Examination of Homeopathic Preparations in Cell-Free Systems

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Abstract

This pilot study using cell-free systems ascertained the following: that Ubiquinon Injeel® and Ubiquinon Injeel® forte have different effects, that these Injeels® and conventional ubiquinon dilutions have different effects, and that the differences in effects of the single potencies included in the Injeels® do not follow a linear pattern.

The issues raised by this article merit further research.

Extracto

Este estudio preliminar utilizando sistemas "sin células" a demostrado:, que hay diferencias en los efectos de Ubiquinon-Injeel® e Ubiquinon-Injeel® forte, que hay diferencias en los efectos entre los Injeels® y las diluciones convencionales de ubiquinon, y que las diferencias en los efectos de las potencias incluidas en los Injeels® no siguen un modelo lineal.

Las temas planteadas en este artículo merecen investigación adicional.

Introduction

Critics of homeopathy demand suitable proof of the efficacy of minute and infinitesimal quantities of active substances and will acknowledge the scientific basis of successive potentization only if it can be proved that a homeopathic potency and a conventionally produced dilution have different effects even though they contain identical amounts of the active ingredient.

In the case of single potencies, such proof is easy to obtain using cell-free test systems.[1-5] Cell-free systems-in this case, buffered enzyme solutions-were used as indicators of the effects of the ubiquinon preparations. Every reasonable effort was made to ensure the purity of the enzymes. What can we conclude from cell-free systems? Since such test systems contain only an enzyme and a homeopathic single or mixed remedy, there can be no doubt as to what causes any detected change in the enzyme's activity. Because cell-free test systems isolate individual functions from the context of the total organism, results obtained from such systems cannot be extrapolated to the organism as a whole. In view of our objectives, however, this limitation is an asset rather than a disadvantage. Critics of our work-who are not the same as the above-mentioned critics of homeopathy-often charge us with reductionism, but they are targeting a circumstance that is inherent in the test system and deliberately selected by the investigators.

This article presents a pilot project intended to test the success of similar methods with regard to mixed potencies such as the Injeels.® The authors acquired and tested the following preparations: (1) Ubiquinon in all the single potencies included in the Injeels,® (2) Ubiquinon Injeel® and Injeel® forte, (3) conventional dilutions corresponding to the single potencies, (4) conventional dilutions corresponding to the Injeels,® (5) the solvent used in each preparation, and (6) potentized solvent to compare to each potency.

In addition, we plan to study preparations succussed for different numbers of strokes (10, 50, or 100) at each level of potentization. Our initial work, however, was done with preparations produced exclusively with 10 strokes per potentization level. This preliminary report investigates only Injeels,® the corresponding conventional dilutions, and (in some cases) single potencies with regard to their effect on different cell-free systems. A more comprehensive study is planned that will present the data from all of our experiments.

Resumen

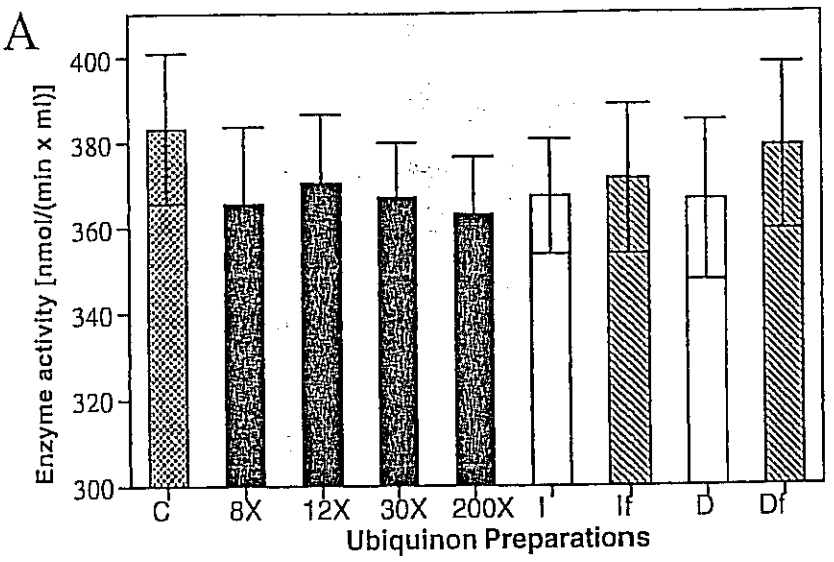
Los que critican la homeopatía requieren una prueba apropiada de la eficacia de las cantidades infinitesimales de sustancias activas. Además, pueden aceptar el fundamento científico de la potenciación sucesiva solamente si se puede verificar que una potencia homeopática y una dilución convencional tienen efectos distintos, aunque contengan cantidades idénticas del ingrediente activo.

