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Dedicated to Prof. Ioan-Iovitz Popescu's 75th Anniversary

THE EFFECT OF SIMVASTATIN TREATMENT ON HIPERLIPEMIC HAMSTERS

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Abstract. The effect of simvastatin treatment on the evolution of atherosclerotic process in hiperlipemic hamsters, by measuring the serum parameters (total cholesterol – TC, triglicerides – TG, LDL – cholesterol, HDL – cholesterol, total peroxyl radical trapping potential – TRAP, thiobarbutiric acid reactive substances – TBARS, and angiotensin converting enzyme – ACE), was studied. The main result of our studies is the dramatic reduction of lesions on the cardiovascular system after two months of applied hypercholesterolemic diet combined with one more month of the same diet and treatment with simvastatin. We interpret the results by using standard statistical analysis (for correlation effects), a kinetic approach and near equilibrium thermodynamics (for time dependence of measured parameters).

Key words: atherosclerosis, hyperlipemic hamsters, concentration of serum parameters, cardiovascular lesions, simvastatin, kinetic theory, near equilibrium thermodynamics.

1. INTRODUCTION

Among the most efficient drugs for the treatment of hypercholesterolemia are statins, both for reducing the progression and inducing the reducing the regression of atherosclerosis. Mainly, the statins act as inhibitors of the 3-hydroxy-methylglutaryl- coenzime A (HMG-Co A) and in this way limit the synthesis of cholesterol in the cell [1–3].

However, there are many experimental data indicating that statins can also interfere with some other major events involved in the formation or reduction of the atherosclerotic lesions, independently or not of their hypo-cholestrolemic potential. Among these events can be mentioned the oxidative modification of LDL, together with the increase of triglycerides (TG) levels, and formation of lipid peroxides etc [4–7].

Since the liver controls the hypercholesterolemia all the statins have the liver as target-organ and are present in a low concentration in the perpheral circulation. For example, simvastatin concentration in the liver is higher than 80% while the ciorculating one is not more than 5%.

The aim of our work was to study the effect of simvastatin treatment on the evolution of atherosclerotic process in hiperlipermic hamsters (HL) by measuring the following serum parameters: total cholesterol (TC), TG, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), lipid peroxides expressed as the total peroxyl radical trapping potential (TRAP) and as thiobarbituric acid reactive substances (TBARS), and angiotensin converting enzyme (ACE), at different stages of hyperlipemic diet and drug administration. To this purpose, we used the Golden Syrian hamsters, previously employed to investigate vascular changes in atherogenesis [8–11], as long as it is well known the similarities to the human aspects of atherosclerosis [11, 12]. Also, we measured at the end of treatment the lesions areas localized on different segments of the cardiovascular system. More precisely, our goal was to see how important is the duration of treatment, if there is any interaction (correlation) between the above mentioned components (serum parameters) (*i.e.* TC, TG, LDL-C, HDL-C, TRAP, TBARS, ACE) during the hyperlipemic diet and simvastatin action.

Our main results are: (1) the area of lesions after two months of applied hypercholesterolemic diet combined with one more month of the same diet and treatment with simvastatin is strongly reduced; (2) there is an important interaction (correlation) among all measured parameters; (3) the concentration of all above components is depending of time in the studied interval of there months. These obtained results suggest us to describe/interpret them using a kinetic approach and near equilibrium thermodynamics.

2. MATERIAL AND METHODS

Animals. The animals studied were divided in two groups: (i) the control group having 10 hamsters fed standard chow supplemented with 3% cholesterol and 15% butter, and (ii) the treated group with 10 hamsters fed the same diet as for controls for 8 weeks, then treated daily by gavage (for 4 weeks) with 0.3 mg simvastatin/kg body, simultaneous with the hyperlipemic diet.

The control group was fed with hyperlipemic diet for 3 month and, in the third month also water. The treated group received the same diet for 3 months and in the third month, also was added 0.3 mg simvastatin per kg weight daily.

Serum. Blood was collected from the retro-orbital plexus of hamsters (fated overnight), every 2 week into the experiment. Blood was allowed to clot, and after a 5 minute centrifugation at 2,000xg serum was collected.

Biochemical assays. Serum total, LDL and HDL cholesterol, triglycerides were measured with enzymatic kits from Sigma. They were expressed as mg of each on dl of sample. Lipid peroxides were determined as thiobarbituric acid reactive species (TBARS) and expressed as equivalent nmols malondialdehyde (MDA) [13]. Total peroxyl radical-trapping potential (TRAP) in serum was

determined by an adapted protocol [14], and expressed as free radicals, in μ moles, trapped by I liter of serum. Angiotensin I converting enzyme activity was assayed by using the method from [15], with hippuril-L-leucine as substrate.

Light microscopy protocol. The vasculature of anesthetized hamsters was washed out of blood by perfusing PBS under pressure trough the abdominal aorta using venal cava as outlet. Afterwards, a mixture of 4% paraformaldehyde, 0.075M lysine in 0.01 MNa periodate (PLP) was perfused (Nakane citrate). After 10 minute fixation in situ, the aortic arch and heart were excised coronary arteries and aortic valves were dissected out and together with the aortic arch were immersed in 10% paraformaldehyde. In order to obtain cryosections from the aortic valves, specimens were immersed in PBS at 4°C containing successively 5%, 10% and 20% sucrose (occasionally 10% glycerol) for 15 minutes at room temperature 1h and 10h, respectively, at 4°C. Specimens were frozen in isopentane cooled with liquid nitrogen and stored at – 70°C. Cryosectionns (5–10 μm) were cut on a Harris cryostat at – 30°C and mounted on slides previously coated with 2% gelatin.

