antibody in solution not being added (P). No bead-bound radioactivity was found, this indicating that it is the PM immunocomplex which actually binds to antibody J, and therefore the overall process includes the formation of the PM immunocomplex, followed by its binding to the J antibody coating the bead.

### 4.2. Influence of initial labelled ($M_0$) and unlabelled ($Q_0$) antigen concentrations on reaction kinetics and equilibrium

| t (min) | 0 | 10 | 30 | 60 | 120 | 180 | ∞ | $M_0$ (cpm) | $(Q)_0$ (ng/mL) |
|---|---|---|---|---|---|---|---|---|---|
| $Z_1$ | 247,5 | 1181,5 | 2760,7 | 4957,2 | 7711,1 | 8515,9 | 14364,5 | 15929,0 | 0,000 |
| $Z_2$ | 29,1 | 873,7 | 1972,4 | 3920,8 | 5752,0 | 6887,5 | 11750,0 | 12790,5 | 0,000 |
| $Z_3$ | 112,8 | 681,4 | 1715,5 | 2863,1 | 4203,1 | 5103,7 | 8866,6 | 9463,7 | 0,000 |
| $Z_4$ | 154,1 | 535,3 | 1190,5 | 1978,0 | 2431,0 | 3448,7 | 5819,7 | 6310,6 | 0,000 |
| $Z_5$ | 213,4 | 438,2 | 530,5 | 881,2 | 1391,3 | 1927,1 | 2952,9 | 3409,3 | 0,000 |
| $Z_6$ | 274,0 | 1023,5 | 2337,4 | 3132,2 | 4431,0 | 4971,6 | 6964,6 | 15929,0 | 0,533 |
| $Z_7$ | 47,4 | 778,9 | 1727,1 | 2810,2 | 3840,5 | 4864,0 | 5506,9 | 12790,5 | 0,533 |
| $Z_8$ | 107,3 | 597,2 | 1384,9 | 2062,8 | 2731,5 | 3018,1 | 4335,6 | 9463,7 | 0,533 |
| $Z_9$ | 181,7 | 519,7 | 913,0 | 1495,5 | 1995,1 | 1965,9 | 2968,5 | 6310,6 | 0,533 |
| $Z_{10}$ | 215,1 | 407,9 | 384,9 | 593,9 | 1057,0 | 1126,3 | 1475,1 | 3409,3 | 0,533 |
| $Z_{11}$ | 230,0 | 824,5 | 1121,7 | 1329,2 | 1622,3 | 1644,6 | 1483,5 | 15929,0 | 5,917 |
| $Z_{12}$ | 17,7 | 519,7 | 1090,8 | 1320,0 | 1293,5 | 1430,0 | 1222,5 | 12790,5 | 5,917 |
| $Z_{13}$ | 56,1 | 477,7 | 815,7 | 893,0 | 800,1 | 856,0 | 774,4 | 9463,7 | 5,917 |
| $Z_{14}$ | 136,4 | 440,5 | 596,5 | 709,0 | 671,5 | 672,6 | 521,2 | 6310,6 | 5,917 |
| $Z_{15}$ | 199,1 | 361,0 | 215,9 | 250,1 | 392,6 | 431,1 | 266,6 | 3409,3 | 5,917 |
| $Z_{16}$ | 155,5 | 1210,5 | 3075,0 | 5287,5 | 7286,1 | 9494,1 | 14602,5 | 17463,0 | 0,000 |
| $Z_{17}$ | 0,00 | 845,8 | 2292,1 | 4297,8 | 6702,5 | 7310,0 | 12756,5 | 14146,0 | 0,000 |
| $Z_{18}$ | 35,2 | 695,7 | 1683,5 | 3130,8 | 4709,7 | 5193,4 | 9363,6 | 10352,0 | 0,000 |
| $Z_{19}$ | 17,2 | 396,8 | 1116,0 | 2096,5 | 3063,4 | 3781,6 | 6132,8 | 7036,0 | 0,000 |
| $Z_{20}$ | 30,3 | 262,0 | 497,4 | 825,2 | 1612,1 | 1964,0 | 3228,6 | 3635,0 | 0,000 |
| $Z_{21}$ | 88,5 | 1020,5 | 2115,1 | 3663,9 | 4735,9 | 5504,7 | 6851,3 | 17463,0 | 0,533 |
| $Z_{22}$ | 56,5 | 618,5 | 1746,4 | 2876,8 | 3468,5 | 3786,0 | 5539,4 | 14146,0 | 0,533 |
| $Z_{23}$ | 22,0 | 518,5 | 1178,4 | 1897,5 | 2716,0 | 3008,4 | 4273,4 | 10352,0 | 0,533 |
| $Z_{24}$ | 7,3 | 370,7 | 947,0 | 1391,0 | 1929,1 | 2116,7 | 3094,5 | 7036,0 | 0,533 |
| $Z_{25}$ | 28,0 | 191,6 | 352,9 | 577,4 | 934,6 | 1128,3 | 1482,9 | 3635,0 | 0,533 |
| $Z_{26}$ | 76,5 | 335,5 | 509,1 | 754,6 | 966,7 | 918,7 | 1113,5 | 17463,0 | 5,917 |
| $Z_{27}$ | 0,00 | 145,1 | 351,5 | 568,6 | 697,5 | 769,5 | 955,5 | 14146,0 | 5,917 |
| $Z_{28}$ | 20,4 | 147,5 | 351,2 | 405,0 | 368,9 | 391,8 | 702,3 | 10352,0 | 5,917 |
| $Z_{29}$ | 0,00 | 89,0 | 245,0 | 421,0 | 371,0 | 419,4 | 437,5 | 7036,0 | 5,917 |
| $Z_{30}$ | 18,9 | 64,6 | 41,1 | 37,7 | 125,0 | 136,4 | 196,2 | 3635,0 | 5,917 |

