CCII. VITAMIN C REQUIREMENTS OF THE GUINEA-PIG.

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VAN EEKELEN *et al.* [1933], Hess & Benjamin [1934] and Johnson & Zilva [1934] have shown that human beings do not excrete ascorbic acid or excrete it only to a limited extent when it is ingested by them unless they are fully "saturated" with the vitamin. The last-named workers were at the same time able to show by means of detailed quantitative experiments that certain individuals may be so depleted of the vitamin as to take a considerable time to reach the condition of saturation when consuming daily doses of 100–150 mg. of vitamin C. Yet such individuals not only fail to display any symptoms even of latent scurvy but appear to be in perfect bodily and mental vigour. Similar results have since been obtained by a number of workers. Some of these are, however, inclined to attribute to this lack of vitamin C "saturation" a clinical significance which in the opinion of the writer is not warranted by the existing knowledge of the subject. Experiments on guinea-pigs are given below which throw some further light on the matter.

The chief aim of this investigation was to ascertain the relationship of the intake of ascorbic acid to the vitamin C content of the "selective" organs and other tissues of these animals, to their susceptibility to scurvy, to the urinary excretion of ascorbic acid and to their general well-being.

Storage of vitamin C and susceptibility to scurvy.

That guinea-pigs of about 300 g. maintained on a good diet with cabbage *ad lib.* under favourable hygienic conditions show little individual variation in susceptibility to the disease is well known to workers in experimental scurvy. The tissues of these animals, especially the "selective" tissues such as the adrenals, anterior lobe of the pituitary, the liver, the small intestine etc. contain vitamin C in quantities which cannot be raised even by further administration of high doses of ascorbic acid either *per os* or by injection. In other words, when the tissues of growing guinea-pigs are supplied with vitamin C to their maximum capacity, the animals vary little in the time taken by them to succumb to scurvy.

The following experiments were designed in order to obtain more detailed knowledge concerning the influence of the bodily store of vitamin C in the guinea-pig on the time of survival on a scorbutic diet. For this purpose chosen animals, as used for customary vitamin C testing, were placed on the scorbutic diet employed in this laboratory. During the first 10 days the various groups received *per os* daily doses of 0.5, 1, 2, 3, 10 and 20 mg. of ascorbic acid respectively. At the end of this time the daily dose was discontinued and the animals were allowed to remain on the scorbutic diet until they succumbed. It is seen from Fig. 1 that the time taken by group 1 (daily dose 0.5 mg.) to succumb to scurvy after receiving the last dose of ascorbic acid (average time about 20 days) was

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Fig. 1. All the animals except those in group 8 died of scurvy, uncomplicated by any other disease.

less than that taken by the control group 9. The animals in group 2 also died of the deficiency earlier than the control group of animals, but the difference here is less marked than in the case of group 1. The remaining groups appear to the writer not to have shown any significant deviation in their behaviour on the deficient diet from that of the animals which consumed very generous supplies of vitamin C in the form of cabbage during the period preceding the deprivation of the vitamin. It is of interest to note that when the guinea-pigs were depleted of vitamin C previous to the period of dosage with 0.5 mg. of ascorbic acid the time taken by the animals to succumb to scurvy (group 7) was almost the same as when no previous depletion took place (group 1). Furthermore, a dose of 0.5 mg. administered daily after exhausting the animals of their vitamin C store by maintaining them on a scorbutic diet for 10 days was sufficient to prevent the production of macroscopic lesions after 37 days when the animals were killed by chloroform (group 8). The import of these results will receive comment in a subsequent section when they will be considered in conjunction with the other information obtained in this investigation.

The influence of the quantity of ascorbic acid consumed on the concentration of the vitamin in the tissues.

The original aim was to establish the minimum daily dose of ascorbic acid necessary to be consumed *per os* by the guinea-pig in order that the animal should excrete it in appreciable amounts in the urine and to correlate this dose with the concentration of the vitamin in the tissues. This could not be ascertained by direct experiment, since it was found that the guinea-pig had to consume extremely high doses of ascorbic acid before any appeared in the urine. Recourse was, therefore, made to injection after which, as has been shown already by the writer [Zilva, 1935, 1, 2], urinary excretion of the vitamin quickly follows. Analyses of the tissues of a number of guinea-pigs which received single graded injections were therefore carried out and compared with those of groups of animals receiving similar doses *per os*.