Morphometric analysis. The thoracic aorta from all hamsters fixed in 10% paraformaldehyde was opened longitudinally, stained for lipids with Oil Red O and mounted in Aquamont between glass slide and glass coverslip Images of the aorta were taken with a Hammamatsu camera attached to a Nikon light microscope. Morphometric analysis of the lipid deposits was performed with a specific computer progam – LUCIA, rented from NIKON Co.

3. RESULTS

The concentration values of measured serum parameters (at different intervals of time) are given in Table 1 and Table 2 and the variation of the treated group with respect of the control one at the end of treatment are in the Table 3.

Then by using standard statistical analysis [16, 17], the correlation coefficients *r* were calculated between: (a) drug administration and lesion reduction $r_{M,L}$; (b) drug administration and serum parameters $r_{M,i}$, where *i* can be TC, TG, LDL-C, HDL-C, TRAP, TBARS, ACE; (c) measured serum parameters and extension of lesions $r_{i,L}$; (d) $r_{i,j}$ between different serum parameters.

To make all the mentioned calculations, we used the following assumptions: (1) the drug is the variable and the lesions are the final effects; (2) the effect of the drug on each of above parameters can be considered separately; (3) the effect of each parameters is manifested both on the lesions and the other parameters (components).

This way to use statistical method is justified as long as we analyze the experimental facts such as they come out from experiments.

Table 1

Serum parameters of hamsters

Serum parameters hamsters																
Number & group	CHOL (mg/dl)				TG (mg/dl)				LDL-C (mg/dl)				HDL-C (mg/dl)			
/weeks	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week
HL3mo+H2O																
1	87.0	538.0	583.0	330.0	160.0	400.0	293.0	300.0	27.0	205.0	226.0	215.0	44.0	92.0	98.0	75.0
2	81.0	573.0	560.0	360.0	153.0	254.0	204.0	315.0	30.0	249.0	260.0	243.0	42.0	74.0	78.0	70.0
3	81.0	426.0	405.0	409.0	114.0	149.0	137.0	258.0	26.0	260.0	300.0	266.0	48.0	91.0	96.0	80.0
4	65.5	277.5	380.0	273.0	133.0	343.0	346.0	273.0	29.0	137.0	230.0	177.0	41.0	85.0	109.0	93.0
5	68.0	887.5	1,223.0	706.0	145.0	1,595.0	1,722.0	651.0	28.0	350.0	450.0	284.0	39.0	77.0	73.0	82.0
6	65.0	367.0	450.0	369.0	148.0	422.0	660.0	404.0	27.0	173.0	228.0	184.0	40.0	97.0	109.0	92.0
7	70.4	584.0	583.0	434.0	226.0	740.0	505.0	340.0	25.0	252.0	308.0	261.0	47.0	75.0	100.0	86.0
8	88.1	570.0	375.0	280.0	259.0	850.0	274.0	193.0	27.0	213.0	257.0	192.0	49.0	71.0	68.0	68.0
9	64.4	230.0	285.0	379.0	188.0	265.0	431.0	326.0	27.0	115.0	118.0	227.0	39.0	112.0	103.0	75.0
10	68.2	484.0	478.0	520.0	125.0	637.0	613.0	850.0	29.0	226.0	233.0	241.0	43.0	69.0	60.0	70.0
Mean	**73.86**	**493.70**	**532.20**	**406.00**	**165.10**	**565.50**	**518.50**	**391.00**	**27.50**	**218.00**	**261.00**	**229.00**	**43.20**	**84.30**	**89.40**	**79.10**
SD	**9.39**	**186.88**	**262.04**	**127.95**	**46.15**	**426.18**	**455.83**	**202.80**	**1.51**	**67.30**	**84.31**	**36.60**	**3.71**	**13.70**	**17.96**	**9.06**
HL3mo+ Simvastatin																
11	96.1	387.0	629.4	437.3	194.0	359.0	405.0	346.0	45.3	271.0	380.0	267.0	40.0	70.0	68.0	96.0
12	81.2	411.0	563.7	416.5	180.0	362.0	503.0	388.0	36.0	294.0	314.0	245.0	39.0	70.0	67.0	109.0
13	90.0	476.4	409.6	460.1	105.0	334.0	232.0	299.0	42.7	343.0	242.0	301.0	46.0	61.0	89.0	104.0
14	75.0	396.0	412.0	436.2	111.0	336.0	283.0	302.0	32.2	253.0	247.0	290.0	43.0	81.0	93.0	82.0

(continues)

Table 1 (continued)

