The retention of ascorbic acid by guinea-pig tissues

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(*Received* 12 *November* 1970 – *Accepted* 10 *May* 1971)

1. Male and female guinea-pigs with high tissue concentrations of L-xyloascorbic acid (ascorbic acid, AA) and male guinea-pigs with high tissue concentrations of D-araboascorbic acid (isoascorbic acid, isoAA) were given a scorbutogenic diet and the rates of depletion from various tissues were measured for both isomers.

2. The loss of AA from the brain and eye lens was considerably less rapid than the loss from the adrenal glands, spleen and aqueous humour. After 14 d on the AA-free diet the AA concentrations in the brain and eye lens were 24 and 27% respectively of the initial concentrations; the corresponding values for the aqueous humour, adrenal glands and spleen were 3, 4 and 5%. There was no apparent sex difference in the rate of loss of AA.

3. The loss of isoAA was much more rapid than that of AA in the spleen, adrenal glands and aqueous humour; in the brain and eye lens the depletion patterns of the two isomers were similar.

The wide range in the concentration of L-xyloascorbic acid (vitamin C, AA) in guinea-pig tissues has long been recognized. Over a broad range of dietary intakes organs such as the adrenal glands and spleen have concentrations many times that of others such as the kidney and aorta (Penney & Zilva, 1946; Hughes, Hurley & Jones, unpublished observations).

Tissue concentrations of AA will be influenced by both the rate of uptake (or 'fixation') of AA and the 'retention capacity' of the tissue for AA.

The factors that influence the passage of AA across biological membranes and its deposition in the tissues have been characterized in some detail (Raiha, 1958; Hughes & Maton, 1968; Hughes & Hurley, 1969). Much less, however, is known of the 'retention' mechanism, and the factors that are determinants for the rate of loss of AA from tissues. Penney & Zilva (1946) reported that all the organs examined by them conformed to the same basic depletion pattern. The experiments described below were designed to extend the original observations of Penney & Zilva by examining two organs not included in their survey, namely the brain and eye lens. This was felt to be of interest because of recent indications that both these organs have an anomalous pattern of AA retention (Hughes & Jones, 1971). Furthermore, there are suggestions that in both the eye lens and the brain AA has important biochemical involvements (Heath, 1962; Pauling, 1968). Depletion studies with D-araboascorbic acid (isoascorbic acid, isoAA) were included in order to assess the biochemical specificity of the retention mechanism.

EXPERIMENTAL

Animals and diet

Albino guinea-pigs, initial body-weight 300 g, were used. They received the semi-synthetic pelleted scorbutogenic diet previously described (Hughes & Hurley, 1969) and were housed in groups of five in galvanized zinc cages.

Administration and determination of ascorbic acids

The guinea-pigs received the unsupplemented scorbutogenic diet for 10 d to deplete the tissues of the greater part of the AA already present. On the 11th day, and for a further 14 d, AA or isoAA was given daily as a 1 % supplement in the drinking water – the only satisfactory method of producing high tissue concentrations of isoAA (Hughes & Jones, 1970). The supplemented water was replaced by a fresh supply daily. Supplementation ceased on the 25th day (= day 0 of the depletion period). On each of days 0, 2, 6, 9, 14 and 20 of the depletion period and, because of the rapid depletion, on day 4 in the isoAA group, five animals were killed and the organ concentrations of AA (or isoAA) were measured. Killing was by stunning followed by decapitation and exsanguination; selected organs were rapidly dissected out, dried between filter paper and weighed and the AA content was determined.

The 2,6-dichlorophenolindophenol method was used for the determination of the ascorbic acids in the adrenal glands, spleen and brain (Bessey, 1938; Hughes, 1956) and the 2,4-dinitrophenylhydrazine method for their determination in the aqueous humour and lens (Raiha, 1958; Hughes, Hurley & Jones, 1971). Recovery tests had previously indicated that in both these methods AA and isoAA were quantitatively equivalent.

Plan of experiment

Three groups of thirty guinea-pigs were used. Groups 1 (males) and 2 (females) received AA and group 3 (males) received isoAA. The group of female animals was included to compare the depletion rates in the two sexes; there is evidence of a sex difference in the concentration of AA in certain tissues (Brook & Grimshaw, 1968; Hughes & Jones, 1971).

RESULTS

The depletion curves for the three groups are given in Fig. 1, the $\log_{10}$ of the tissue concentration being plotted against time of depletion.

The depletion curves were of an exponential type and those for the adrenal glands, spleen and aqueous humour were identical to the ones obtained by Penney & Zilva (1946). On the other hand, the slopes for the brain and eye lens (organs not examined by Penny & Zilva) were of a quite different pattern and indicated a much slower rate of depletion (Fig. 1 *A*, *B*). After 14 d on the scorbutogenic diet these organs still retained 24 and 27 % respectively of their original concentrations of AA.

