Beyond the dichotomy in vivo - in vitro: in silico

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Abstract. From the beginnings of the biochemistry as discipline, the dichotomy between *in vivo- in vitro* conditions has been in the center of their methodological discussions. With the growing influence of computer simulations - sometimes called "*in silico*" conditions-, a new methodological problem is added to biochemistry. However, "simulation" could be seen as a core concept that is in fact used in the *in vivo - in vitro* dichotomy. In this sense, *in silico* dimension could be considered as a natural extension of the classical dichotomy. From the way in which simulation is used on *in vivo-in vitro* dichotomy, we also suggest that the general idea of simulation proposed by Hartmann have to be redefined. The intuitive idea of simulation resting on "imitation" relationship, as a "process that imitates another process" (Hartmann 1996), has to be complemented by others methodological concepts like "isolation" or "disruption".

Introduction

From the beginnings of the biochemistry as discipline, methodological considerations have been in the center of their discussions. Among these methodological considerations, one of the most important has been the tension between in vivo and in vitro conditions. How to evaluate and refine these conditions has been a central challenge of biochemistry. Significant methodological advances in biochemistry have been motivated in the necessity of achieving more reliability for in vivo and in vitro experiments. With the growing influence of computer

simulations, a new condition- sometimes called in silico- comes into biochemistry. It is natural to assume that, with this condition, new methodological problems appear. However, beside some particular problems derived from new methodologies, 'simulation' could be seen as a core concept that is actually used on in vivo - in vitro dichotomy. This use is consistent with a general concept of simulation proposed by Hartmann. According to Hartmann, a simulation is a "process that imitates another process" (Hartmann 1996). Despite this initial agreement, we will argue that, if we take into account biochemical practices, Hartmann's simulation idea has to be complemented by others concepts beyond "imitation" relationship, like "disruption" or "isolation".

In the section I of the paper, we will trace some sources of simulation idea in biochemistry. In this section, we will also start to show how biochemists use of simulation idea is to some extent different from Hartmann's proposal. In section II, we will continue this discussion in the context of computer simulation in biochemistry. In this section, we will argue that we can make, in biochemical computer simulations context, a similar statement about simulation concept modification.

I. Simulation in biochemistry

The concept of simulation has been used by biochemists long time before computers arrived[1]. Expressions like "in vitro simulation" or "in vivo simulation" are commonly used in experimental contexts (Bugnon et al. 1998). This use is based on the methodological concern of getting reliable experimental conditions. A 'reliable' experimental condition is usually understood in this context as a situation that resembles, in some aspects, another one. In this sense, we

[1] We suggest here only that biochemists have been used simulation concept, not that they have been used this particular term or expression.

can think that simulation idea could rest on “imitation” relationship. This first intuition could be traced from historical origins of biochemistry.

Biochemistry uses several methodological concepts from chemistry. Among them, “isolation” and “purity” are pointed out by historians as central (Fruton 1972). The ideal in this case was “to isolate” a substance or compound and, in this way, be able to study this substance or compound in its “pure” state. This common chemical methodology was called later “in vitro” when it was adopted by biochemistry, because it was understood as related with conditions of a living organism or process – in vivo conditions-. This in vitro approach could be considered a consequence of a “mechanistic” and “antivitalistic” position (Kohler 1973). Physiologists assumed that vitalistic ideas were wrong, and then they expected that in vitro conditions gradually substitute any vestige of in vivo conditions. It was supposed that a particular compound or pathway, separated from its “vital” context, could conserve their properties or main behavior. Therefore, this supposition was operating every time in vitro results were related with in vivo conditions. But, shortly those suppositions exhibit their limits, specially when physiologist compared in vitro outcomes with results from alive organisms. Because, an “analytical” approach produces imprecise results, biochemists tried to ‘imitate’ some especially complex processes. For example, by the middle of XIX century, physiologists started to develop a technique called “perfusion”, that later became widely used in intermediary metabolism studies (Holmes 1991). In this method an organ was maintain in physiological conditions that are close to an organism alive by pumping blood to a particular organ. This way, biochemists tried to ‘imitate’ vital conditions in order to obtain better experimental results.