Serum parameters hamsters																
Number & group	CHOL (mg/dl)				TG (mg/dl)				LDL-C (mg/dl)				HDL-C (mg/dl)			
/weeks	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week
15	105.1	544.3	571.5	411.4	161.0	692.0	493.0	320.0	38.3	248.0	305.0	185.0	54.0	85.0	95.0	129.0
16	56.0	290.6	274.6	278.0	43.0	166.0	105.0	133.0	24.7	203.0	153.0	164.0	37.0	71.0	99.0	97.0
17	84.0	420.0	465.0	387.0	60.0	232.0	250.0	330.0	35.0	335.0	411.0	187.0	36.0	60.0	80.0	96.0
18	96.0	430.0	435.0	442.0	98.0	315.0	276.0	374.0	27.0	361.0	450.0	257.0	32.0	69.0	71.0	86.0
19	86.0	385.0	360.0	344.0	106.0	297.0	230.0	392.0	29.0	211.0	211.0	234.0	41.0	80.0	69.0	98.0
20	63.0	211.0	250.0	269.0	159.0	417.0	350.0	366.0	30.0	278.0	278.0	122.0	54.0	72.0	80.0	129.0
Mean	**83.24**	**395.13**	**437.08**	**388.15**	**121.70**	**351.00**	**312.70**	**325.00**	**34.02**	**279.70**	**299.10**	**225.20**	**42.20**	**71.90**	**81.10**	**102.60**
SD	**15.22**	**91.86**	**124.99**	**68.66**	**50.29**	**139.02**	**125.29**	**75.35**	**6.72**	**54.04**	**92.87**	**58.35**	**7.30**	**8.14**	**12.20**	**15.90**

Table 2

Serum parameters of hamsters (continued)

Serum parameters hamsters													
Number & group	TRAP (uM)				TBARS (nmoles eq MDA/ml)				ACE (U/ml)				LESIONS
/weeks	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	% area
HL3mo+H2O													
1	980.0	750.0	693.0	660.0	6.00	7.19	7.23	12.10	36.0	65.0	73.0	60.0	0.872
2	1,100.0	814.0	768.0	700.0	6.30	6.32	6.37	10.11	54.0	63.0	68.0	65.0	0.930
3	1,007.0	773.0	715.0	605.0	5.73	6.80	7.36	11.50	54.0	60.0	77.0	59.0	1.028
4	1,036.3	699.3	645.8	657.4	6.15	4.76	14.73	13.02	55.02	51.6	63.8	64.9	0.929
5	998.0	682.8	607.8	606.1	5.48	8.66	7.20	12.96	54.2	58.3	77.4	62.2	1.679

(continues)

Table 2 (continued)

Serum parameters hamsters													
Number & group	TRAP (uM)				TBARS (nmoles eq MDA/ml)				ACE (U/ml)				LESIONS
/weeks	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	0 week	8 week	10 week	12 week	% area
6	984.5	730.4	582.5	616.0	5.15	3.75	12.03	19.58	57.1	46.8	71.3	55.8	0.381
7	915.5	746.3	667.3	636.5	8.03	7.14	13.74	11.54	37.1	63.6	82.7	59.8	1.799
8	954.4	720.0	708.1	585.2	6.27	7.28	10.98	13.65	44.5	48.8	61.7	68.2	0.322
9	974.0	734.0	680.9	647.1	7.80	8.24	11.06	14.93	43.5	57.2	58.7	52.5	0.951
10	896.0	705.8	694.5	665.7	7.97	8.23	12.76	14.68	47.3	58.0	72.1	56.8	1.658
Mean	**984.57**	**735.56**	**676.29**	**637.90**	**6.49**	**6.84**	**10.35**	**13.41**	**48.29**	**57.23**	**70.57**	**60.42**	**1.055**
SD	**58.06**	**38.20**	**53.80**	**34.86**	**1.06**	**1.55**	**3.07**	**2.63**	**7.75**	**6.28**	**7.57**	**4.77**	**0.514**
HL3mo+ Simvastatin													
11	951.3	690.0	739.2	739.5	6.70	5.98	7.97	8.85	55.8	73.5	89.5	56.0	0.091
12	1,041.0	654.0	674.9	690.1	5.00	7.89	7.70	9.24	52.7	63.2	89.8	51.2	0.081
13	969.3	642.9	727.3	764.2	6.20	6.96	7.48	6.55	46.5	71.7	76.0	50.1	0.021
14	897.5	713.0	717.0	782.8	6.30	6.05	5.90	10.56	43.0	70.1	67.4	43.0	0.331
15	933.4	771.4	739.1	758.1	9.40	7.31	9.18	10.04	61.0	55.4	87.7	52.3	0.012
16	945.0	760.0	771.7	790.8	8.00	7.95	11.10	5.51	84.0	95.0	104.1	86.7	0.512
17	1,100.0	802.0	743.0	692.0	6.21	7.20	8.30	6.90	47.0	50.0	65.0	60.0	0.025
18	891.0	730.0	681.0	793.0	8.03	9.15	8.00	6.50	50.0	58.0	71.0	60.0	0.300
19	984.0	1,002.0	735.0	776.0	5.14	6.11	7.60	6.21	45.0	50.0	55.0	51.0	0.075
20	873.0	680.0	682.0	699.0	5.80	6.03	7.71	8.92	56.0	52.0	49.0	47.0	0.315
Mean	**958.55**	**744.53**	**721.02**	**748.55**	**6.68**	**7.06**	**8.09**	**7.93**	**54.10**	**63.89**	**75.45**	**55.73**	**0.176**
SD	**69.96**	**104.04**	**31.99**	**41.06**	**1.40**	**1.06**	**1.33**	**1.79**	**11.93**	**14.12**	**17.28**	**12.11**	**0.174**

All the calculations for correlation coefficients were done only at the end of the treatment stage. The obtained values for r are given in Table 4.

Looking in the Table 1 and Table 4, it can be noted the following:

(1) The extension of lesion for the treated group was much smaller than for that of control group (about 83% lower), which points out the very beneficial effect of the drug on reducing the atherosclerosis;

(2) The concentration values of all measured parameters were different at various stages of administered diet and treatment with simvastatin. This dependence of time shows us that the equilibrium or (rather) stationary state was not completely reached after 3 months. On the other hand the corresponding variations were not very large (as a matter of fact, in most of cases, they were quite small), such that we can suppose that all involved processes were near-equilibrium.