The depletion of isoAA is included in Fig. 1 *C*, and a quantitative comparison of the depletion rates of AA and isoAA in male guinea-pigs is given in Table 1.

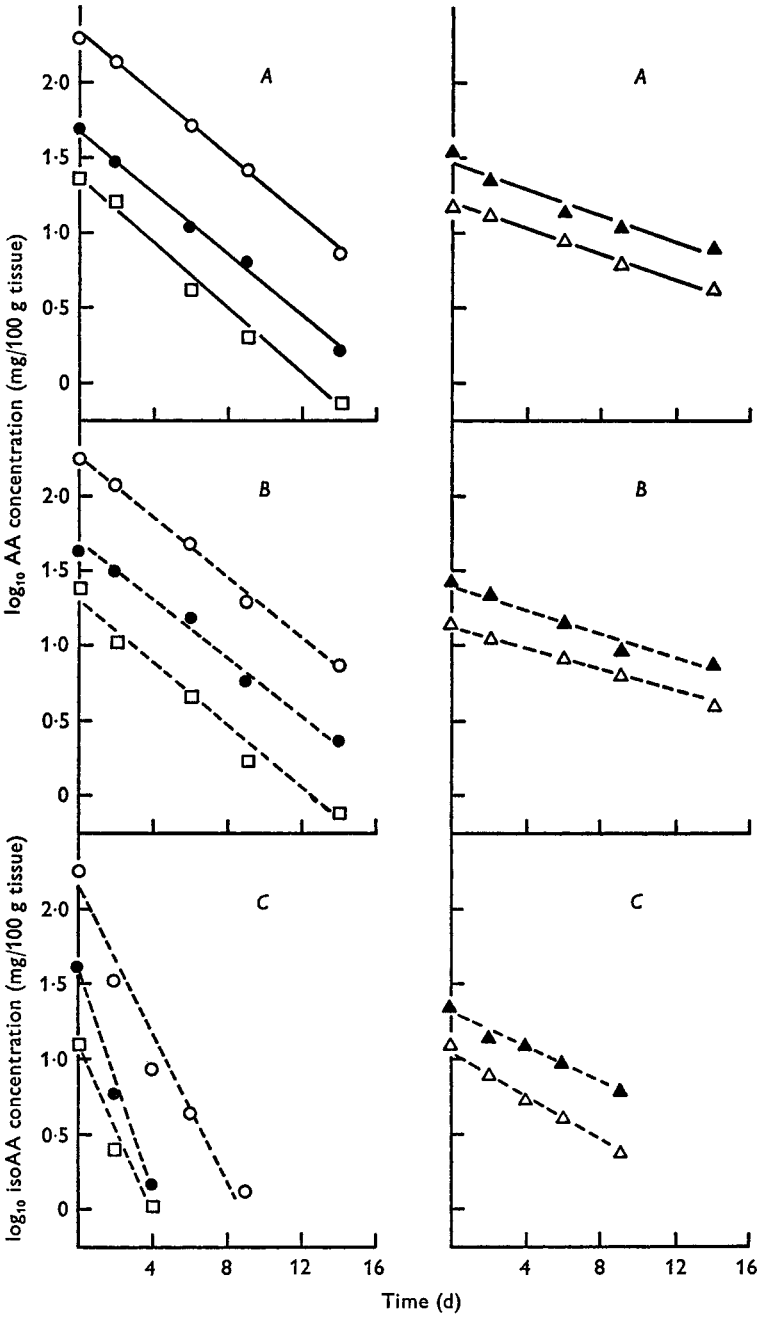

Fig. 1. Loss of L-xyloascorbic acid (AA) (*A*, *B*) and D-araboascorbic acid (isoAA) (*C*) from adrenal glands (○), spleen (●), aqueous humour (□), brain (▲) and eye lens (△) in male (– – –) and female (——) guinea-pigs when placed on a scorbutogenic diet.

Table 1. *Loss of ascorbic acid (AA) and isoascorbic acid (isoAA) from tissues of male guinea-pigs, when placed on a scorbutogenic diet*

(Each value is the mean (with standard error) for five animals; (*a*) concentration in mg/100 g tissue; (*b*) percentage of initial concentration remaining)

