Effect of Ascorbic Acid on Serum Cholesterol Levels and on Die-Away Curves of ^{14}C-4-Cholesterol in Baboons

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SUMMARY

Fourteen young male baboons (*Papio ursinus*) were divided into two groups. All the animals received the same dietary regimen during a 2½-month adaptation period. During the next 3 months one group received 250 mg and the other 20 mg vitamin C daily. For the last 2½ months of the experiment no vitamin C was given to the first group, and that of the second group was increased to 350 mg daily. Simultaneously with the switch-over, ^{14}C-4-cholesterol was administered. A classical two-pool system for the kinetic behaviour of cholesterol in the body was confirmed. Vitamin C treatment did not alter the serum cholesterol levels significantly, but the production rate was repressed. It was also shown that vitamin C was depleted from the body in a typical two-pool fashion.

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Myasnikova[1] was the first to show that vitamin C had the ability to influence serum cholesterol levels of patients. She observed that the intravenous administration of high doses of vitamin C to patients with high levels of serum cholesterol resulted in a distinct decrease, whereas in patients with low values it produced increased serum cholesterol levels.

Tyapina[2] studied the influence of 500-mg intravenous doses of vitamin C on the cholesterol levels of patients suffering from hypertension and atherosclerosis. She observed a hypercholesterolaemic effect within a few hours and a decrease in blood cholesterol after 10 days.

Spittle[3] made the chance observation that she could vary her own serum cholesterol level by means of her vitamin C intake. She reported that the serum cholesterol levels of healthy people under 25 years of age fell after dietary administration of 1 g of vitamin C per day. The same treatment of atherosclerotic patients showed a rise in serum cholesterol, which she attributed to mobilisation of arterial cholesterol by the dietary vitamin C. In an attempt to repeat these findings, Anderson *et al.*[4] found that in 41 subjects aged 18 - 24 years, vitamin C caused an increase in serum cholesterol rather than a decrease.

In a review Ginter[5] discussed the normalising effect of vitamin C on serum cholesterol, but also pointed out that too often methodological errors were committed. A common fault was the administration of vitamin C together with therapeutic measures which resulted in reactions falsely ascribed to vitamin C administration.

Schaffer[6] argued strongly in favour of the preventative effect that vitamin C has on the development of atherosclerosis. Bronte-Stewart *et al.*[7] found that in vitamin C-deficient patients, fats known to elevate serum cholesterol under normal conditions failed to produce a response in serum cholesterol levels. Administration of vitamin C caused an increase in serum cholesterol values. Oral administration of vitamin C resulted in a faster increase in serum cholesterol than intramuscular administration. They also reported that vitamin C failed to influence the level of serum cholesterol of healthy individuals.

Chronic ascorbic acid deficiency lowered catabolism of cholesterol in guinea pigs.[8] Cholesterol-fed guinea pigs also had a raised vitamin C consumption.[9]

It is known that baboons, like other primates, need dietary vitamin C, and therefore appear to be appropriate research models in experimentation regarding vitamin C and cholesterol metabolism. Previous studies have indicated spontaneous intimal lesions in free-living baboons.[10] We also found that during the initial stress of captivity, known to increase serum cholesterol,[11] oral administration of vitamin C tended to lower serum cholesterol.[12]

It was decided to study the effect of dietary vitamin C on serum cholesterol levels and the die-away curves of ^{14}C-4-cholesterol from the serum pool of young male baboons (*Papio ursinus*) in an effort to resolve the controversy regarding the interaction between vitamin C and cholesterol metabolism.

MATERIALS AND METHODS

In order to vary the serum vitamin C levels and to determine whether this might influence serum cholesterol

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levels, 14 young male baboons were kept on diets[11] which varied only in their ascorbic acid content.

All baboons were first kept on 1 - 2 mg vitamin C/kg body weight/day for 2½ months to adapt to captivity (period 1).