(3) Also, from Table 4 it results clearly that all the studied components interact among themselves and these coupling effects cannot be neglected in most of the cases. More than that, since the sign of almost all correlation coefficients was in the right direction, it is possible to asses the contribution of each parameter to the formation or reduction of lesion extension.

4. THE KINETIC APPROACH

Because our experiments (done at a macroscopic level) show that the studied phenomena are rather dynamics and the coupling between different parameters exists, a kinetic description could be seen as a step for understanding the inner hamster processes – as a results of diet and drug action.

Based on the fact that our experimental parameters are slowly varying with respect time, one more approximation was considered as justified, namely the linear approach (or near-equilibrium description).

To formulate the kinetic theory, we imagine a bio-chemical picture in order to take into account the coupling of different parameters and with the hope the dependence on time might be explained [18, 19].

The bio-chemical picture is as follows:

If at time $t = 0$ (initial time) a diet D is administered to the animals, then there is a bio-chemical reaction:

$$\sum_i A_i + D \rightleftharpoons \sum_i A_i^{(1)}, \tag{1}$$

where A_i represents the parameter (component) i, D is for the diet and $A_i^{(1)}$ it the same components with their concentration modified due to the diet D.

At another time t_0, if we administer the same diet D (as a continuation) to the control group, the reaction is:

$$\sum_i A_i^{(1)} + D \rightleftharpoons \sum_i A_j^{(2)}. \tag{2}$$

Also, at $t = t_0$ the second group received the drug M along the diet D and the reaction is:

$$\sum_i A_i^{(1)} + D + M \rightleftharpoons \sum_i A_i^{(3)}. \tag{3}$$

The corresponding values of $A_i^{(1)}$, $A_i^{(2)}$ depend of the action of diet D and $A_i^{(3)}$ is a function of diet D and drug M.

Now if we note the corresponding concentration by X_i, X_D, X_M then, in the linear approach (near-equilibrium) one can write the following set of coupled kinetic equations for the variation of concentration in time, as a results of mutual interaction, for all components including the diet and or the drug:

$$\frac{dX_p}{dt} = \sum_q \alpha_{pq} k_q \qquad p, q = i, D, M, \tag{4}$$

where α_{pq} are the coupling coefficients given for our bio-chemical model by:

$$\alpha_{pp} = -\sum_{q \neq p} k_{pq} \qquad \text{(diagonal terms)}, \tag{5}$$

$$\alpha_{pq} = k_{qp} \qquad p \neq q \qquad \text{(off} - \text{diagonal terms)}, \tag{6}$$

In (5) and (6) k_{pq} are the rate constants and in general: $k_{pq} \neq k_{qp}$.

The set of equations (4) satisfies the Onsager principle as can be easily proved [20].

By solving the system of equations (4), one can obtain $X_p(t)$ and explain the observed variation of the above parameters, inside of 3 months interval. Unfortunately, because our system of coupled equations is formed by at least 9 coupled equations, to find the corresponding solutions $X_p(t)$ is a tremendous task, especially we need to know all values of k_{pq}, which is a problem at this stage of our research.

However using the relations (5) and (6) one gets:

$$\sum_p \frac{dX_p}{dt} = 0. \tag{7}$$

The relation (7) tell us that in the process of interaction between components among themselves and with the diet and drug, the mass is conserved such that:

$$\sum_p X_p = C_p = \text{const.} \tag{8}$$

The relation (8) is a very useful one in many respects. One of this, very important, is that we can obtain the effective concentration of drug X_M (the variation of this) which interacts with the hamster and the diet D. To find X_M we write instead of (7), equivalently:

a) For the control group:

$$\sum_i \frac{dX_i}{dt} + \frac{dX_D}{dt} \to \sum_i \frac{dX_{i,f}^{(D)}}{dt} = 0, \tag{9}$$

which leads to:

$$\sum_i X_{i,f}^{(t,D)} = C_D = \text{const.} \tag{10}$$

and

b) For the treated group (with simvastatin)

$$\sum_i \frac{dX_i}{dt} + \frac{dX_D}{dt} + \frac{dX_M}{dt} \to \sum_i \frac{dX_{i,f}^{(D+M)}}{dt} = 0 \tag{11}$$

or

$$\sum_i X_{i,f}^{(t,D+M)} = C_{D+M} = \text{const.} \tag{12}$$

where f refers to the final moment t_f when the hamsters are sacrificed to measure the extension of lesions.

After a little algebra from (10) and (12), if the control and treated groups are enough homogeneous and the direct interaction between diet and drug is very weak (a realistic assumption), one obtains [18–19]:

$$X_M \simeq \sum_i X_{i,f}^{(D)} - \sum_i X_{i,f}^{(D+M)}, \tag{13}$$

which gives us the desired effective concentration of the drug used actively during the treatment at the moment t_f. (For more details, see [18]).

To estimate X_M, via (13), we can use either the solution of (4) or experimental values.

It has to be noted that i in the relation (4)–(13) is not limited in principle, to the above 7 parameters (measured by us), but it is possible to include all relevant components that may cause some effects on atherosclerosis. However, if only few parameters are considered, than X_M from (13) tell us just the corresponding part of drug consumed (or necessary to be consumed) in the interaction with those components.