All the injections, unless otherwise stated, were performed intramuscularly (femoral muscle) without an anaesthetic. The injected animals were previously depleted of vitamin C by maintenance on a scorbutic diet for 6 days but the other groups received their dose orally for 15 days whilst subsisting on a scorbutic diet without being previously depleted. Details of the method of analysis have already been described in a previous communication [Zilva, 1935, 1].

It is seen from the figures in Table I that, considering the limitations of the method, the injection of a single dose of ascorbic acid brings about in the tissues of the guinea-pig a concentration of the vitamin similar to that found when the animal receives orally an equal dose daily for 15 days whilst on a scorbutic diet. Consequently the necessary concentration of ascorbic acid in the tissues following injection which permits its excretion in the urine can be reached by the same dose given *per os* for 15 days. It is further seen that a dose of 5–8 mg. was required by the guinea-pig on a scorbutic diet before even a trace of the vitamin could be detected in their tissues and finally that the highest concentration approximately equal to that obtained by the injection of 50 mg. was only attained when doses of about 20 mg. were employed.

Results obtained by Jacobsen [1935], Giroud *et al.* [1935], Fox & Levy [1936] and De Caro [1936], in connexion with the deposition of ascorbic acid in the tissues agree in principle with those given above. Owing, however, to the different experimental procedures adopted by these workers, especially by the former, direct comparison of the quantitative data is made difficult.

Table I.

Wt. of animal g.	Scorbutic diet days	Method of administration	Quantity found										Excreted in urine mg.	Gain in wt. g.	
			Small intestine		Large intestine		Liver		Carcass						
			Total mg./g.	mg./g.	Total mg./g.	3 mg. <i>L</i> -ascorbic acid.	mg./g.	mg./g.	Total mg./g.	Adrenal mg./g.	Total mg./g.	Pituitary			
270	6	Intramuscular injection	0.05	0.6	0.03	0.3	0.06	0.7	0.1	0.01	1.3	0 to trace	0	10	
280	6	"	0.04	0.5	0.02	0.3	0.06	0.5	0.0	0.00	0.0	"	0	20	
277	6	"	0.06	0.9	0.05	0.5	0.05	0.6	0.1	0.01	1.8	0	0	17	
282	6	"	0.07	0.8	0.02	0.2	0.06	0.7	0.0	0.01	1.0	0 to trace	0	22	
Average 277	6	—	0.06	0.7	0.04	0.3	0.06	0.6	0.1	0.01	1.0	—	0	17	
3 mg. <i>L</i> -ascorbic acid daily.															
350	15	<i>Per os</i>	0.08	1.2	0.06	0.7	0.05	0.8	0.0	0.10	2.3	0	—	70	
295	15	"	0.05	0.7	0.04	0.5	0.06	0.7	0.2	0.01	0.9	Trace	—	25	
310	15	"	0.06	0.7	0.04	0.5	0.05	0.6	0.0	0.00	0.0	+	—	45	
305	15	"	0.05	0.7	0.04	0.4	0.05	0.5	0.0	0.00	0.5	0	—	40	
Average 315	15	—	0.06	0.8	0.05	0.5	0.05	0.7	0.1	0.01	0.9	—	—	45	
5 mg. <i>L</i> -ascorbic acid.															
290	6	Intramuscular injection	0.05	0.6	0.05	0.5	0.14	1.5	0.0	0.00	0.0	0	0	25	
290	6	"	0.05	0.6	0.05	0.5	0.05	0.6	0.1	0.01	2.0	Trace	0	5	
275	6	"	0.07	1.1	0.07	0.8	0.09	0.9	0.2	0.01	1.7	0 to trace	0	10	
285	6	"	0.10	1.2	0.05	0.5	0.08	0.9	0.2	0.01	2.4	+ to +	0	18	
Average 285	6	—	0.07	0.9	0.06	0.6	0.09	1.0	0.1	0.01	1.5	—	0	15	
5 mg. <i>L</i> -ascorbic daily.															
315	15	<i>Per os</i>	0.08	1.2	0.05	0.6	0.05	0.6	0.0	0.01	1.6	Trace to +	—	50	
335	15	"	0.06	0.9	0.05	0.5	0.03	0.5	0.0	0.01	1.2	+	—	70	
310	15	"	0.07	1.0	0.04	0.4	0.05	0.6	0.3	0.01	2.1	+	—	45	
Average 320	15	—	0.07	1.0	0.05	0.5	0.04	0.6	0.1	0.01	1.6	—	—	55	

Table I (cont.).