En el caso de las potencias sencillas, una tal prueba est fácil a obtener, empleando sistemas "sin células."[1-5] Los sistemas sin-células - en este caso soluciones enzimáticas - sirvieron como indicadores de los efectos de las preparaciones conteniendo ubiquinon. Hicimos cada esfuerza razonable para verificar la puridad de las enzimas. ¿Y qué se puede concluir de los sistemas sin-células? Como los tales sistemas contienen solamente una enzima y un remedio homeopático, sencillo o en combinación, no hay duda sobre lo que causa los cambios en la actividad de la enzima. Como los sistemas sin-células aíslan las funciones particulares del contexto del organismo total, no se puede extrapolar los resultados emanantes al organismo entero. Sin embargo, en vista de nuestros objetivos, esta limitación sirve como una ventaja y no una desventaja. Los que critican nuestra obra - no específicamente los mismos críticos de la homeopatía mencionados arriba - nos acusan de reduccionismo pero en este caso se enfocan sobre una circunstancia intrínseca al sistema de prueba que fue

escogido deliberadamente por los investigadores.

Este artículo presenta un diseño para verificar el éxito de métodos semejantes con respecto a las potencias mezcladas, tal como los Injeels®. Los autores obtuvieron y pusieron a prueba las preparaciones siguientes: (1) Ubiquinon en todas las potencias sencillas incluidas en los Injeels®, (2) Ubiquinon Injeel® e Injeel® forte, (3) Diluciones convencionales que correspondan a las potencias sencillas, (4) Diluciones convencionales que correspondan a los Injeels®, (5) El solvente empleado en cada preparación, (6) El solvente potentizado como comparanza a cada potencia.

Además, vamos a estudiar las preparaciones sacudidas (con sucusión) con cantidades distintos de sacudidas (10, 50, o 100) a cada nivel de potenciación. Hicimos nuestro primer trabajo, sin embargo, con preparaciones producidas exclusivamente con 10 sacudidas a cada nivel de potenciación. Este artículo preliminar investiga solamente los Injeels®, las diluciones correspondientes convencionales, y en unos casos, unas potencias sencillas con respecto a sus efectos sobre varios sistemas "sin células." Vamos a hacer un estudio más amplio para presentar todos los resultados de nuestros ensayos.



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Statistics

	C	8X	12X	30X	200X	I	If	D	Df
Control (C)		x		x	x			x	
8X	x								
12X									
30X	x								
200X	x								
Injeel (I)									
Injeel forte (If)									
Dilution corresponding to Injeel (D)	x								
Dilution corresponding to Injeel forte (Df)									

Figure 1: Determining the catalytic activity of glutathione S-transferases in the presence of different ubiquinon preparations. The cytosolic extract was incubated with the necessary assay components and the ubiquinon preparations listed above for 10 minutes at 32°C. Chart A shows average values (adjusted to the control value) and positive and negative standard deviation. C represents the untreated control. Chart B presents the results of statistical evaluation (n = 45). X denotes significant differences (P<.01).

Methods

Active Agents

The active substance we chose was ubiquinon in potencies of 8X, 12X, 30X, and 200X, all produced using 10 succussions per potentization level. Two mixed potencies or potency chords produced according to the same protocol were also used-Ubiquinon Injeel,® which contains the potencies 12X, 30X, and 200X, and Ubiquinon Injeel® forte, which contains the potencies 8X, 12X, 30X, and 200X. Also tested were mixed dilutions corresponding to Injeel® and Injeel® forte. The dilutions differed from the Injeels® only in that they were not potentized but produced according to a conventional mixing procedure. All of these preparations and solutions were supplied by Biologische Heilmittel Heel GmbH, Baden-Baden, Germany. Our study used a blinded test format; all preparations were coded prior to use but decoded before the statistical analysis.

