Effect of the Administration of Xanthine on the Appearance of Xanthine Oxidase Activity in the Liver of Growing Rats

In the new-born rat, some hepatic enzymes are already present at the adult levels, while others are still absent and appear at various times after birth[1-3].

The starting signal regulating the appearance of the enzymatic activities during postnatal development is not known. As a working hypothesis, one may assume the existence of a repression-derepression system analogous to that found in micro-organisms.

In normally fed rats, liver xanthine-oxidase (E.C. 1.2.3.2.) activity appears after the twentieth day of life, while in protein malnutrition the time of appearance is delayed[4]. It is therefore possible to formulate the hypothesis that the appearance of the enzyme activity depends on the presence of an adequate amount of xanthine in the cell; the administration of xanthine might therefore anticipate the appearance of the enzyme activity.

Methods. Sprague-Dawley rats, 12–30 days of age, were used for these experiments. The samples were always from litter mates. Individual differences in xanthine-oxidase activity are actually negligible among rats from the same litter. Xanthine in physiological saline (24 mg/100 g body weight) was administered daily by i.p. injection. Control rats were injected with physiological saline. Upon histological examination of the liver, damages due to xanthine administration have never been evidenced. In some cases actinomycin D, cycloheximide or cortisone were administered.

When established, rats were sacrificed by decapitation, livers were rapidly removed, washed in 0.9% saline, pooled and homogenized in ice cold 0.039*M* phosphate buffer at pH 7.3 (1.5 wt/v) for 45 sec in a Potter homogenizer fitted with a motor-driven Teflon pestle.

Xanthine-oxidase activity was determined directly in the homogenate with a Warburg respirometer, as described by AXELROD and ELVEHJEM[5]. The enzyme activity was expressed in terms of μl of oxygen consumed per g of fresh liver or per mg of P of deoxyribonucleic acid (DNA-P)/h.

The effect of actinomycin D and cycloheximide administration was controlled respectively on the basis of the rate of incorporation of orotic acid-6-^{14}C into total RNA and of L-leucine-^{14}C and of L-phenylalanine-^{14}C into protein. 2.5 μC of orotic acid-6-^{14}C were injected into each actinomycin D-treated and control rat. The animals were sacrificed 20 min later, livers were rapidly removed, washed in 0.9% saline and homogenized (1:3 wt/v) in ice cold 0.01*M Tris* buffer at pH 7.3. RNA was extracted with the hot sodium chloride method[6].

L-Leucine-^{14}C (5 μC/100 g body weight) and L-phenylalanine-^{14}C (10 μC/100 g body weight) were injected i.p. into cycloheximide-treated and control rats, 30 min before sacrifice. The acid-insoluble precipitate was prepared from liver homogenate by adding 1 vol. of 10% TCA containing 1% casaminoacids.

The radioactivity was determined with the aid of a Nuclear Chicago low background counter.

RNA was assayed by the SCHNEIDER orcinol procedure[7]. DNA-P was determined by the method of WEBB and LEVY[8].

Results and discussion. In Table I it can be seen that while in control rats the xanthine-oxidase activity is present only after the twenty-second day of life, in rats injected for at least 2 consecutive days with xanthine,

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Table I. Effect of the administration of xanthine on the time of appearance of xanthine-oxidase activity

Age (days)	Days of treatment	Xanthine-treated[a]			Control[b]		
		No. of rats	O_2 μl/g wet liver/h	O_2 μl/mg DNA-P/h	No. of rats	O_2 μl/g wet liver/h	O_2 μl/mg DNA-P/h
12	5	13	0	0	10	0	0
13	–	–	–	–	4	0	0
14	5	3	0	0	3	0	0
15	2	5	110 (0–255)[c]	363 (0–885)	–	–	–
16	3	33	113 (0–229)	413 (0–874)	2	0	0
17	1	4	0	0	2	0	0
17	2	4	0	0	2	0	0
17	3	5	127 ± 53.6	625 ± 385	2	0	0
17	4	36	155 ± 14.9	602 ± 64	10	0	0
17	5	15	198 ± 26.8	652 ± 103	12	0	0
17	9	12	254 ± 50.3	841 ± 169	12	0	0
21	–	–	–	–	5	0	0
22	–	–	–	–	2	80 ± 13.2	269 ± 58
24	–	–	–	–	2	82 ± 21.3	313 ± 86
25	5	8	313 ± 9.2	1507 ± 69	4	270 ± 17.2	1106 ± 75
28	–	–	–	–	9	259 ± 22.5	890 ± 111
30	5	3	371 ± 28.6	1161 ± 92	3	277 ± 62.6	816 ± 206