This brief description seems to fit well with a general idea of simulation proposed by Hartmann (1996), who define simulation in terms of "imitation" and "process". A simulation is then "a process that imitates another process" (p.83). Indeed, simulation could be seen as class

of model -a dynamic model-. When a simulation is carry out by a computer, it is called a "computer simulation". This general point of view is followed by Humphreys when he tries to characterize computer's simulations (Humphreys 2004). Beside this last consideration, we can see Hartmann's simulation concept as a general characterization that includes not only computer programs but also scientific practices. However, when we inspect scientific practices to see how is understood the relationship between in vivo- in vitro we realize that the "imitation" part of simulation idea have to be complement by others concepts. To develop this suggestion, we have to come back to perfusion example.

Perfusion techniques for intermediary metabolism research were widely used until the beginning of the XIX century. Some important knowledge about intermediary metabolism was acquired. However, persistently failures in this biochemical area started to show the limits of this technique (Holmes 1991). Those failures produce two main consequences. First, the growing awareness of the complexity of biochemical field research. Second, the necessity of changing an input-output schema in order to get reliable results in intermediate metabolism studies (Fruton 1999:360-361). Moreover, it was not clear that perfusion could be the technique that allows this change. By using perfusion technique in intermediate metabolism, physiologist could determine in a quantitative way "the overall rates of metabolism, the relative proportions of carbohydrates, fat, and protein metabolized" (Holmes 1992:152) among others things. However, as Holmes has point out, perfusion technique was unable to detect the intermediate stages of a metabolic process, because the intermediate metabolites were produced in small quantities. A new technique or methodological tool was needed. Otto Warburg developed a method that allowed conserving tissues in such a way that similar results to perfusion method could be obtained but with a better observational capacity. This technique depended on the possibility of cutting the tissues "slices" between certain limit: around 0.2 mm in ordinary air

and 0.5 mm in pure oxygen (Holmes 1991:163). Metabolic reactions in those tissues slices were later measured with an instrument called micromanometer, also developed by Warburg.

This schematic history from perfusion to slides tissues techniques could be seen as a successful account for in vitro methods. However, it could be seen also as a sophistication of the classical dichotomy. Tissues slide methods could be understood as a more precise technique that suppose an in vivo approach. This technique was originally developed by Warburg for study some hypothesis about the mechanism of cancer. By 1931, Krebs, a former Warburg's collaborator, started to apply this technique to intermediate metabolism. But, it was considered problematic to apply this technique, because it was believed, or at least supposed, that there is had to be a link between the structure of the cell and metabolic process. Because the tissue slices technique depends on cutting the cells, biochemists like Warburg did not trust in this technique. It is controversial if this supposition is a vestige of vitalistic ideas, but it show anyway the profound concern about simulation of in vivo conditions. Of course, Krebs spend several weeks testing how accurate and reliable tissue slice technique could be – by replicating well know experiments in intermediary metabolism-. This way Krebs could establish tissue slice technique and micromanometer as a reliable simulation of in vivo conditions.

Nevertheless, the better simulation approach representing by tissue slices technique compared with perfusion technique is not resting only on an imitation relationship, but in a sort of isolation or disruption relationship. Cutting tissues and putting them in a glass container were considered a very artificial way to study metabolic reactions. But, this kind of technique do not represent a triumph of in vitro approach, as we could suppose. Their design and use was motivated by the goal of achieving a better in vivo description. In this sense, we can see biochemical simulation concept- consisted on imitation and disruption relationship - as a consequence of in vivo-in vitro tension. In fact, tissues slide technique resembles less than

perfusion technique, but it simulates better. Therefore, it could be said that this result is partially allowed by isolation.