Time after last dose (*d*)	Adrenal glands				Spleen				Aqueous humour				Brain				Eye lens			
	AA		isoAA		AA		isoAA		AA		isoAA		AA		isoAA		AA		isoAA	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
0	172·0 ±16·0	—	187·9 ±6·3	—	46·2 ±1·2	—	41·0 ±2·0	—	26·6 ±1·6	—	15·7 ±1·3	—	33·7 ±1·1	—	23·1 ±1·1	—	15·7 ±0·2	—	13·6 ±1·0	—
2	118·5 ±6·2	69	33·5 ±2·4	18	31·9 ±2·3	69	5·8 ±0·4	14	10·3 ±0·3	39	2·5 ±0·2	16	28·0 ±0·7	84	16·8 ±0·7	73	12·3 ±0·3	79	8·3 ±0·6	61
6	49·6 ±2·9	29	4·2 ±0·8	2	15·4 ±1·1	33	< 1·0	< 2	4·6 ±0·4	18	1·1 ±0·1	7	15·1 ±0·3	45	10·8 ±0·8	47	9·0 ±0·3	57	4·3 ±0·3	32
9	20·3 ±3·6	12	1·3 ±0·1	< 1	6·1 ±1·0	13	—	—	1·9 ±0·1	7	0·8 ±0·04	5	9·2 ±0·5	27	6·6 ±0·4	29	6·8 ±0·2	43	2·6 ±0·1	19
14	7·6 ±1·8	4	—	—	2·5 ±0·1	5	—	—	0·8 ±0·05	3	—	—	8·2 ±0·2	24	—	—	4·3 ±0·2	27	—	—
20	1·8 ±0·3	1	—	—	< 1·0	< 2	—	—	—	—	—	—	4·9 ±0·1	15	—	—	2·8 ±0·2	18	—	—

After only 2 d of depletion isoAA in the adrenal glands and spleen had fallen to 18 and 14% respectively of its initial concentration; the corresponding values for AA at this stage were 69 and 69%. Measurements of isoAA were not made after the 9th day as it was by then virtually absent from certain tissues.

DISCUSSION

The rapidity with which a deficiency disease appears in animals receiving a diet deficient in an essential nutrient will in part depend upon the ability of the tissues to retain the nutrient. AA is lost rapidly from the tissues of guinea-pigs receiving a scorbutogenic diet, and this no doubt has a determinant role in the rapid onset of scurvy in the species (Penney & Zilva, 1946; Shanklin & O'Dell, 1966).

This rapid rate of depletion has been confirmed in our experiments; after 14 d on the scorbutogenic diet the concentrations of AA in the aqueous humour, spleen and adrenal glands had fallen to 3, 5 and 4% respectively of their concentrations at the commencement of the depletion period (Table 1).

Since there was no discernible difference between the patterns of AA depletion in male and female guinea-pigs (Fig. 1), different rates of depletion are an unlikely explanation of the sex differences in tissue concentrations of AA for human plasma and guinea-pig eye lens (Brook & Grimshaw, 1968; Hughes & Jones, 1971).

The demonstration that both the lens and brain of near-scorbutic guinea-pigs still contain significant concentrations of ascorbic acid confirms an earlier observation by Ginter, Bobek & Gerbelová (1965) and lends credence to the hypothesis that AA has possible metabolic involvements in these organs (Heath, 1962; Hughes & Jones, 1971). There is accumulating evidence from other studies that the behaviour of the brain and eye lens as regards fixation and retention of AA is anomalous in more respects than one (Martin, 1961; Hughes & Jones, 1971). Organs in which AA has metabolic involvements could well have developed greater-than-average retention capacities towards AA. Of interest in this respect is the recent suggestion that mental activities are related to cerebral concentrations of AA (Pauling, 1968) and the report that the administration of AA to patients suffering from mental depression produced a definite improvement (Walker, 1968). Certainly the whole question of the relationship between AA and the activity of the nervous system would appear to merit further investigation.

Recent studies have indicated that normal growth of guinea-pigs can be maintained by isoAA, provided that a sufficiently high concentration is present in the tissues (Fabianek & Herp, 1967; Hughes & Jones, 1970). The results summarized in Fig. 1*C* and Table 1, however, indicate an important point of difference between the two isomers. IsoAA is lost from the tissues at a much greater rate than AA.

As with AA, the retention capacity of the lens and brain towards isoAA was of a quite different order from that of other organs (Fig. 1C). After 9 d the brain and lens still contained 29 and 19% respectively of their original concentrations (Table 1). Here too the behaviour of the brain was anomalous, the pattern of depletion of isoAA being not significantly different from that of AA (Table 1). Recently, Pelletier (1969) has reported that guinea-pig tissues lose isoAA more rapidly than AA; he also found

that the retention of AA by the brain was significantly greater than by other organs examined.

Two points of interest emerge from this study. Firstly, it appears that depletion patterns for both isomers fall into two groups – those of rapidly depleting organs (≡ low retention capacity) and those of slowly-depleting ones (≡ high retention capacity). Further investigations are necessary before one can relate type of depletion to the metabolic significance of AA in any specific organ. Secondly, the retention capacity of a tissue for ascorbic acid appears to be characterized by a certain degree of structural specificity that permits a more efficient retention of the naturally occurring isomer.

This work was made possible by generous financial support from Beechams Products (UK) (PRJ) and the Medical Research Council (RJH).

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Printed in Great Britain