After this period 7 baboons were kept on 20 mg vitamin C/day and the other 7 received 250 mg vitamin C/day for another 3 months (period 2). Subsequently the animals receiving 20 mg/day were given 350 mg/day, whereas the other 7 baboons receiving 250 mg vitamin C/day until then were put on a diet depleted of vitamin C (period 3). The average body weight of the baboons was 13,0 kg, and varied from 7 to 16,5 kg.

Simultaneously with the last 2½ months' treatment ^{14}C-4-cholesterol (16,8 μCi) (Amersham, England) in a physiological saline ethanol solution was injected intravenously into each baboon. Blood samples obtained by venepuncture were allowed to clot, and serum samples were collected after separation by centrifugation in the cold. Radioactive determinations in the serum were done by liquid scintillation (Instagel, Packard) counting, and estimations by the tomatine method showed that more than 95% of the radioactivity resided in cholesterol.

Cholesterol was determined by the method of Zak and Ressler.[14] Vitamin C was determined by the method of Roe and Kuether[15] as modified by Schaffert and Kingsley.[16]

The calculation of the half-life values for the cholesterol pools ($t_{\frac{1}{2}}$), the pool sizes (M_A) and the kinetic constants, were done according to the method of Goodman and Noble.[17]

RESULTS AND DISCUSSION

The 14 baboons were kept on a standard diet[11] supplemented with fruit and vegetables to give an intake of about 1 - 2 mg vitamin C/kg body weight/day during the period of adjustment to their new environment. During the initial adjustment period of 2½ months (period 1) the vitamin C requirement apparently increased, as the serum vitamin C level decreased from an average value of 1,45 mg/100 ml to 0,79 mg/100 ml (Table I). Day[18] reported the requirement of vitamin C for another primate, the Rhesus monkey, to be 2 mg/kg body mass/day.

While the vitamin C requirement increased during the adjustment period, a simultaneous significant increase in serum cholesterol was observed, from an initial value of 100 mg/100 ml to 124 mg/100 ml. St Clair *et al.*[19] reported considerable increases in the serum cholesterol of squirrel monkeys during transportation and acclimatisation.

During the next 3 months 7 baboons received 250 mg vitamin C/kg/day while the control group received about 1 - 2 mg vitamin C/kg/day (period 2). At the average intake level of 19 mg/kg/day the serum vitamin C level rose to the initial value of the free-living baboons. With the average intake of 2 mg vitamin C/kg/day of the control group during period 2 the serum level eventually dropped to 0,51 mg/100 ml. The oral administration of an average of 19 mg vitamin C/kg/day did not cause any significant change of the average serum cholesterol of the 7 treated baboons when compared with the 2 mg/kg/day of the control group (Table I, period 2).

The regimens for the two groups of baboons were changed during period 3 so that the previous control group now received an average of 27 mg vitamin C/kg body weight/day, whereas the group previously receiving 19 mg vitamin C/kg body weight/day now received no vitamin C. As could be expected, the serum vitamin C level of the 7 baboons receiving 27 mg vitamin C/kg/day rose dramatically to 1,22 mg/100 ml. The withdrawal of vitamin C caused a sharp drop in serum vitamin C levels. If a semilogarithmic plot is drawn of the serum vitamin C levels against time, our data comply with a classical two-pool system for vitamin C in the body.

Since we did not use radio-isotope tracers, calculation of all the parameters of a two-pool system is not permissible. We can, however, calculate the $t_{\frac{1}{2}}$ values of the two pools. The $t_{\frac{1}{2}}$ value of the first fast turning-over pool is 7 days, with the $t_{\frac{1}{2}}$ value of the second slower turning-over pool 156 days. Burns[20] reported $t_{\frac{1}{2}}$ values of 15 and 3 days for man and guinea pigs, respectively. The $t_{\frac{1}{2}}$ value of 7 days for the pool falls well within