This intuition is present in Roger Strand characterization of in vivo- in vitro relationship. Strand tried to carry out a characterization of the in vivo- in vitro relationship from a perspective near to the biochemical practices (Strand, Fjelland, Flatmark, 1996). When he describes in vitro studies he point out that they have to be different from in vivo "in order to overcome the in vivo methodological problems" (p. 2). An in vitro study consisted on a simulated situation, in an artificial way, of a "target phenomena". When the "target phenomena" is supposed to be so complex or unknown that the in vitro result became unreliable, then another kind of simulation is carried out: in vivo studies. In vivo studies use an organism or process that is close or similar to the target phenomena. In general, an experiment is called "in vivo" when is carried out in animals alive. The majority of in vivo and in vitro simulations try to prevail over some methodological limitations[2]. This prevailing is only possible because some complex phenomena are sometimes "simplified", "isolated" or "disrupted" from his original context. Therefore, simulation idea, in a biochemical context, could be seen, at least, as a two-folded concept, as long as "imitation" and some kind of "isolation" relationships are needed.

It is important to stress that in vivo and in vitro condition does not always work like model and target phenomenon, because both conditions can be seen as simulations. In biochemical practices, in vivo conditions are used in different ways. When an in vivo experiment or simulation is carrying out, the target phenomenon could be another in vivo process or an in vitro experiment. The target phenomenon depends on the experimenter goals. For this reason,

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[2] Of course, in vitro simulations are sometimes used to overcome ethical problems, but here we want to stress only the methodological role of simulations.

when we refer to in vivo or in vitro conditions, we have to be aware that their function is case-dependent. Anyway, we can say that in the majority of biochemical experimental contexts, the in vivo-in vitro dichotomy is working.

II. Biochemical computer simulations

In the previous section, we have suggest some extensions to simulation concept, at least when it is applied to biochemical practice. But, if this extension of simulation concept rest mainly on some biochemical practices, it is not directly evident that it could be applied to computer simulations in biochemistry. Therefore, in this section we try to show how the extension of simulation concept could be also used to understand computer approaches in biochemistry. In order to achieve this goal, we are going to discuss some particular examples that belong to the same area than the examples discussed in the previous section.

Computer simulations are now widely used for study metabolic reactions. Some computer approaches are more concern with abstract aspects of biochemical kinetics (Haunschild, Freisleben et al. 2005) or with general aspects of the cell (Tomita, Hashimoto et al. 1999). Others computer approaches are more concern with particular aspects of metabolic process. In this last group, there are programs like METAMOD, Gepasi[3] or Scamp that tries to simulate different aspects of biochemical reaction dynamics. This type of computer program can be considered as an aid in model building and testing, because one of their main tasks is to translate biochemical formulas into mathematical equations.

For example, the user of Gepasi program has to supplies the system with information about stoichiometric structure of the pathway, kinetics of the reactions that are to be considered and the initial concentration of chemical species. Then Gepasi build the differential

[3] The entire project behind *Gepasi* has been recently reformulated by Pedro Mendes with a program for simulating biochemical networks called *Copasi* -COmplex PAthway Simulator-. (More information could be found at http://www.copasi.org)

equations that characterize the system. This way Gepasi could describe the temporal evolution of reactions. Gepasi is also generally used to study steady-state properties of pathways. In particular, this program has been used to study enzymes interaction in biochemical pathways.

The others programs cited above are used in similar contexts than Gepasi. METAMOD is a software package for study steady-state metabolic pathways. This package allows also study control analysis of metabolic pathways. Scamp is a general-purpose simulator of metabolic reaction.

We are going to analyze a couple of examples about how this kind of computer program is used by biochemists. Those follow examples rest mainly on studies about metabolic channelling and drug design made by Cornish-Bowden and collaborators (Cf. Cornish-Bowden 1991 and Cornish-Bowden 2003).