Enzyme Preparations

The enzymes used in the cell-free test systems were extracted from rat livers processed at 4 °C. To extract the cytosolic fraction,[6] the rat livers were first homogenized in 5 mL of buffer A (30 mmol of potassium phosphate buffer, pH 8.3) per gram of liver tissue, using a cell homogenizer, and then centrifuged for 10 minutes at 1500*g*. The effluent was then centrifuged again at 105,000*g* for 60 minutes (using the Swing-out Rotor AH-629 manufactured by Sorvall, Bad Homburg, Germany). Aliquot portions (2.5 mL) of the resulting effluent were each applied to a Sephadex G25 column equilibrated with buffer A and then eluated with 3.5 mL of

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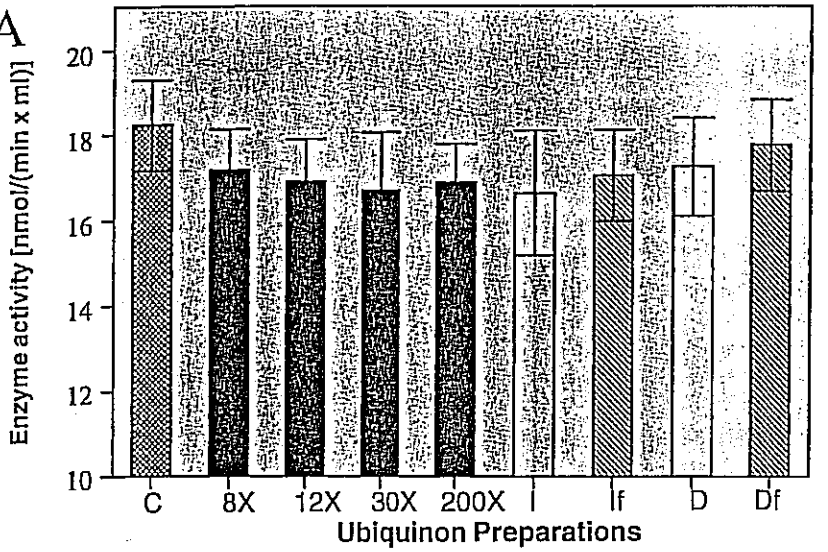

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Statistics

	C	8X	12X	30X	200X	I	If	D	Df
Control (C)		x	x	x	x	x	x	x	
8X	x								
12X	x								x
30X	x								x
200X	x								x
Injeel (I)	x								x
Injeel forte (If)	x								
Dilution corresponding to Injeel (D)	x								
Dilution corresponding to Injeel forte (Df)			x	x	x	x			

Figure 2: Catalytic activity of acid phosphatase in the presence of different ubiquinon preparations. The enzyme was incubated with the ubiquinon preparation and the synthetic substrate p-nitrophenyl phosphate at 30°C; after 5 minutes the reaction was stopped and the quantity of enzymatically formed p-nitrophenyl was determined. Chart A shows average values (adjusted to the control value) and positive and negative standard deviation. C represents the untreated control. Chart B presents the results of statistical evaluation (n =72). X denotes significant differences (P<.01).

buffer A. This step served to remove any endogenous substrates. After adjusting its protein concentration to 2 mg/mL with buffer B (0.25 mol of saccharose, 10 mmol of TRIS hydrochloride, pH 6.5), the elute was used to determine the activity of glutathione S -transferases (GSTs) in the cytosolic fraction. For use in determining the activity of xanthine oxidase and of the total xanthine oxidase/dehydrogenase system, the protein concentation of the eluate was adjusted to 5 mg/mL with buffer A. The BIO-RAD version (BIO-RAD, Munich, Germany) of Bradford's protein quantitation method[7] was used to determine the quantity of protein. Bovine γ globulin served as the reference substance.