[a] Xanthine was administered daily at the dosage of 24 mg/100 g body weight by i.p. injection, up until the day before the sacrifice. [b] Control rats were injected with physiological saline. [c] No. in parentheses represent the range of values.

the activity becomes detectable as early as the fifteenth day of life.

Moreover, prolonged xanthine treatment results in a further increase of activity, even at an age at which definite activity is already present. Any attempt to cause the appearance of xanthine-oxidase activity in the first 14 days of life was always unsuccessfull.

The effect of administration of xanthine is not mediated through the adrenal glands; in fact, 3 consecutive injections of cortisone (2.5 mg/100 g body weight 3 times every 4 h) did not cause any appearance of xanthine-oxidase activity in 16-day-old rats.

Table II shows that 4 days after the interruption of xanthine administration xanthine-oxidase activity slowly decreases, so that at the twenty-second day of life the enzyme activity is still higher than the normal value but lower than the value found before suspending xanthine administration.

This result indicates that a continuous administration of xanthine is essential in order to maintain the level of xanthine-oxidase activity induced by the injection of substrate.

As shown in Table III, actinomycin D treatment reduces the synthesis of RNA to approximately $^1/_3$, and cycloheximide almost completely inhibits the amino acid incorporation into proteins.

Table IV shows that actinomycin D is not able to prevent the effect of xanthine on xanthine-oxidase activity, a finding that would demonstrate that the action of xanthine is not at the transcriptional level. On the contrary, cycloheximide partially prevented the effect of xanthine.

On the basis of this last result it is possible to formulate 2 hypotheses: the first, that xanthine carries on its action

Table II. Evaluation of residual xanthine-oxidase activity[a]

Age[b] (days)	Xanthine-oxidase activity				Residual xanthine-oxidase activity $O_2\mu l$/mg DNA-P/h
	Control		Xanthine treated[c]		
	No. of rats	$O_2\mu l$/mg DNA-P/h	No. of rats	$O_2\mu l$/mg DNA-P/h	
17	2	0	2	443	–
22	2	269	2	378	109

[a] By 'residual xanthine-oxidase activity' is meant the difference between the level of enzymatic activity remaining 4 days after the interruption of xanthine administration and the level which is normally present at the same age. [b] Rats were all part of the same litter. [c] Xanthine was administered daily at the dosage of 24 mg/100 g body weight, from 12–16 days old, by i.p. injection. After the sixteenth day of life, the administration of xanthine was stopped also for rats to be sacrificed at the twenty-second day of life. Control rats were injected with physiological saline.

Table III. Inhibition of RNA and protein synthesis by actinomycin D and cycloheximide

Experiment No.	No. of rats[a]	Treatment	Tracer used	RNA cpm/μg RNA-P/0.2 ml	Protein cpm/mg liver
1	1	none	Orotic acid-6-^{14}C	691	–
	1	ACT D[b], 5 h	Orotic acid-6-^{14}C	188	–
	1	ACT D, 10 h	Orotic acid-6-^{14}C	131	–
2	1	none	L-Leu-^{14}C	–	29.0
	1	CHX[c], 5 h	L-Leu-^{14}C	–	0.3
	1	CHX, 10 h	L-Leu-^{14}C	–	0.2
3	1	none	L-Phe-^{14}C	–	121.3
	1	CHX, 5 h	L-Phe-^{14}C	–	0.8
	1	CHX, 5 h	L-Phe-^{14}C	–	0.9