TABLE I. SERUM CHOLESTEROL AND VITAMIN C LEVELS OF 14 YOUNG MALE BABOONS TREATED WITH DIFFERENT DIETARY LEVELS OF VITAMIN C

		Vitamin C treatment																	
		Period 1 (1 - 2 mg/day)						Period 2 (250 mg/day)							Period 3 (0 mg/day)				
	Date	27/2	14/3	29/3	10/4	24/4	8/5	22/5	7/6	19/6	3/7	17/7	1/8	10/8	30/8	11/9	26/9	11/10	23/10
Vitamin C	Aver.	1,46	0,58	0,72	0,67	0,73	0,78	1,35	1,12	1,26	1,28	1,48	1,48	1,37	0,43	0,27	0,26	0,24	0,18
	SD	0,30	0,17	0,20	0,18	0,10	0,13	0,13	0,16	0,19	0,16	0,12	0,16	0,12	0,08	0,02	0,03	0,02	0,05
		1 - 2 mg/day						20 mg/day							350 mg/day				
	Aver.	1,44	0,73	0,73	0,54	0,76	0,79	0,56	0,55	0,55	0,64	0,69	0,57	0,51	1,22	1,22	1,29	1,35	1,27
	SD	0,21	0,18	0,15	0,19	0,12	0,18	0,12	0,16	0,10	0,21	0,19	0,19	0,07	0,14	0,14	0,20	0,30	0,10
		1 - 2 mg/day						250 mg/day							0 mg/day				
Cholesterol	Aver.	94	108	112	117	107	116	125	119	117	129	119	128	147	130	128	119	96	85
	SD	13	15	21	12	8	11	16	15	5	23	10	8	20	14	11	17	13	12
		1 - 2 mg/day						20 mg/day							350 mg/day				
	Aver.	106	119	125	127	121	131	126	133	127	128	139	125	141	131	132	129	109	96
	SD	27	11	12	21	12	6	10	14	18	36	26	9	25	12	30	17	7	9

this range. If only one kinetic vitamin C pool were present in the body, our young baboons with an average vitamin C level of about 1,4 mg/dl would have a serum level of less than 0,1 mg/dl after 28 days of no vitamin C intake. At this level clinical scurvy would normally arise. In another experiment, however, we observed that baboons receiving no vitamin C for 8 months did still not show any clinical signs of scurvy. Our observations therefore substantiate the existence of a second slower turning-over pool for vitamin C.

The average serum cholesterol of the 7 baboons receiving no dietary vitamin C decreased faster (62 mg/100 ml total) in 74 days (period 3) than that of the 7 baboons receiving 27 mg/kg/day (45 mg/100 ml total) during the same period, although the difference did not seem to be significant.

Cholesterol labelled with ^{14}C in position 4 was injected intravenously during the last dietary switchover of the vitamin C regimen (period 3). By assuming a two-pool system for cholesterol the average values of the specific radioactivity of cholesterol were plotted semilogarithmically against time. The data obtained conformed with a two-pool system for body cholesterol.[17] From these plots all the relevant parameters were calculated (Table II).

The $t\frac{1}{2}$ values of the first pool of the two groups of baboons differed only slightly. A $t\frac{1}{2}$ value of 8,5 days was found for baboons receiving no vitamin C, compared with 9,5 days for baboons treated with vitamin C. The size of the first pool, M_A, can be calculated with a reasonable degree of certainty. It was indicated that the size of the first pool M_A of the vitamin C-treated animals was slightly larger than that of the baboons receiving no vitamin C, namely 10,61 g versus 9,95 g. The first pool normally comprises the free cholesterol of serum, cholesterol esters of serum, red blood cell cholesterol and liver cholesterol.[21-23] It is also likely that cholesterol from the spleen, kidney, lung and intestines equilibrates sufficiently fast to comprise part of pool M_A.[22]