There has been a discussion in biochemical literature about the in vivo function of a special interaction between enzymes called “metabolic channelling” (Mendes, Kell et al. 1996). This kind of enzyme interaction occurs when a common intermediate between two consecutives enzymes is directly transferred from the catalytic influence of the first enzyme to the “catalytic center” of another enzyme (Milani, Pesce et al. 2003). When a multi-enzyme complex is in favorable physiological conditions, there is the possibility that the product of the reaction catalyzed by one enzyme passed directly to the next reaction as a substrate of the next enzyme. Certainly, it is not enough to show the possibility that this metabolic channelling take place. In this point, we can notice how in vivo- in vitro tension is working. Even if we can prove that channelling occurs in vitro, it is not follow that this process will be happened in vivo. A way in which we can approach to this problem is by asking about the value for the organism of the supposing channelling. Then we can propose different hypotheses about this state of affairs.

The next step is to build models to describe alternative situations suggested by the hypotheses. By means of computer simulation, those models can be build and tested.

Cornish-Bowden investigate different hypothesis about metabolic channelling using computer models (Cornish-Bowden 1991)[4]. In particular, he tries to test a common assumption about metabolic channelling. It is usual to suppose that channelling decrease free concentration of intermediate metabolites. Cornish-Bowden examines this assumption by simulating the behavior of a set of four enzyme-catalyzed reactions. Let considering a fixed concentration of the starting material and the intermediary metabolites. It can also be supposed that the concentration of one of the intermediaries is too high. It is supposed that the concentration of this metabolite could be a risk for the health of the organism, but the values of the flux and the others metabolites are considered adequate for the needs of the organism. The problem is, then, how to decrease the concentration of this metabolite – keeping the original values of initial concentration and the steady-state flux -. The coefficients of the all metabolites and the flux used in this simulation are arbitraries, but they are consistently employed in all the simulation process (p.104).

An obvious way to solve this task is bypassing the “problematic” intermediary with a channelling. In a model where all others parameters remain unchanged– initial material and the kinetic of the reactions-, it is found by simulation that the concentration decreasing in the problematic intermediary is very slight. So “it is obvious that introducing a channel into a model without making any other changes to it does not provide a mechanism for decreasing the concentration of the by-passed intermediate” (p.105).

[4] For this particular research, it is used METAMOD (Cornish-Bowden 1991). But, the same results could be obtained with *Gepasi*.

Cornish-Bowden's conclusion have been questioned by Pedro Mendes en several articles. Mendes showed how channelling process could decrease free metabolites concentration by means of some parameters variation. Then, the discussion has been centered on the legitimacy of changing parameters in biochemical simulations. But, in any case, from those simulations is clear that changing parameters in the model could affect the reversibility of channelling reaction. And reversibility of channelling could be a good explanation why concentration do not decrease, because a reversible reaction could "inhibit the ability of any channel to decrease a pool"[5] (Mendes 1992:259).

Through the simulation of different models, it is possible to test hypotheses about channelling mechanism. But, in any case suppositions that imply considerations about in vivo conditions are present. For example, failures in in vitro experiments seem to suggest that channelling of dynamics complexes are related with a in vivo property of the cell[6]. A hypothesis to explain this failure could be that stabilization of dynamics complexes are bound to the "intact cells" (Mendes 1994:16). It is interesting to notice, that we found in this case a similar concern about cell integrity, than the expressed in the case of tissue slices technique. That similarity reveals an analogous preoccupation about in vivo conditions. It is obvious that a reliable computer simulation has to consider this knowledge.

The other example that we take into account, involves testing models for gycolysis of Trypanosome brucei in the context of drug design (Cornish-Bowden 2003). The Trypanosome brucei is a parasitic protozoan that causes African sleeping sickness. This organism has a very simple metabolism. It has not a tricarboxylate cycle (TCA), so it excretes pyruvate directly on the host. Because this organism does not have TCA, with a glycolysis description we can almost

[5] 'Pool' is a name used to refer to the intermediate metabolism concentration.