Determining Glutathione S-Transferase Activity

The activity of GSTs in the cytosolic fraction was determined using the method described by Habig et al,[8] which we converted to a microtiter plate format [2]: 100 µL of potassium phosphate buffer (0.2 mol, pH 6.5),100 µL of the test solution (or, in the case of the control, water) and 30 µL of GSH (glutathione, 10 mmol) were pipetted onto a microtiter plate, and 50 µL of CDNB (1-chlorine-2,4-dinitrobenzol, 6 mmol, dissolved in 95% ethanol) were added. Then 20 µL of cytosol extract (protein concentration 0.13 mg/mL)-or, in the case of the control, 20 µL of buffer B-was added to start the reaction. The course of the reaction was monitored at 32°C for 10 minutes at a wavelength of 340 nm. Readings were taken with a temperature-controlled microtiter plate reader (ATTC 340, SLT Labinstruments, Crailsheim, Germany). A measuring period featuring measurements between minute 3 and minute 7 was used to calculate ΔE/min, which was then multiplied by a factor of 2,333 to yield activity per volume in nmol x min^{-1} x mL^{-1} (for 0.02 µL cytosol extract). The molar decrease coefficient for CDNB is 9.6 $mmol^{-1}$ x cm^{-1}.

Determining the Activity of Xanthine Oxidase and of the Total Xanthine Oxidase/Dehydrogenase System

The activity of xanthine oxidase and of the total xanthine oxidase/dehydrogenase system was ascertained by measuring enzyme-catalyzed urate formation.[8] 100 mL of the cytosolic fraction (final protein concentration, 0.5 mg/mL) was incubated for 30 minutes at 30°C with 300 mL of the ubiquinon preparation (or, in the case of the controls, water) and 600 mL of 0.17-mol TRIS hydrochloride buffer (pH 8.1) containing 100 mmol of xanthine with or without 0.17mmol of NAD.

At the end of the incubation period, the reaction was stopped by adding 10 volumes of ice-cold ethanol. After 5 minutes of centrifugation at 4°C and 1,000*g*, the effluent was incorporated by rotation under vacuum. The resulting precipitate was resuspended in 800 mL of 51.2% acetonitrile/0.1% trifluoroacetyl acid (pH 2.5) and recentrifuged (for 10 minutes at 4,000*g*). Column chromatography was performed on 100 mL of the effluent using a Knauer high-performance liquid chromatography instrument. A 250.0 x 4.0 mm Spherisorb S ODS 2 (particle size

5 mL, Knauer, Berlin, Germany) was used as the column with the appropriate prep column/anterior column. The enzymatically formed urate was eluated from the column with 51.2% acetonitrile/0.1% trifluoroacetyl acid (pH 2.5) at a flow rate of 0.5 mL/min. Urate absorption was measured at a wavelength of 292 nm.

The peak area was used to quantitate the enzymatically formed urate according to the reference equation urate (nmol/mL) = 1.705 x peak area - 1.886; correlation coefficient: 0.999. Urate in various concentrations served as the reference substance.

Determining Acid Phosphatase Activity

Acid phosphatase derived from potatoes was supplied by Boehringer-Mannheim. The catalytic activity of this enzyme was determined by measuring the quantity of p-nitrophenyl formed. A microtiter plate format was used for the experiments[9]: 100 mL of the ubiquinon test solution was added by pipette to 100 mL of the synthetic substrate (5.5 mmol of p-nitrophenyl phosphate in 0.1 mol of citrate buffer, pH 5.6), and the reaction was started by adding 20 mL of the enzyme (diluted 1:200 with 10 mmol of acetyl sodium, pH 5.6). After 5 minutes of incubation at 30°C, the reaction was stopped by adding 100 mL of sodium hydroxide (1.0 N), and the quantity of p-nitrophenyl formed was measured at a wavelength of 405 nm using a temperature-controlled microtiter plate reader. The quantity of p-nitrophenyl was determined by applying the reference equation p-nitrophenyl [nmol x mL-1] = 64.82 x OD 405 nm - 3.373; correlation coefficient 0.998. P-nitrophenyl in various concentrations served as the reference substance.