Wt. of animal g.	Scorbutic diet days	Method of administration	Quantity found										Excreted in urine mg.	Gain in wt. g.	
			Small intestine			Large intestine			Liver			Carcass			Pituitary
			mg./g.	Total	mg./g.	Total	mg./g.	Total	mg./g.	Total					
20 mg. <i>L</i> -ascorbic acid.															
295	6	Intramuscular	0.12	1.6	0.10	1.0	0.16	1.8	0.7	0.03	5.1	+ to + +	1.6	5	
305	6	injection	0.09	1.3	0.10	1.1	0.10	1.2	0.8	0.03	4.6	+ to + + +	3.7	5	
275	6	"	0.07	0.9	0.07	0.8	0.09	0.9	0.9	0.02	3.1	+ to + + +	1.7	0	
285	6	"	0.15	2.0	0.08	0.8	0.11	1.4	0.9	0.03	4.3	+ + + +	3.6	15	
Average 290	6	—	0.11	1.5	0.09	0.9	0.12	1.3	0.8	0.03	4.3	—	2.7	6	
20 mg. <i>L</i> -ascorbic acid daily.															
340	15	<i>Per os</i>	0.18	2.9	0.11	1.2	0.20	3.0	1.0	0.05	8.1	+ +	—	60	
340	15	"	0.13	2.3	0.09	1.2	0.11	1.7	0.8	0.03	4.5	+ +	—	50	
320	15	"	0.17	2.2	0.09	0.8	0.11	1.6	0.7	0.04	6.0	+ + +	—	45	
330	15	"	0.29	3.5	0.15	1.6	0.23	3.2	1.6	0.04	5.8	+ + + to + + + +	—	45	
Average 332	15	—	0.19	2.7	0.11	1.2	0.16	2.4	1.0	0.04	6.1	—	—	50	
25 mg. <i>L</i> -ascorbic acid.															
320	6	Intramuscular	0.20	2.2	0.12	1.2	0.17	2.4	1.0	0.03	5.8	—	4.0	20	
315	6	injection	0.18	2.2	0.14	1.4	0.13	2.0	1.0	0.04	6.6	+ + + +	6.2	40	
270	6	"	0.19	2.3	0.10	0.9	0.12	1.1	0.8	0.03	4.1	+ to + + +	5.8	5	
277	6	"	0.17	1.7	0.14	1.4	0.13	1.4	0.9	0.03	4.4	+ to + + +	5.6	12	
Average 296	6	—	0.19	2.1	0.13	1.2	0.14	1.7	0.9	0.03	5.2	—	5.4	19	
50 mg. <i>L</i> -ascorbic acid.															
290	6	Intravenous injection	0.14	1.9	0.10	1.2	0.16	2.1	0.9	0.04	5.1	+ + + +	13.0	18	
272	7	—	0.04	0.6	0.02	0.3	0.07	0.9	0.1	0.00	0.0	Trace	—	10	

*The influence of the quantity of ascorbic acid in the tissues
on its excretion in the urine.*

Reference was made above to the fact that extremely high doses of ascorbic acid must be administered to guinea-pigs *per os* before it appears in the urine. This is illustrated by the following details. Guinea-pigs previously depleted of vitamin C by subsisting for 5 days on a scorbutic diet failed to excrete any ascorbic acid in the urine after receiving 9 daily doses of 50 mg. The administration of as much as 300 mg. to animals not even previously depleted failed to cause any appreciable excretion. Only when the dose was raised to 500 mg. did the vitamin appear in the urine and then in a comparatively small amount, namely 8 mg. Similarly a dose as high as 1 g. was followed by the excretion of as little as 36 mg. Euler [1935] also failed to find appreciable excretion by the guinea-pig after similar doses given *per os*.

As seen from Table I, ascorbic acid is much more readily excreted by the kidney after it is injected. It was found in fact that the vitamin began to be voided in the urine after a single injection as soon as its concentration in the tissues of the animal approached the maximum. In these experiments this was achieved when a daily dose of 20 mg. was consumed by the guinea-pigs for 15 days or after a single injection of the same dose in vitamin C-depleted animals.

