Regulation of Ascorbic Acid and of Xylulose Synthesis in Liver Extracts

THE EFFECT OF STARVATION IN VARIOUS ANIMALS

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1. The effect of starvation on the synthesis of ascorbic acid and of xylulose from glucuronolactone and from gulonate has been studied with liver extracts from cats, rabbits, hamsters, mice and guinea pigs. 2. The synthesis of ascorbic acid from glucuronolactone is decreased in all species except cats, and that from gulonate is decreased in hamsters and mice only. 3. The synthesis of xylulose from glucuronolactone was decreased in all species except cats and mice, whereas from gulonate it was enhanced in all the species examined.

The adaptive changes of the synthesis of ascorbic acid and of xylulose in rat-liver extracts during starvation have been reported (Stirpe & Comporti, 1965). In the present paper the effects of starvation on the synthesis of these substances in liver extracts from animals of other species are described. The main aim of this study was to ascertain whether the changes observed in rats were limited to this or to some species, or were a general consequence of starvation, since it is known that an enzyme can undergo adaptive changes in some species but not in others (Rosen & Nichol, 1964). The study was also intended as a screening to select the best suitable animal species for future work on this subject. Guinea pigs were included among the species examined, to study the formation of xylulose in an animal not able to synthesize ascorbic acid.

EXPERIMENTAL

Animals. Grey rabbits, albino mice, golden hamsters and albino guinea pigs were purchased from a breeding farm (Stefano Morini, S. Polo d'Enza, Reggio Emilia); cats were bred in the Laboratory. All animals were kept for at least 1 week in our animal room before being used for the experiments. They were maintained on the following diets: rabbits, oats and green vegetables; mice, stock diet used by Stirpe & Comporti (1965), bread and green vegetables; hamsters, stock diet and green salad; cats, rice cooked in milk and minced cooked meat; guinea pigs, bran and green vegetables. The starvation period was 24 hr. for all animals except hamsters; these were starved for 48 hr. to ensure consumption of the food stored in the cheek pouches. All animals except cats were stunned with a blow on the

head and killed by exsanguination; cats were exsanguinated under anaesthesia with Nembutal (50 mg./kg. body wt., given intraperitoneally). It was previously checked in rats that Nembutal does not affect the syntheses to be studied, nor the changes induced by starvation.

Biochemical determinations. Liver extracts were prepared in 0.15 M-KCl as described by Stirpe & Comporti (1965). The procedure for studying the synthesis of ascorbic acid and of xylulose, as well as all the analytical procedures, were also as described by Stirpe & Comporti (1965).

RESULTS

Glucuronolactone and potassium gulonate only were used as substrates for the synthesis of ascorbic acid and of xylulose, since the changes observed with these substrates in starved rats were very similar to those obtained with glucuronate and gulonolactone.

In the normal controls of all the species examined the amount of ascorbic acid formed from glucuronolactone was lower than the quantity synthesized by rat-liver extracts (Stirpe & Comporti, 1965), minimal values being observed in cats (Table 1). Lower values, as compared with rats, were also observed with gulonate in liver extracts from cats and rabbits. Starvation lowered the synthesis of ascorbic acid from glucuronolactone and from gulonate in hamsters and mice, from glucuronolactone only in rabbits, and was ineffective in cats.

The synthesis of xylulose in fed animals was comparable with that observed in rats. During starvation the amount of xylulose formed from glucuronolactone was decreased in all species but cats, whereas the quantity formed from gulonate was increased in all the animals examined, although to different extents.

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Table 1. *Synthesis of ascorbic acid and of xylulose in fed and in starved animals*

Incubation mixtures contained (in a final volume of 2.5 ml.): 50 μ moles of potassium phosphate buffer, pH 7.4; 4 μ moles of NAD; 4 μ moles of ATP; 75 μ moles of nicotinamide; 5 μ moles of $MgCl_2$; 20 μ moles of substrate; 0.7 ml. of liver extract. Incubation was in air at 37° for 2 hr. with glucuronolactone and for 1 hr. with gulonate. Results are mean values $\pm$ S.E.M. The numbers of experiments are given in parentheses.

Species	Treatment	Ascorbic acid formed		Xylulose formed	
		From glucuronolactone (μ mole/mg. of N/2 hr.)	From gulonate (μ mole/mg. of N/hr.)	From glucuronolactone (μ moles/mg. of N/2 hr.)	From gulonate (μ moles/mg. of N/hr.)
Cat	Control (2)	0.012	0.031	1.26	0.97
	Starved* (3)	0.014 $\pm$ 0.002	0.026 $\pm$ 0.014	1.46 $\pm$ 0.07	1.61 $\pm$ 0.74
Rabbit	Control (5)	0.050 $\pm$ 0.013	0.040 $\pm$ 0.013	1.07 $\pm$ 0.16	0.96 $\pm$ 0.32
	Starved* (5)	0.015 $\pm$ 0.002	0.047 $\pm$ 0.005	0.71 $\pm$ 0.06	3.40 $\pm$ 0.34
Hamster	Control (6)	0.074 $\pm$ 0.008	0.104 $\pm$ 0.006	1.67 $\pm$ 0.17	1.72 $\pm$ 0.24
	Starved* (2)	0	0.064	0.58	1.99
	Starved† (4)	0	0.067 $\pm$ 0.030	0.81 $\pm$ 0.09	2.25 $\pm$ 0.23
Mouse	Control (6)	0.030 $\pm$ 0.011	0.137 $\pm$ 0.006	0.82 $\pm$ 0.12	0.64 $\pm$ 0.11
	Starved* (6)	0.001	0.075 $\pm$ 0.003	0.77 $\pm$ 0.06	1.91 $\pm$ 0.08
Guinea pig	Control (6)	—	—	1.24 $\pm$ 0.10	1.24 $\pm$ 0.10
	Starved* (6)	—	—	0.39 $\pm$ 0.05	2.39 $\pm$ 0.17

* For 24 hr.

† For 48 hr.

DISCUSSION

Decreased synthesis of ascorbic acid as a result of starvation is not found in all the species examined, no changes being observed in cats in the synthesis from both substrates, and in rabbits in the synthesis from gulonate.

The overall effect of starvation on the synthesis of xylulose in the species examined was similar to that observed in rats, the only exceptions being observed in cats and mice with glucuronolactone as substrate. As far as allowed by the number of species examined, it may be concluded that only the enhancement of xylulose synthesis from gulonate is a common effect of starvation. Starvation affects

the synthesis of xylulose independently from that of ascorbic acid, as indicated by the experiments with guinea pigs.

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