Statistics

The values obtained for each enzyme system (activity per volume in nmol x min^{-1} x mL^{-1}) were subjected to single-factor variance analysis (balanced design). Subsequently, multiple mean comparisons (Scheffé test) were performed. In addition, Duncan's multiple-range test was applied to the values obtained for the xanthine oxidase enzyme system. A probability of error of 1% (P<.01) was chosen as the limit of significance.

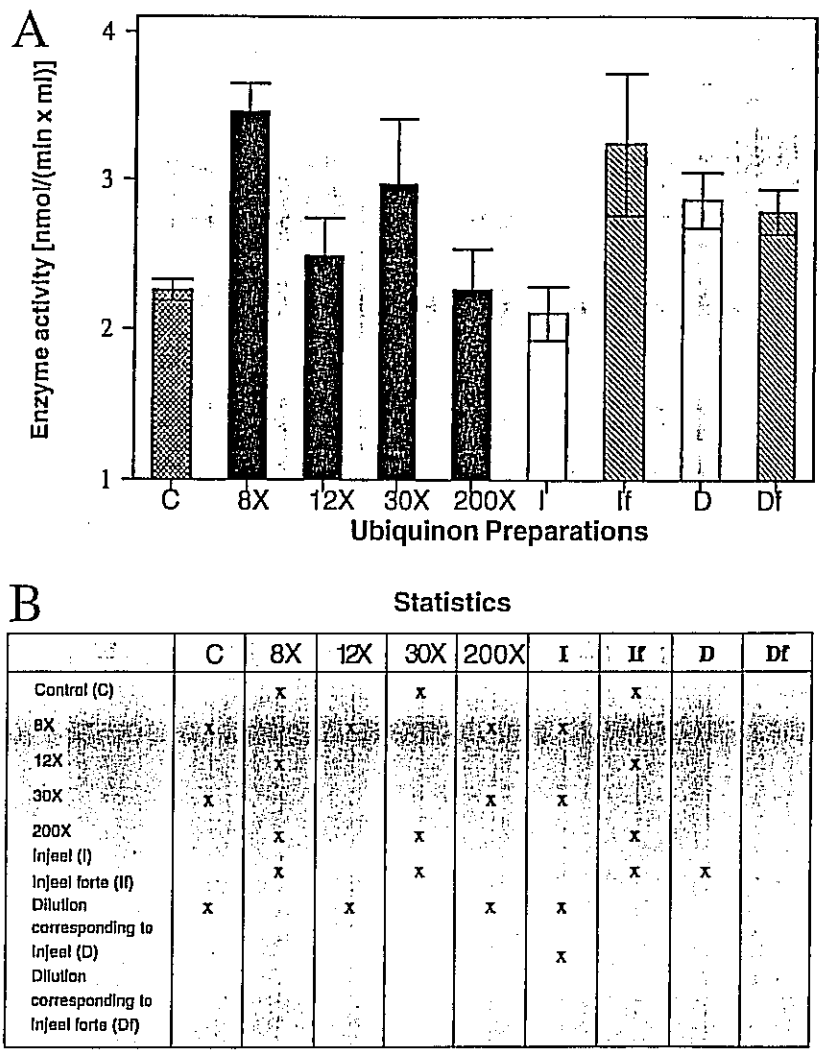

Figure 3: Activity of the total xanthine oxidase/dehydrogenase system in the presence of different ubiquinon preparations. The cytosolic extract was incubated with the necessary assay cofactors xanthine and nicotinamide adenine dinucleotide (NAD+) and the ubiquinon preparations listed above for 30 minutes at 30oC. Chart A shows average values (adjusted to the control value) and positive and negative standard deviation. C represents the untreated control. Chart B presents the results of statistical evaluation (n = 8). X denotes significant differences (P<.01).