It is to be noted that comparatively little ascorbic acid is excreted in the urine when 20 mg. are injected but the quantity excreted increases with the rise in the dose. The amount of the vitamin voided is, therefore, controlled not only by the concentration in the tissues but also by the size of the dose injected. If the dose is too small no significant quantities of the vitamin will appear in the urine.

Vitamin C depletion of the tissues and prolonged growth of guinea-pigs.

As a point of interest in connexion with the problem of the influence of vitamin C storage on the welfare of the guinea-pig, it is appropriate to record here the history of these animals covering the major part of their life while subsisting on vitamin C supplies which, although fully protective, do not permit of their storage in significant quantities in the tissues. A number of such guinea-pigs were examined in this connexion and a few representative cases are described here in a summarized form. All the animals to be discussed were females since the main purpose of the experiment to which they belonged necessitated the choice of this sex. The sex factor does not, however, affect the present issue. These guinea-pigs were kept on a scorbutic diet offered *ad lib.* which consisted of oats, bran, middlings, fish meal, barley meal and autoclaved full-cream milk made up from a dried powder. Besides the basal diet the animals received daily 10 ml. of decitrated lemon juice which was approximately equivalent to 5 mg. of ascorbic acid.

B 61. This guinea-pig was under observation on the above diet from 28 July 1933 to 8 November 1934. Its initial weight was 305 g. and it reached a maximum weight of 765 g. after 313 days. The animal was killed by stunning and bleeding at the end of 467 days and its tissues were examined for the presence of ascorbic acid. No significant quantities of the vitamin could be detected. Macroscopic signs of scurvy were not found at the post-mortem examination.

B 77. This guinea-pig was under observation on the above diet from 31 July 1933 to 7 July 1934. Its initial weight was 310 g. and it reached a maximum weight of 970 g. after 221 days. The animal was killed by stunning and bleeding at the end of 465 days. No significant quantities of ascorbic acid were found in

its tissues and no macroscopic signs of scurvy were found at the post-mortem examination.

B 87. This guinea-pig was under observation on the above diet from 7 September 1933 to 4 May 1936, i.e. for 969 days, when it died of pneumonia. No macroscopic signs of scurvy were found at the post-mortem examination. Its initial weight was 340 g. and it reached the maximum weight of 985 g. after 649 days.

B 99. This guinea-pig was under observation on the above diet from 15 January 1934. Its initial weight was 255 g. It is still alive and growing, having reached the weight of 875 g.

The above typical experiments show that the animals could survive for long periods and reach body weights similar to those attained by fully "saturated" guinea-pigs subsisting on a diet containing cabbage *ad lib.* without having any appreciable store of vitamin C in their tissues.

The influence of vitamin C depletion in guinea-pigs on the complement of their serum.

A number of years ago it was shown by the writer [Zilva, 1919] that guinea-pigs existing on a scorbutic diet and receiving low doses of the vitamin, insufficient to protect them from scurvy but high enough to prolong the duration of the disease for several months, when immunized with a typhoid organism yielded sera with as high amboceptor and agglutinin titres as those obtained

Table II.

Diet	Wt. of guinea pig g.	Complement dilution							
		1/12	1/16	1/24	1/32	1/48	1/64	1/96	1/128 1/192
5 days on scorbutic diet	310	4	4	3½	3	2½	2	1	trace -
" "	315	4	4	3½	3½	3	2	1	trace -
" "	275	4	4	3½	3½	3	2	1	trace -
" "	315	4	4	3½	3	2	1	trace	- -
7 days on scorbutic diet	340	4	3½	3½	3	2½	2	1	trace -
" "	270	4	4	3½	3	2½	2	1	trace -
Mixed diet	450	4	4	3½	3	2	2	1	trace -
7 days on scorbutic diet	315	4	4	3½	3½	3	2	2	trace -
Mixed diet	310	4	3½	3½	3	3	1	1	- -
7 days on scorbutic diet	342	4	4	3½	3	2½	2	trace	trace -
Mixed diet	320	4	4	4	3½	3	2	2	1 -
10 days on scorbutic diet	310	4	3½	3	3	2½	2	1	- -
Mixed diet	315	4	3½	3	3	2	1	1	- -
10 days on scorbutic diet	350	3½	3½	3½	3	3	2	2	1 -
Mixed diet	305	4	4	3½	3	2½	2	1½	trace -
10 days on scorbutic diet	335	4	3½	3	2½	2	1	trace	- -
Mixed diet	335	4	3½	3	3	2	1	trace	trace -
19 days on scorbutic diet	380	4	4	3½	2½	2	1	trace	- -
" "	330	4	4	3½	3	2½	1	trace	- -
Mixed diet	310	4	3½	3	2½	2	1	trace	- -
20 days on scorbutic diet	320	3½	3	2	1	trace	-	-	- -
" "	325	4	3½	3	2	1½	1	trace	- -
Mixed diet	305	4	3½	2	1½	1	trace	trace	- -
7 days on scorbutic diet	440	4	3½	2	1½	1	trace	-	- -
" "	470	4	3½	2	1½	1	trace	-	- -
Mixed diet	440	4	4	2	1½	1	1	-	- -
7 days on scorbutic diet	425	4	3½	3	2½	2	1	trace	- -
" "	450	4	3½	3	2½	2	1	trace	- -
Mixed diet	450	4	3½	3	2½	2	1	trace	- -