Now, by using the average values of the measured serum parameters, booth for control and treated group at the moment when animals were sacrificed, from (13) one obtains for X_M:

$$X_M = 0.60 \text{ nmol/}L, \tag{14}$$

which seems to be a realistic value, about 87% of administered drug of one day. This value should be compared with the concentration of simvastatin in the liver (which is over 80%). At this moment the value of (14) has not to be taken too literally but rather as the order of magnitude for consumed drug.

Also, we have to note that the relation (13) will be a good approximation only if the two groups of animals are identical or as much as it is possible homogeneous otherwise the results might have no relevance and we need to use the formula (12) in the paper [18].

Coming back to the set of equation (4), we look to the solution of the form [21]:

$$X_p = Ae^{-rt}, \tag{15}$$

where r satisfies the equation given by the characteristic determinant:

$$\det | r\delta_{pq} - \alpha_{pq} | = 0. \tag{16}$$

For some values of coupling coefficients α_{pq}, respectively of rate constants the roots r of (16) may be conjugate complex quantities:

$$r = \alpha + i\beta \qquad r^* = \alpha - i\beta. \tag{17}$$

In that case the solution (15) can be written as [21]

$$X_p = [A_p \cos\beta t + B_p \sin\beta t]e^{-\alpha t} + \bar{X}_p \tag{18}$$

where the constants A_p and B_p, $\bar{X}_p$ can be determined from the initial condition. Also $\bar{X}_p$ constants are satisfying the relation $\sum_q \alpha_{pq}\bar{X}_p = 0$.

5. AN APPROXIMATION FOR THE SOLUTION OF (18)

Whenever $\beta t \le 1$, by using the series expansion of $\sin\beta t$ and $\cos\beta t$, from (18), up to the second order in t one obtains:

$$X_i(t) \simeq (a_i + b_i t + c_i t^2)e^{-\alpha t} + \bar{X}_i, \tag{19}$$

where $a_i = A_i$, $b_i = \beta B_i$ and $c_i = -\beta^2 A_i/2 = -\beta^2 a_i/2$.

Using the initial condition and stationary state condition, we have:

$$- \text{ for } \quad t = 0: \quad a_i + \bar{X}_i = X_i(0); \tag{20}$$

$$- \text{ for } \quad t \to \infty: \quad \bar{X}_i = X_i(\infty).$$

Looking to our experimental values in Table 1, it was observed that for TC, TG, LDL-C, HDL-C, TBARS and ACE there is a time t_m when the average concentration of these parameters reach a maximum value and for TRAP a minimum one. These facts give us the possibility to search the extremum of (19) with respect the time and to determine α_i in the exponential. Then:

$$\alpha_i = \frac{bi + 2c_i t_m}{(a_i + b_i t_m + c_i t_m^2)}. \tag{21}$$

Because the average value over time $\bar{X}_i$ of (19) is

$$\bar{X}_i = \lim_{t\to\infty} \frac{1}{t} \int_0^t x_i(t)dt = X_i(\infty),$$

the value of $X_i(\infty)$ can be approximated (good enough) with the average value over the corresponding time interval of experimental data of Table 1.

The value of β can be chosen such that to ensure a good convergence of our series expansion of (18), *i.e.* the contribution of the next term of ~ t^3 in (19) to be neglected (or to be less than 5% for $t_m = 10$ units of time). This corresponds to $\beta \approx 0.056$ and $b_i < 3\alpha_i/t_m$. By this choice of β the c_i is calculated from $c_i = -\beta^2\alpha_i/2$.

The constant b_i can be determined either by introducing the solutions (19) in the set of equations (4) or considering b_i as a free parameter and fitting the solution (19) with the experimental data. Since at this stage of our research, the coupling coefficients α_{pq} are difficult to be calculated for all seven serum parameters, the fitting with the experimental data is used and we estimated b_i such that the sum of square errors to be minimum. In this way α_i of (22) is determined too.

The resulted values of α_i, b_i, c_i α_i and $\bar{X}_i$ by using the above procedure are given in the Table 5. In the Table 6 are presented the calculated values for the concentration $X_i(t)$ of serum parameters, via relation (19), with the constants α_i, b_i, c_i α_i and $\bar{X}_i$ of Table 5, and compared with the average experimental values of Table 1. In the Tables 5 and 6 the units are the same as in the Table 1.

The solutions of (19) given in the Table 6, are perfect compatible with the set of equations (4) and they explain well the variation with respect to time, of the measured serum parameters.

Of course the theoretical results can be improved going to higher order terms in the series expansion of (18).

From the examination of the theoretical data of Table 6 it results two things: a) after one or two weeks the variation of hamster serum parameters is important. For example, TC and TG are about two-three times higher than the corresponding values for $t = 0$; b) for $t \geq 16$ weeks (8 weeks of treatment with simvastatin, in our case) the all seven serum parameters become constant with a high precision which points out that the stationary state was reached. Both these results are in good agreement with the measured parameters.

6. THE PRODUCTION OF ENTROPY *S*

In the frame of the same bio-chemical picture and near equilibrium, the production of entropy can be written as:

$$\frac{dS_{ext}^{(D)}}{dt} + \frac{dS_{int}}{dt} \rightarrow \frac{dS_{int,f}^{(D)}}{dt} \tag{22}$$

for control group, and

$$\frac{dS_{ext}^{(D)}}{dt} + \frac{dS_{int}}{dt} \rightarrow \frac{dS_{int,f}^{(D)}}{dt} \tag{23}$$

for treated group, where *ext* refers to diet *D* and drug *M* and *int* to the hamsters parameters.