**Table 1.** Influence of $M_0$ y $Q_0$

Table 1 shows that for a given value of Q, with increasing concentration of M, increases the amount of radioactive immunocomplex for all times. The influence of unlabeled antigen Q, increasing its concentration, reduces the amount of radioactive immunocomplex for all time, which can be attributed to the partial occupation of the antibody binding sites by Q.

The data in Table I have been fitted to **Eq.4** in the General Model, save for the $Z_0$ unspecific activity term, which has been taken as equal to $p \cdot M_0$. These are its parameters and coefficients:

| $P_{01}$ | b | c | $k_{d1} \cdot 10^4$ | $P_{02}$ | i | j | $k_{d2} \cdot 10^4$ | p | r | AIC |
|---|---|---|---|---|---|---|---|---|---|---|
| 42610 | 545926 | 69245 | 0.001389 | 16280 | 302253 | 20135 | 0.000283 | 0.006 | 0.995 | 3470 |

which can be reduced to:

$$Z = \frac{a \cdot M_0}{Q_0 + c'}\left[1 - \exp\left(-t \cdot k_{D1}' \cdot (Q_0 + c')\right)\right] + \frac{e \cdot M_0}{Q_0 + j'}\left[1 - \exp\left(-t \cdot k_{D2}' \cdot (Q_0 + j')\right)\right] + p' \cdot M_0$$

**(Eq.4a)**

its parameters and coefficients being:

| a | c' | k'd1 | e | j' | k'd2 | p' | r | AIC |
|---|---|---|---|---|---|---|---|---|
| **0.0246** | 0.0908 | 0.201 | 0.1196 | 0.1938 | 0.01681 | 0.00529 | 0.996 | 3432 |

**Eq.4a** suggests an apparently irreversible biexponential behaviour attributable to the bonding with two binding site types. The apparent kinetic parameters are independent from M, which can be explained by admitting that the labelled reagent is in default with respect to the P antibody in solution. The influence of M is seen in the equation as only affecting equilibrium terms (they precede those between square brackets). The amount of labelled antigen bound at equilibrium is directly proportional to M.

The influence of the unlabelled antigen (Q) is seen in the fact that it appears in the kinetic and equilibrium terms. When $Q_0$ is increased, the apparent rate constant rises, this causing $t_{1/2}$ to decrease. This can be explained by admitting that, as a consequence of the partial occupation of the antibody's binding sites by Q, less time is needed to occupy half of them. The amount of labelled antigen bound at equilibrium decreases for the same reason. As an overall result, Z diminishes for all times.

The consistency between the observed values (Table I) and those calculated by **Eq.4a** is shown in **Figure 1**.

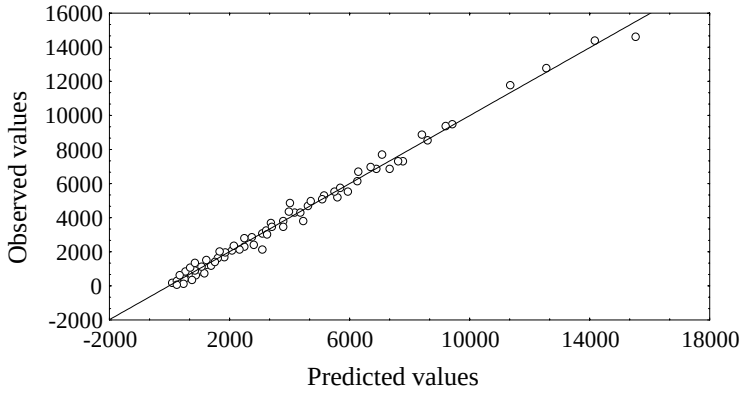


**Fig.1**. Observed Values (Tabla 1) vs. Predicted Values (Ec.4a)

Observed Value = 15.1 + 0.997·Predicted Value r = 0.996

The results at infinite time, corresponding to equilibrium, fit in with the following equation

$$Z_e = \frac{0.0246 \cdot M_0}{Q_0 + 0.0908} + \frac{0.1196 \cdot M_0}{Q_0 + 0.1938} \qquad r = 0.998$$

## 5. CONCLUSIONS

1. In the formation of the PMJ immunocomplexes, an apparently irreversible biexponential behaviour is found, corresponding to two binding site types.

2. As a result of an increased concentration, the influence of the labelled antigen shows in an increased amount of radioactive immunocomplex at all times, while the apparent rate and dissociation equilibrium constants remain unaltered.

3. The influence of the unlabelled antigen is seen in the fact that, when its concentration is increased, the amount of radioactive immunocomplex drops for all times, which can be put down to the partial occupation, by Q, of the antibody's binding sites, whereas the apparent rate and dissociation equilibrium constants rise.

4. Equilibrium data do not allow us to distinguish single site from double site binding models. However, a distinction was possible between both models when kinetic data were used.

5. Experimental results were satisfactorily fitted to the theoretical model.

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