[6] Channelling could occur in static complexes of enzymes or in dynamic complexes. The term 'dynamic' in this last case came from the short life of enzymes in the complexes (Mendes 1994: 12-13).

exhaust all its metabolic activity (p. 513). In addition, there are detailed experimental studies about the kinetics and the enzyme parameters values of this organism. In this sense, it can be avoided an arbitrary determination of model's parameters. As we have seen in the previous example, this arbitrary determination could be a source of disagreement.

One kind of problem in drug design is to identify satisfactory targets that can modify reaction's metabolism. Because the target in this case involves a way to kill or at least to alter significantly the trypanosome brucei, a desirable goal could be to change the metabolism of this organelle. A way in which metabolism can be changed is by increasing fluxes. However, increasing metabolic fluxes is very difficult. Decreasing them could be better, but it is still difficult. A better place to look for is metabolite concentration because there are less stable than fluxes. In fact, alter metabolite concentrations is the mechanism working in some herbicides and antimalarial drugs (p. 510). This mechanism supposes inhibiting enzymes. We can consider two main inhibition types: competitive and uncompetitive[7]. A competitive inhibition is working when an inhibitor and the substrate compete for the same site and their influence in the flux, when the concentration of metabolites is not fixed, is in general very slight. On the contrary, when we have an uncompetitive inhibition in those conditions, their influence in the flux is important. Variations in concentrations of substrate have a very different effect when we consider a competitive or an uncompetitive inhibitor. In the first case, variations in concentration of substrate "nullified" the inhibitor while in the case of uncompetitive inhibitor those variations "potentiate" the inhibitor (p. 511).

From the previous considerations, a potential target of drug design could be a reaction that involves an uncompetitive inhibition. "Stoichiometric constrains" on metabolite

[7] There are other main types like reversibles or irreversibles inhibitions and there is also a division between noncompetitive and uncompetitive inhibitions. But, here we are only concern with competitive and uncompetitive inhibitions (Cf. Leskovac 2003:73ss).

concentration, select only four – of many- targets as useful[8]. In addition, one of this targets is not "intuitive obvious" for a biochemist. This particular target is the pyruvate transporter in the Glycolysis of trypanosome brucei[9] (pp. 513-514). By selecting pyruvate transporter as an available target, a successful computer simulation has been accomplished.

Those cases are examples of computer simulations in biochemistry. How are they related with simulations in biochemical practices developed in the previous section?. Is there a significant difference between the way in which simulation idea is used in biochemical practices and in computational programs?. Both questions can be addressed together.

However, there is a previous methodological problem. Where to look for the answer to those questions?. We can distinguish- at least- between a computational and a theoretical model or between restrictions that are due to computational aspects or biochemical aspects of the simulation. By setting the problem in this way, it seems that it is not difficult to make a sharp and clear distinction between both kinds of models. For example, in a program like Gepasi, we can separate mathematical methods used to solve the equations from the biochemical data provided by the user. An additional intuition related with this point can reinforce the separation between models. The mathematical methods used by programs like Gepasi are very common and widely used in several scientific areas[10]. Furthermore, the computational model could correspond to general aspect of the simulation while biochemical model could represent particular and specific aspects of simulation. This way, we have to look for the characterization of computational simulation only in the first group. However, it seems

[8] According to Cornish-Bowden, those "stoichiometric constrains" rest mainly on "algebra", because he uses Gaussian elimination to analyze networks that represent stoichiometric relationship (Cornish-Bowden 2002).

[9] This target is also interesting because some experimental observation had shown that inhibiting it in vivo bring about a concentration rise.

[10] This particular consideration can be understood by appealing to Humprheys' *templates* concept. (Cf.. Humphreys 2004).

that this strategy could have, as a consequence, a basic misunderstanding of computer simulations in biochemistry.