TABLE II. AVERAGE KINETIC PARAMETERS CALCULATED AFTER INTRAVENOUS INJECTION OF ^{14}C-4-CHOLESTEROL INTO 7 YOUNG MALE BABOONS WITH DIETARY DEPLETION OF VITAMIN C AND 7 YOUNG MALE BABOONS RECEIVING 27 MG VITAMIN C/KG/DAY

Parameters	−Vitamin C	+ Vitamin
$t\frac{1}{2}_A$	9,5 days	8,5 days
$t\frac{1}{2}_B$	69,0 days	85,5 days
C_A	2 413 dpm/mg	1 752 dpm/mg
C_B	1 310 dpm/mg	1 730 dpm/mg
α	0,0793	0,0815
β	0,0105	0,00811
M_A	9,95 g	10,61 g
PR_A	300 mg/day	172 mg/day
k_{AA}	−0,055	−0,045
k_{BB}	−0,035	−0,045
K	0,728	0,869

$t\frac{1}{2}_A$ = half-life of first pool A; $t\frac{1}{2}_B$ = half-life of second pool B; C_A and C_B = constants; $\alpha = \frac{0{,}693}{t\frac{1}{2}_A}$, $\beta = \frac{0{,}693}{t\frac{1}{2}_B}$; M_A = the size of pool A = $\frac{R_A}{C_A + C_B}$;

$k_{AA} = \frac{-\alpha M_A C_A - \beta M_A C_B}{R_A}$; $k_{BB} = -(\alpha + \beta + k_{AA})$ = total rate of removal of cholesterol from pool B.

$PR_A = \frac{R_A \alpha \beta}{\alpha C_B + \beta C_A}$ = production rate of cholesterol in pool A.

$k = \frac{R_A}{1{,}44 . t\frac{1}{2}}$ = turnover rate.

R_A = amount of isotope injected into pool A.

Our findings on the $t\frac{1}{2}$ values and size of the first pool M_A for the two groups of baboons indicate that vitamin C treatment increases the turnover of this pool. Our estimates for the pool size of 9,95 - 10,61 g for the first pool M_A, per baboon of 13,2 kg body weight, was higher than 6,7 g reported by Wilson[24] for baboons (23,2 kg), and also higher than the 0,309 - 1,843 g of the much smaller squirrel monkeys.[25]

The $t\frac{1}{2}$ values of the second pool were significantly higher for the vitamin C-treated baboons (85,5 days) than for the vitamin C-deficient animals (69,0 days). These values were also considerably higher than the 23 - 37 days reported for baboons by Wilson,[24] but were more within the range of the 35 - 67 days reported by Eggen *et al.*[26]

Vitamin C apparently depressed the production rate (PR_A) of cholesterol of the first pool, A, from 300 mg/day of the control group to 172 mg/day for the vitamin C-treated group. Wilson found the PR_A of baboon cholesterol to be 629 mg/day, which was more than twice as high as the values reported here. The turnover rate, k, for the baboons receiving vitamin C was also 19% higher than that of the control group.

Kritchevsky[27] reported half-life values for baboon cholesterol between 31 and 51 days, which is shorter than the values reported for the second pool, B, in this article. They calculated the daily synthesis rate of cholesterol as 47 mg/kg/day, which would be equivalent to 611 mg/day/baboon in our experiment, which would be more than twice as high as the values reported here.

Our results seem to indicate that vitamin C represses the *in vivo* cholesterol synthesis and increases the turnover rate of the fast miscible pool, but decreases the rate of removal from the slow miscible pool.

CONCLUSIONS

High oral doses of vitamin C did not significantly lower the serum cholesterol levels in baboons. Withdrawal of all dietary vitamin C did not result in a concurrent rise in serum cholesterol.

Body cholesterol of baboons is metabolised according to a two-pool system, as in man. A similar two-pool system was found for body vitamin C.

Vitamin C repressed the production rate (PR_A) of cholesterol *in vivo* and increased the turnover rate, k, of the fast miscible pool, but decreased the rate of removal from the slow miscible pool.

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