Results and Discussion

Presumably, in the cell-free systems described here, homeopathic single and mixed potencies act like the "allosteric effectors" whose effects on cyclic adenosine monophosphate (cAMP) are described in biochemistry texts. As we have shown elsewhere,[10-12] homeopathic remedies stimulate individual enzymes *in situ*–that is, in the context of the cell-in very different ways. *In situ*, even neighboring enzymes in the same subcellular compartment are influenced differently. Their activity is

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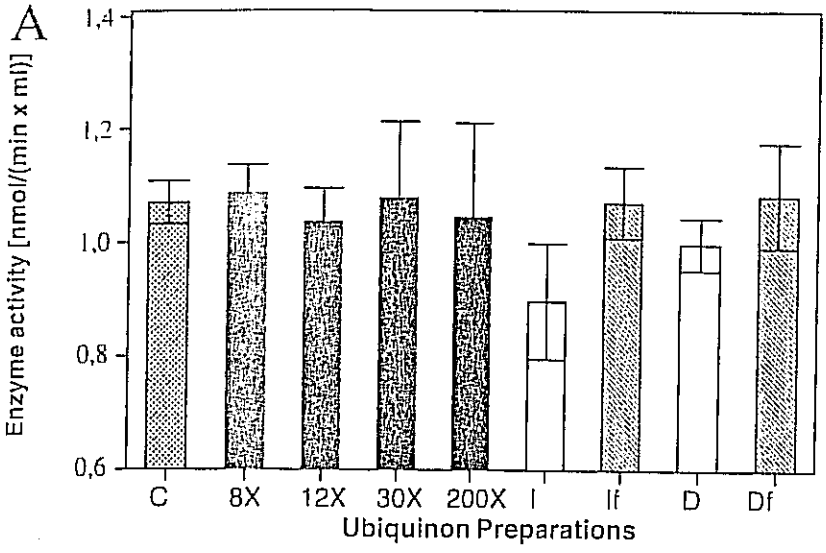

B

Statistics

	C	8X	12X	30X	200X	I	If	D	Df
Control (C)						X			
8X						X			
12X						X			
30X						X			
200X						X			
Injeel (I)	X	X	X	X	X		X	X	X
Injeel forte (If)						X			
Dilution corresponding to Injeel (D)						X			
Dilution corresponding to Injeel forte (Df)						X			

Figure 4: Catalytic activity of xanthine oxidase in the presence of xanthine and different ubiquinon preparations. Enzyme activity was determined by measuring the quantity or urate synthesized. Chart A shows average values (adjusted to the control value) and positive and negative standard deviation. C represents the untreated control. Chart B presents the results of statistical evaluation (n = 8). X denotes significant differences (P<.01).

controlled-or, to put it in cybernetic terms, regulated-in different ways. On the basis of this model and of the results already mentioned, we can imagine how the tremendously complex effects expressed in the drug picture of a homeopathic remedy occur. Without earlier studies of intact organisms, it would be impossible to judge the significance of the results we obtained using cell-free systems. On the other hand, in more complex systems than the cell-free model, it would be impossible to prove that homeopathic substances are effective, because direct effects are difficult–if not impossible, given the minute quantities of active agents involved–to prove in the intact organism.[1,2,3] These considerations must preface our discussion so that the significance of the results can be assessed.

Admittedly, it is not easy to explain the effects of the mixed potencies present in Injeels® in terms of the allosteric effector model, which is based on the interaction of a distinct homeopathic substance with the enzyme biomolecule. Can the three or four homeopathic potencies in an Injeel®-each of which has its own specific effect, as the results of tests with single potencies demonstrate–exert a combined additive effect, or are their individual effects active in different domains of the enzyme molecule? There is a great deal to be said in favor of the latter assumption, which will also be easier to confirm using enzyme-kinetic methods and procedures that access protein conformation (the three-dimensional arrangement of the biomolecules in space).

Looking first only at our results with the Injeel® and Injeel® forte, we see that these substances may either depress enzyme activity, as is the case with GSTs (Figure 1) and acid phosphatase (Figure 2), or stimulate it, as is the case with the total xanthine oxidase/dehydrogenase system (Figure 3) in comparison to the untreated control.

Looking at the effects of Injeels® in comparison with those of nonpotentized mixtures (conventional dilutions), we find that the effects of Injeel® and Injeel® forte may work in the same direction as the effects of the conventional ubiquinon mixtures, but on a quantitatively different level, as is the case with acid phosphatase, GST, and xanthine oxidase (Figure 4), or the effects of Injeels® and dilutions may work in opposite directions, as in the total xanthine oxidase/dehydrogenase system. Since the potentizing process (which in this case involved succussion for 10 strokes at each level) constitutes the only difference between Injeels® and conventional dilutions, it can be assumed that potentization causes the difference in their effects.