It could be argued that what we call a computer simulation in biochemistry includes the "pure" computational model and the theoretical or biochemical model. It is clear that this statement is not enough. However, what is implicit in the previous statement could be considered a deeply reason: there are some biochemical restrictions, which are not only part of the data provided by the user but also part of the computer program construction. We do not want to imply that a sharp separation between computational and biochemical model is always useless. But, we want to stress that this last perspective could not be the best strategy when we want to study biochemical computer simulations

Gepasi, Scamp and METAMOD were constructed with biochemical restrictions in their programming. Maybe the main assumption used in the computer programs cited, is the theoretical restriction imposes by Metabolic Control Analysis (MCA). With this name is called now a method that in another time was identify as a "theory". The main concern of this method is to get a good description of the pathway kinetics (Nielsen 1997). In particular, this method permits to analyze how the control of fluxes and intermediate concentrations in a metabolic pathway is conducted by different enzymes. An important aspect of this technique is that it is assumed that the control of the flux is not due to a unique rate-limiting step. Instead of that, is assumed that control flux is "spread" among the enzymes. From this context, we can suggest that we have to consider theoretical restrictions when we try to evaluate computer simulations in biochemistry.

We have emphasized the role of in vivo- in vitro dichotomy when we analyzed biochemical simulations and computer simulations of metabolic pathways[11]. But, this point can be based on general considerations about the role of MCA. A major part of MCA history could be understood as a pursuit of a better comprehension of in vivo conditions. Technical concepts like elasticity or control coefficient, that are parts of MCA technique, can be considered a way to describe properties that were not reduced to particular mechanism. As Mendes seems to imply, to describe and characterize 'systemic properties', could be considered as a methodology for describing and characterizing in vivo conditions (Mendes 1994). In this sense, it is evident that this kind of computer simulations could be characterized by an 'imitation' relationship. This imitation part could be seen not only by the simulation successful result, but also, as we have seen, by the way in which the computer model is build. In those particular cases, a main concern of biochemists is to get a good "imitation" of the dynamical aspects of the reaction. Furthermore, to use some theoretical assumptions to construct the computational model is a vehicle to achieve this purpose.

However, the same theoretical resources can be used to postulate a "disruption" or a kind of abstraction from a particular target. We can see this aspect of biochemical simulations from theoretical considerations that suppose the in vivo- in vitro dichotomy. Methodological assumptions used in the construction of the computer programs cited, emphasize the behavior of the whole pathway, considering the enzyme mechanism as a "black box"[12]. For example, it could be suggested that, in the biochemical case mentioned above, is operating a mechanism like cooperative feedback inhibition. A feedback inhibition is a very common mechanism that can be understood as a transfer of flux control from an end-product in a step to the pathway of

[11] Of course, the statements made here are only related with a particular kind of simulations of metabolic kinetics.

[12] The perspective of MCA requires some knowledge about *in vivo* kinetics properties of enzymes. And most of the information available now is from *in vitro* experiments. Cfr. Wang, L., I. Birol, et al. (2004)

reactions – generally the first committed step in the pathway-. Nevertheless, this kind of mechanism is practically ignored by the previous computer simulations, because of the assumptions implicit in MCA. Those simulations take a perspective where the behavior of fluxes and metabolite concentration are mainly "systemic properties" (Cornish-Bowden, Cardenas et al. 2004). This particular aspect is based on using MCA technique. Moreover, this strategy is not seen as part of the system limitations, but as contributing to the simulation task.

Additionally, there is another aspect that reveals a form of "disruption" in biochemical computer simulations. In metabolic computer simulations, all reactions are treating as reversible unless it is completely certain that a reaction have to be considered as reversible and this assumption rest largely on MCA[13]. For example, when reactions like pyruvate transport are taken as reversible, the distribution of flux control change radically from a step that not have any control to the second most important step in the reaction (Cornish-Bowden and Cardenas 2003:514).

Conclusions

If the description of biochemical simulations presented in this paper is correct, then we have arguments to redefine the intuitive and general idea of 'simulation' presented by Hartmann. In particular, this idea of simulation has to be complemented by other concepts beside imitation relationship. We propose that simulation idea, at least in biochemistry, use relationships like 'disruption' or 'isolation'.