from animals receiving cabbage *ad lib*. Nor was there any diminution in the complement activity of the blood of these animals subsisting on the restricted diet for prolonged periods. Recently Marsh [1936] asserted that in guinea-pigs depleted of vitamin C for 7 days or less the complement, as determined by a standardized haemolytic serum, either disappeared or suffered reduction. No experimental details were given.

Table II gives data of complement titrations obtained with sera from guinea-pigs which have been deprived of vitamin C for 5, 7, 10, 19 and 20 days respectively. It is seen, as would have been expected from the writer's earlier work, that no significant difference between the titres of the sera of these animals and those of control animals receiving a mixed diet with cabbage *ad lib*. could be observed.

I am indebted to Dr D. B. Steabben for checking the readings of the above titrations.

DISCUSSION.

The minimum prophylactic daily dose which is capable of protecting growing guinea-pigs of about 300 g. subsisting on a vitamin C-deficient diet from macroscopic scorbutic lesions lies in the region of 1 mg. [Zilva, 1932; Hirst & Zilva, 1933; and others] and according to Goettsch [1928] and others about double this quantity is required to obviate the development of microscopic lesions in the pulp of the teeth of these animals. The above results show that only traces of the vitamin are found in the "selective" tissues of guinea-pigs, when a daily dose of 5-8 mg.—a dose 2-3 times as great as that which prevents the production of microscopic lesions—is given for 15 days. Furthermore, to attain the maximum concentration in the tissues such as is obtained by the injection of 50 mg. of ascorbic acid in a vitamin C-depleted animal, ten times the protective dose of 2 mg. is necessary. It was found in addition that on administering the protective dose (2 mg.) to guinea-pigs on a scorbutic diet for 10 days, the time taken by these animals to succumb to the disease was not significantly less than that taken by guinea-pigs which received a daily dose of 20 mg. during the preliminary period and even by guinea-pigs which consumed cabbage *ad lib*. previous to being placed on a deficient diet. Yet in both the latter groups the concentration of ascorbic acid in the tissues was at a maximum before the vitamin was withheld from the diet. The accumulated vitamin C in the body of the guinea-pig therefore does not act as a store in the true sense of the word.

The difference between the dose necessary fully to protect the guinea-pig from scurvy and the one which brings about the maximum "saturation" of its tissues with the consequent elimination of a part of it in the urine, is therefore great. No evidence could, however, be obtained which indicated that guinea-pigs receiving daily doses ranging from the minimum protective dose to that necessary to bring about the "saturation" of their tissues with the vitamin, or even to that necessary for discrete traces of ascorbic acid to appear in the "selective" tissues, deviated from their "saturated" fellows in any discernible feature. If guinea-pigs in this zone of "unsaturation" are in a condition inimical to their well-being it is of a character too subtle to be detected by the methods employed in this investigation. Slight microscopic changes in the teeth of guinea-pigs can be observed after they have subsisted for 7-10 days on a scorbutic diet [Zilva & Wells, 1919], by which time we now know that their tissues have lost the best part of their vitamin C, but as was seen tissues of animals receiving doses capable of preventing these microscopic changes contain ascorbic acid in minimum amounts and consequently very marked depletion of

guinea-pig tissues of vitamin C is no indication of the presence of even these microscopic lesions.