In (22) and (23),

$$\frac{dS_{int}}{dt} \geq 0$$

(as for isolated systems),while

$$\frac{dS_{ext}}{dt} < 0$$

or,

$$\frac{dS_{ext}}{dt} > 0,$$

both for diet and drug.

For us, the most interesting situation is when the production of entropy [22] corresponds to a stationary state, because in that case the effect of the drug is considered as a good one. But in this case, due to Prigogine, the production of entropy has to be minimum *i.e.*:

$$\frac{d}{dt}\left[\frac{dS_{int,f}^{(D+M)}}{dt}\right]_{t=t_s} = 0, \qquad \frac{d^2}{dt^2}\left[\frac{dS_{int,f}^{(D+M)}}{dt}\right]_{t=t_s} > 0, \tag{24}$$

where t_s is the moment when the stationary state is reached.

Indeed, following [22–23], the production of entropy of (22) and (23), in its final form, for our *bio-chemical model* can be written as [18–19]:

$$\frac{dS_{int,f}}{dt} = \frac{AJ}{T} = R\left[k_{12}\prod_k (X_k)^{\nu_k} - k_{21}\prod_l (X_l)^{\nu_l}\right]\ln K\frac{\prod_k (X_k)^{\nu_k}}{\prod_l (X_l)^{\nu_l}}, \qquad (25)$$

where A is the chemical affinity, J – the current, T – the temperature, R – the ideal gases constant, k – rate constant, ν_k and ν_l – the stoichiometric coefficients for *reactants* and *products* respectively and K – the constant of chemical equilibrium.

The productions of entropy of (25) satisfies the conditions (24) only if there is interaction between components which is in agreement with the kinetic approach described above and also with our experimental data.

To see that (24) is satisfied by using (25) there are two possibilities: (a) to use the solution for $X_i(t)$ of (18) or (19), and (b) to use experimental values. The last one, for simvastatin treatment, is approximately fulfilled, which means that after three months of diet and treatment, the stationary state is almost obtained:

As a matter of fact, from condition

$$\frac{d^2}{dt^2}\left[\frac{dS_{int,f}^{(D+M)}}{dt}\right]_{t=t_s} > 0$$

it can be determined the shortest time (critical time, t_s) for getting the stationary state.

Also, since in our case the administered diet and drug doses are constant in time, it can be supposed that:

$$\frac{dS_D}{dt} = C_D = \text{const.} \quad \text{and} \qquad \frac{dS_M}{dt} = C_M = \text{const.} \qquad (26)$$

Then, because dS_{int}/dt, on the left side of (22) and (23) are zero (this corresponds to initial state) by integration, from (26) one obtains:

$$S_{int,f}^{(D+M)} - S_{int,f}^{(D)} = C_M(t - t_0) \qquad (27)$$

and

$$C_M = -C_D, \qquad t > t_0, \qquad \text{(as a condition for stationarity)}, \qquad (28)$$

where t_0 is the starting moment of treatment.

The important result contained in the relation (27) is that the entropy, after treatment, is smaller for $C_D > 0$ than of the control group, which it is the case of normal animals. In our experiments this fact is very well correlated with the extension of lesion for the two groups (see Tables 1 and 3). For a more realistic picture of thermodynamic and kinetic processes, which take place inside hamsters in the course of diet and treatment it is needed (as a next step) to consider a non-linear approach [32].

7. DISCUSSION OF THE RESULTS

As it was mentioned already, the concentration values of all measured parameters (TC, TG, LDL-C, HDL-C, TRAP, TBARS, ACE) and also the extension of lesions are given in the Table 1 for different moments of time.

The examination of Table 1 points out that:

a) the extension of lesion of the treated group (hyperlipemic diet and 0.3 mg simvastatin/kg weight/hamster daily) was strongly reduced as compared to that of the control group – about 83% lower (see Table 3), showing the very beneficial effect of simvastatin on regression of atherosclerosis in hamsters.

b) The concentration values of all measured parameters were different at various stages of administered diet and simvastatin treatment, which means we have to do with the dynamically phenomena. Apparently, the variation with respect time is not very coherent. But it can be seen, in the frame of kinetic approach this is not the case.

c) The variations of the average values of the concentration of the group treated with simvastatin, as compared to the control group, are given in the Table 3. The corresponding variation was in the expected direction in all cases, but for TC and LDL-C is very small. It is not clear, for the moment, why it was so. One possible explanation might be owing to the fact that our group was not large enough and also not very homogeneous. Another possibility is that for TC and LDL-C the transient state may be quite different of the other components (longer for cholesterol and LDL-C), that probably may or may not be seen from the kinetic analysis.

Table 3

The variation of serum parameters concentration after treatment as compared to the control group at the moment when hamsters were sacrificed

Parameter	Concentration variation (%)	Lower/Higher
Lesions	83	Lower
TC	4	Lower
TG	17	Lower
LDC-C	2	Lower
HDL-C	30	Higher
TRAP	17	Higher
TBARS	41	Lower
ACE	8	Lower

The highest influences of simvastatin were on TBARS, HDL-C, TG, and TRAP that is in agreement with [24–25].

Proceeding to a standard statistical analysis, the correlation coefficients between drug, on the one hand, and lesions and all the seven components on the

other hand, were calculated. Also, the correlation coefficients between components themselves and each component and lesions were computed. The obtained values are given in Table 4.