There are some possible criticisms to the main thesis of this paper.

[13] MCA made very different assumptions about irreversible or reversible reaction when it uses technical concepts like "elasticity". This is a property of an enzyme that consisted on the relationship of the variation of the rate regarding the concentration of a metabolite. Cfr. Cornish-Bowden and Cárdenas (2000)

It could be argued that “simulation” is an idea that describes only the “imitation” part of in vivo- in vitro dichotomy. However, we want to follow the way in which biochemists use “simulation” word, so the “extension” of simulation concept could be seen as an attempt to get a simulation concept closer to scientific practices.

Another criticism can be expressed in the following way. Maybe the notion of simulation proposed in this paper could be applied to biochemistry, but from this result, it is not evident that this can be extended to others disciplines. Nevertheless, we do not claim that this concept of simulation have to be extended to others disciplines – although it is a possibility that have to be explored-. This partial objective is not against the main thesis of this paper. If we want to get a better understanding of the scientific meaning of simulation concept, maybe we have to consider partial characterizations.

There is even another possible criticism. “Imitation” seems to be understood in this paper, as if this concept implies that the relationship between the simulation and the target has to be seen in terms of “similarity”. But, from diverse sources this idea has been discredited. So, the original “imitation” relationship do not exclude a “disruption” or “isolation” relationship. This criticism supposed that the discussion about the role of “similarity” in scientific practices – especially in model building- is closed. But, this discussion is far from being closed. However, even in the case that we can interpret “imitation” in that way, we think that made explicit some ‘occult’ relationship could be a desirable objective.

Nevertheless, the maim criticism to the principal thesis of this paper is that the concept of “isolation” or “disruption” could be seen as a common aspect of every model building. According with this objection, when we build a model, we have to simplify some aspects. In this sense, there is a “disruption” between the model and the “original”. Therefore, this idea of simulation is only another aspect of a common and well-known process: model building. I think

that this criticism made an important- and obvious- point: the close relationship between simulation and model building. If biochemical simulations and computer simulations are both models, then this similitude is not surprising. However, we try to show in this paper that 'disruption' relationship came mainly from in vivo- in vitro dichotomy. This could be seen from the examples about perfusion and tissue slices technique in the section I, but also from the cases of biochemical computer simulations. In particular, the technological process from perfusion technique to tissue slices technique articulate a 'disruption' that stresses the general simulation relationship. This way, smashed cells in an isolated vessel allows making better in vivo approximations. The discussion about the possible relationship between cell integrity and synthetic process only express the omnipresence of in vivo-in vitro dichotomy. In the case of biochemical computer simulations, our main concern was to show evidence of the imbricate association between computer and theoretical models. This point can be illustrated by the role of MCA technique in computer restrictions and by the role of more empirical considerations about channelling of dynamic complexes. We can maintain from this evidence, that 'disruption' relationship do not have to be reduced to a general process of model building, but instead this kind of disruption have to be understood as a consequence of a biochemical model resting on in vivo in vitro dichotomy.

At present, we can say that computer simulations are part of scientific practices. This situation not only means the obvious statement that computers are in fact used by biochemist but also that sometimes those computer simulations constitute actual biochemical practices. Additionally, this state of affairs could be seen as another reason why is so difficult –or at least "impractical"- to made a clear distinction between a computational and a "theoretical" model when we analyze a particular example. With this statement, we do not suppose that it is worthless to study "pure" computational aspect of the programs used in biochemistry. Instead,

we want stress that we have to pay close attention to the way in which biochemists use computer simulations. Maybe this is the main reason why disruption is not only part of model building process, but is part of computer simulations in biochemistry. Trying to make a clear and sharp distinction between computational and theoretical models could be result in a 'denaturalization' of actual biochemical practices and consequently there is a risk of missing the particular biochemical context.

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