Great caution must be exercised in interpreting the bearing of the above results on human beings. Obviously systematic experiments such as those described cannot be carried out on man. The only information available in this respect are data recorded by a number of workers in connexion with the urinary excretion of ascorbic acid—results which are not easy to correlate. The comparison is made difficult by the fact that in determining the balance between the intake and output of the vitamin, the amount of ascorbic acid not accounted for varies with the magnitude of the ingested dose, the experimental subject and even with the same individual at different times under similar dietary conditions [cf. Johnson & Zilva, 1934]. Nevertheless, from the available evidence it may be concluded in general that in human beings suffering from declared scurvy the degree of “saturation” with vitamin C¹ is lower than that of the most “unsaturated” subjects who are free from the disease and in good and apparently normal health. This fact emerges particularly from the investigations of Schultzer [1936] and Johnson & Zilva [1934]. The observations of Archer & Graham [1936] in the writer’s opinion also point in the same direction. There is so far, however, no evidence, clinical or laboratory, that such “unsaturated” subjects are in a worse state of health than “saturated” persons. The present investigation carried out on animals more susceptible to scurvy than man also failed to disclose any significant departures of the one type from the other. It was in fact found that “unsaturated” guinea-pigs receiving daily doses of vitamin C just sufficient to prevent the development of macroscopic and microscopic scorbutic lesions succumbed to the disease in almost the same time after the daily dose was discontinued as did the fully “saturated” guinea-pigs. It is also significant to note in this connexion that Schultzer’s patients remained “unsaturated” for a considerable time after the disappearance of the scorbutic symptoms, in spite of receiving such high doses as 600 mg. per day. There seems therefore to be in human subjects also a wide margin of “unsaturation” which has no obvious detrimental effect on the health of the individual. To apply to this zone of “unsaturation” such expressions as “hypovitaminosis C”, “latent or potential scurvy”, “vitamin C sub-nutrition”, “subacute scurvy” etc.—expressions which imply ill-defined pathological conditions—is not justified by our present state of knowledge. Even if some subtle inimical change be associated with this particular state of “unsaturation” the use of such expressions can only tend to obscure the issue.

It is appropriate to add that the failure on the part of an “unsaturated” subject to excrete ascorbic acid in the urine after the administration of a single dose, even a high one, does not yield any information as to the degree of his “unsaturation”.

SUMMARY.

An oral dose of about 500 mg. of ascorbic acid is necessary for growing guinea-pigs (of about 300 g.) subsisting on a mixed diet before the vitamin is excreted in the urine. In contrast to this, excretion is brought about by the injection of a very much lower dose.

Graded doses of ascorbic acid were injected into vitamin C-depleted guinea-pigs and it was found that demonstrable traces of the vitamin appeared in the

¹ The expression “saturation” is used in the sense defined by Johnson & Zilva, i.e. an animal is said to be “saturated” when upon ingesting a constant daily amount of ascorbic acid it excretes a more or less constant daily amount of the vitamin in the urine.

tissues only when a dose between 5 and 8 mg. was employed. The injection of 20 mg. of the acid produced a concentration in the tissues equal to that observed when 50 mg. were injected and this was considered the maximum concentration attainable under the experimental conditions described. Similar results were obtained when corresponding doses were administered *per os* for 15 days to guinea-pigs on a scorbutic diet. Ascorbic acid first appeared in the urine when 20 mg. were injected, i.e. when the maximum concentration of the vitamin in the tissues was reached. As the dose was increased the amount of ascorbic acid excreted also rose.

The time taken by guinea-pigs to succumb on a scorbutic diet after having previously received for 15 days whilst on this diet 2 mg. of ascorbic acid (the protective dose) daily was about the same as that taken by animals which were offered cabbage *ad lib.* during the preliminary period. Previous "saturation" of the tissues therefore does not delay the fatal termination of the disease.

Guinea-pigs on a scorbutic diet receiving daily doses of 10 ml. of decitrated lemon juice (about 5 mg. of ascorbic acid) were observed to live for a number of years during which time they attained very high weights, although the tissues of such animals contained only minimum amounts of vitamin C.

The complement activity of guinea-pig blood suffers no reduction when the animals are depleted of vitamin C or even when they are in a scorbutic condition.

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