If we compare the results obtained using both potentized and nonpotentized forms of Injeel® and Injeel® forte, it is apparent that in all the cell-free systems used here, the effects of Injeel® are more strongly expressed than those of Injeel® forte in terms of reactivating the enzyme system. Since the only difference between Injeel® forte and Injeel® is that the former includes 8X in addition to 12X/30X/

200X, it is obvious to assume that the effect of Injeel® forte might be fine-tuned by varying the proportion of 8X to 12X/30X/200X. Figure 5 models how this might be played out in the catalytic activity of the detection system-in this case, specific enzymes.

When we consider the effects of the single potencies, it becomes apparent that (in comparison with the untreated control) the effect of 12X can be either less (as in the case of xanthine oxidase and total xanthine-oxidase/dehydrogenase) or greater (as in the GSTs) than that of either 8X or 30X. In the potency series we studied, the 12X potency marks a turning point in the effect. Earlier studies of other homeopathic substances, both *in vivo* experiments[10, 11, 16-19] and studies of cell cultures,[20, 21] revealed similar nonlinear effects, which seem to be specific to homeopathy. (It should be noted, however, that the effects of acid phosphatase do not conform to either pattern.)

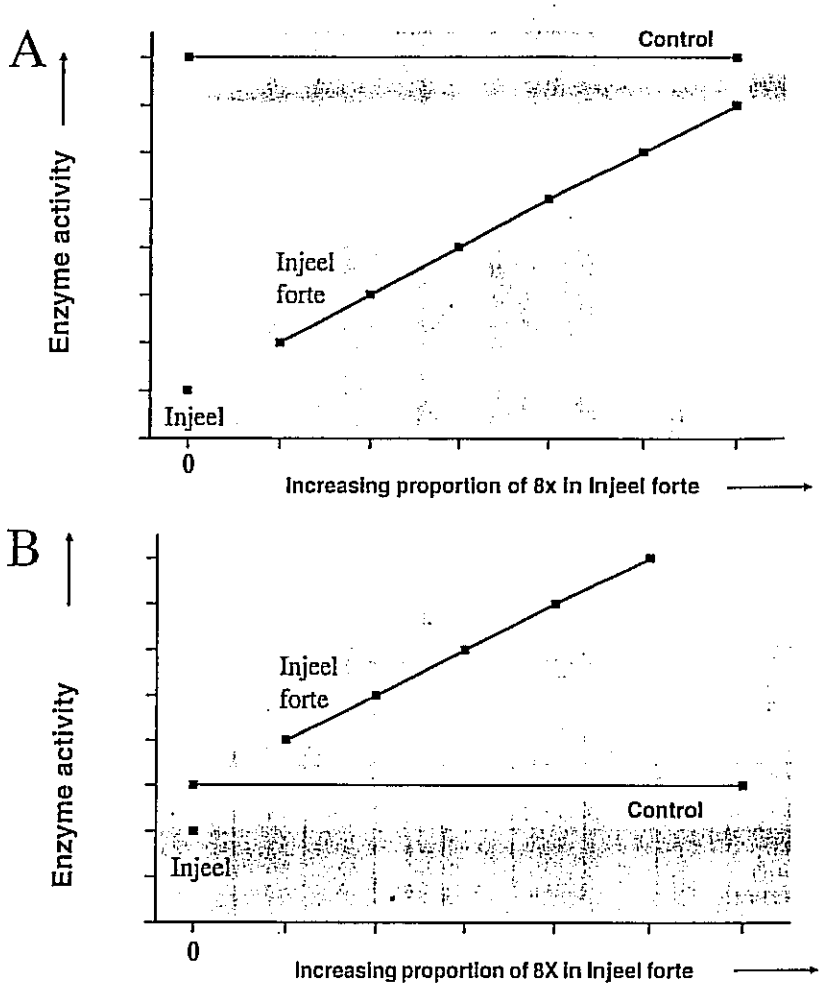

Fig. 5: Model of possible consequences of successive increases in the amount of 8X in Injeel® forte

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