Table 4

Correlation coefficients

r	Drug	TC	TG	LDL–C
Drug	1	–0.09	–0.22	–0.035
TC	–0.09	1	0.695	0.60
TG	–0.22	0.695	1	0.21
LDL–C	–0.035	0.60	0.21	1
HDL–C	0.69	–0.17	–0.17	–0.33
TRAP	0.84	–0.13	–0.195	0.07
TBARS	–0.80	0.11	0.32	–0.07
ACE	–0.22	–0.195	–0.30	–0.24
Lesions	–0.77	0.41	0.50	0.19

r	HDL–C	TRAP	TBARS	ACE	LESIONS
Drug	0.69	0.84	–0.80	–0.22	–0.77
TC	–0.17	–0.13	0.11	–0.195	0.41
TG	–0.17	–0.195	0.32	–0.30	0.50
LDL–C	–0.33	0.07	–0.07	–0.24	0.19
HDL–C	1	0.43	–0.42	–0.345	–0.56
TRAP	0.43	1	–0.76	–0.12	–0.56
TBARS	–0.42	–0.76	1	0.06	0.36
ACE	–0.345	–0.12	0.6	1	0.21
LESIONS	–0.56	–0.56	0.36	0.21	1

From Table 4, it can be seen that there is an important interaction between:

a) *Drug and lesions*. The correlation coefficient was $r_{ML} = -0.77$, which leads to a reduction of lesion extension in a dramatic fortunate way. The corresponding credibility is 98% for $p = 0.01$.

b) *All measured parameters* (TC, TG, C, HDL-C, TRAP, TBARS and ACE) *and simvastatin*. The coupling between drug, on the one hand, and cholesterol and LDL-C, on the other hand, was very small, somewhat similar with the results obtained in [24].

c) *All measured parameters and lesions*. The sign of r_{iL} was that to be expected in all cases. This interaction of parameters and lesions may give the individual contribution to increasing or lowering of lesions extension, which we believe to be an important result.

d) *The parameters coupling* (two by two). These coupling effects cannot be neglected in most of the cases.

Also, from Table 4 it is seen that:

1. TC is well correlated with LDL-C and TG, but in a wrong way with ACE.

2. TG is well correlated with all the other components, except ACE; the correlation of TG with drug and lesions is good too.

3. LDL-C is well correlated with all other parameters, except the sign with TRAP, TBARS and ACE. The correlation of LDL-C with drug is very low, somewhat similar results were obtained in [25].

4. All the other parameters are well correlated among them, except ACE with TC, TG and LDL-C. Also the correlation of LDL-C with TRAP and TBARS and of ACE with TBARS is very low.

Since our experimental data show that there is a time dependence for all studied parameters and also an important coupling among them, in order to find an explanation to these effects, we imagined a *bio-chemical model* composed by components (TC, TG, LDL-C, HDL-C, TRAP, TBARS, ACE in the initial state), diet D and drug M as *reactants* and the resulted value of the above parameters (after diet only or diet and treatment with simvastatin) as *products* (relations (1)–(3)).

Then, the time variation of all involved concentrations, in a near-equilibrium (linear) approximation is described by the coupled set of equation (4), with coupling coefficients given by relations (5) and (6).

For some values of the rate constants the solutions of equations (4) can be written in the form (18) and/or (19). Both these type of solutions may explain the experimental data. In particular, the solution (19) (with empirically determined coefficients) fits quite well the experimental behavior of the concentration during the three months period (see Table 6).

Also, the Table 6 contains two important results:

a) After one or two weeks of hyperlipemic diet, the variation of hamster serum parameters is significant. For example, for TC and TG the concentrations are about two, three times higher than the corresponding values for $t = 0$. Unfortunately, in the range of 1 to 4 weeks we cannot give very relevant experimental values since all the above components were measured sporadically, but for sure they are larger than at $t = 0$.

b) For $t \geq 16$ weeks (8 weeks for treatment in our case) the all seven serum parameters become constant, with a higher precision which points out that the stationary state was reached. Both these results are in good agreement with the measured parameters. Also, at $t = 0$ the calculated values are equal with the experimental ones as results of initial conditions.

However, from equations (4), (5) and (6) we obtained the equation (7) (the equation of the mass conservation in the process of interaction) and by integration the expression (11), *i.e.* the total concentration of serum parameters plus that of diet

Table 5

The values of constants a_i, b_i, c_i, α_i, t_m and $\bar{X}_i$ entering in the solution of eq. (19) as described in the text (the units are the same as in Table 1)

	$\bar{X}_i$	t_m	a_i	b_i	c_i	α_i	Observations
TC	401	10	–318	43.35	0.50	0.322	
TG	340	8	–240	28.00	0.34	0.935	
LDL–C	274	10	–240	25.00	0.376	0.683	
HDL–C	78.55	12	–36.35	6.49	0.057	0.158	
TRAP	741	10	+217	–28.80	–0.34	0.039	
TBARS	7.38	10	–0.70	0.14	0.001	0.200	
ACE	64.5	10	–10.4	1.70	0.016	0.246	

Table 6

The calculated values of serum parameters $X_i(t)$, from relation (19), using Table 5, and compared with the average available experimental data of Table 1 (the units are the same as in Table 1)

Serum parameter	t	$X_{i,\mathrm{calc}}(t)$	$X_{i,\mathrm{exp}}(t)$	$\varepsilon = \frac{X_{i,\mathrm{calc}} - X_{i,\mathrm{exp}}(t)}{X_{i,\mathrm{exp}}(t)} \times 100$	Observations
TC	1	202	–	–	$X_{i,exp}$ not available
	2	281	–	–	$X_{i,exp}$ not available
	4	363	252*)	+44.0	*) The value 252 is for the control group
	8	406	395	+2.8	
	10	408	437	–7.0	
	12	407	388	+4.9	
	16	403	401**)	+0.4	**) The value 401 is the time average value
	20	402	401**)	+0.25	
TG	1	265.5	–	–	$X_{i,exp}$ not available
	2	312	–	–	$X_{i,exp}$ not available
	4	316	303*)	4.3	*) The value 303 is for the control group
	8	340	351	–3	
	10	340	313	+8.7	
	12	340	325	+4.6	
	16	340	340**)	0	**) The value 340 is the time average value
	20	340	340**)	0	
LDL–C	1	165.6	–	–	$X_{i,exp}$ not available
	2	226	–	–	$X_{i,exp}$ not available
	4	273	–	–	$X_{i,exp}$ not available
	8	274	280	–2	
	10	274	299	–8.4	
	12	274	225	+22	
	16	274	274**)	0	**) The value 274 is the time average value
	20	274	274**)	0	

(continues)

Table 6 (continued)

Serum parameter	t	$X_{i,\mathrm{calc}}(t)$	$X_{i,\mathrm{exp}}(t)$	$\varepsilon = \frac{X_{i,\mathrm{calc}} - X_{i,\mathrm{exp}}(t)}{X_{i,\mathrm{exp}}(t)} \times 100$	Observations
HDL–C	1	53.1	–	–	$X_{i,exp}$ not available
	2	61.7	–	–	$X_{i,exp}$ not available
	4	73.5	–	–	$X_{i,exp}$ not available
	8	84.0	71.90	+16.8	
	10	85.6	81.10	+5.5	
	12	86.0	102.60	–16.2	
	16	85.0	78.55**)	+8.3	**) The value 78.55 is the time average value
	20	83.5	78.55**)	+6.3	
TRAP	1	875	–	–	$X_{i,exp}$ not available
	2	821	–	–	$X_{i,exp}$ not available
	4	766	817*)	–6.2	*) The value 817 is for the control group
	8	739	744.5	–0.7	
	10	737.5	721	+2.3	
	12	738	748.5	–1.4	
	16	739.5	741**)	–0.2	**) The value 741 is the time average value
	20	740.5	741**)	–0.0	

at $t = 0$, for all parameters, because of initial conditions (20), $X_{i,calc} = X_{i,exp}$ and they are given only in the Table 1

Table 6 (continued)

Serum parameter	t	$X_{i,\mathrm{calc}}(t)$	$X_{i,\mathrm{exp}}(t)$	$\varepsilon = \frac{X_{i,\mathrm{calc}} - X_{i,\mathrm{exp}}(t)}{X_{i,\mathrm{exp}}(t)} \times 100$	Observations
TBARS	1	6.92	–	–	$X_{i,exp}$ not available
	2	7.10	–	–	$X_{i,exp}$ not available
	4	7.325	7.24*)	+1.2	*) The value 7.24 is for the control group
	8	7.48	7.06	+5.95	
	10	7.49	8.09	–7.4	
	12	7.48	7.93	–5.6	
	16	7.45	7.38**)	+1.0	**) The value 7.38 is the time average value
	20	7.43	7.38**)	+0.6	
ACE	1	57.71	–	–	$X_{i,exp}$ not available
	2	60.26	–	–	$X_{i,exp}$ not available
	4	63.25	–	–	$X_{i,exp}$ not available
	8	64.70	63.89	+1.3	
	10	65.20	75.45	–14	
	12	65.14	55.73	+17	
	16	64.91	64.50**)	+0.6	**) The value 64.50 is the time average value
	20	64.72	64.50**)	+0.3	

and drug is constant. Also, for homogeneous group of animals we obtained the relation (13).

From eq. (13), making use of experimental values, at the final moment t_f, one can estimate X_M; we obtained $X_M = 0.60$ mmol/L, which is about 87% of daily administrate simvastatin. This assessed value of X_M should be compared to the concentration of simvastatin in the liver (over 80%). The agreement seems to be satisfactory, in spite of the linear approach used in this work.

Anyway, in view of this results and solution (19), the linear kinetic approach appears to be a valid description of transient phenomena. By using the same *biochemical picture* and near-equilibrium thermodynamics, we wrote the production of the entropy relations (22)–(25), which satisfies the Onsager and Prigogine principles.

From our analysis, at this stage of the study, it was obtained an important result, namely, the entropy, which after the constant diet and drug administration was smaller for the treated group than for the control one. This means that the *degree of order* is higher for healthy animals. This is a normal result and is very well correlated with the extension of lesions for the two groups.

At this point is useful to mention that our results, both experimental and theoretical, are in good agreement with those of [24–31].

As a final conclusion, it can be said that simvastatin has a good effect on atherosclerosis in HH-hamsters by regress of atherosclerotic lesions and improving the serum parameters. The near-equilibrium kinetic theory is quite satisfactory description of inside hamster's processes. However, from theoretical point of view, a more realistic picture of thermodynamics and kinetic processes, which take place inside hamsters in the course of diet and treatment, is needed. Namely, we have to consider a non-linear approach as was done in [32]. The last remark is that: if F-test is done, the main effect of simvastatin is reflected on the lesion, TRAP, TBARS and HDL, *i.e.* one confirms it is a good anti-oxidant.

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