[a] Rats of each experimental group were part of the same litter. [b] Actinomycin D (Act D) 1 μg/g body weight, was administered i.p. 5 h prior to sacrifice. Orotic acid-6-^{14}C 2.5 μC was injected i.p. 20 min prior to sacrifice. [c] Cycloheximide (CHX) 1 mg/30 g body weight (experiment 2) and 1 mg/15 g body weight (experiment 3), was administered i.p. 5 or 10 h prior to sacrifice. L-leucine-^{14}C 5 μC/100 g body weight, or L-phenylalanine-^{14}C 10 μC/100 g body weight were injected i.p. into the rats 30 min before sacrifice. The radioactivity was determined with the aid of a Nuclear Chicago low back ground counter.

Table IV. Effect of actinomycin D and cycloheximide upon the level of liver xanthine-oxidase

Experiment No.[a]	Age (days)	No. of rats	No. of xanthine injections[b]	Antibiotic used[c,d]	Xanthine-oxidase activity $O_2\mu l$/g wet liver/h	$O_2\mu l$/mg DNA-P/h
1	16	1	3	none	0	0
	17	2	4	none	120	681
	17	2	4	ACT D	156	650
2	16	2	3	none	91	350
	17	2	4	none	187	987
	17	2	4	ACT D	199	1134
3	16	2	3	none	46	162
	17	2	4	none	113	504
	17	2	4	ACT D	118	579
4	16	1	3	none	84	322
	17	2	4	none	152	632
	17	2	4	ACT D	137	583
5	15	2	2	none	76	204
	16	2	3	none	91	396
	16	2	3	CHX	74	254
6	15	2	2	none	0	0
	16	2	3	none	174	597
	16	3	3	CHX	111	389
7	16	2	3	none	229	874
	17	2	4	none	267	1107
	17	1	4	CHX	241	996
8	16	2	3	none	117	289
	17	2	4	none	156	602
	17	2	4	CHX	118	411

[a] Rats of each experimental group were part of the same litter. [b] Xanthine was administered daily at the dosage of 24 mg/100 g body weight by i.p. injection. Rats injected with actinomycin D and cycloheximide and their relative controls, were sacrificed 10 or 12 h after the last injection of xanthine. [c] Actinomycin-D (ACT D) 1 μg/g body weight was administered i.p. 12 h prior to sacrifice. [d] Cycloheximide (CHX) 1 mg/30 g body weight (experiments 5, 6, 8) or 1 mg/15 g body weight (experiment 7) was administered i.p. 10 h prior to sacrifice. The antibiotics were administered between the second or the third day of xanthine treatment, since, as shown in Table 1, the xanthine-oxidase activity markedly increases in this period.

at the translational level, and the second that xanthine acts simply as a stabilizer of the enzyme by decreasing the rate of its degradation, and thereby simulating an enzyme induction. Facts in favour of the second hypothesis are: the decrease of enzymatic activity following interruption of xanthine administration, and the possibility to cause the appearance of xanthine-oxidase activity only after the fourteenth day of life. One might in fact suppose that at this age xanthine-oxidase is already present in non-detectable amounts, and that xanthine exerts its stabilizing effect, which was not possible before, when the enzyme was really absent.

In this respect, xanthine-oxidase of rat liver during the first 30 days of life would behave as xanthine-oxidase of mouse liver. In fact, xanthine-oxidase activity of mouse liver is maintained, in protein depletion, by the administration of xanthine[9]. These same results have not been observed in the protein-depleted adult rat[10] nor in the protein-depleted chick[11,12].

Riassunto. La somministrazione di xantina a ratti di età compresa fra i 12 e i 30 giorni, causa una precoce comparsa di attività xantinossidasica. Tale comparsa è influenzata dalla cycloheximide ma non dal cortisone nè dalla attinomicina D.

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Effect of Chronic Administration of Acetylsalicylic Acid on Lipid Metabolism in Growing Rats

Several in vivo and in vitro investigations have demonstrated the diverse effects of salicylates in biological systems[1,2]. Most of these studies have been confined to adult animals or tissues thereof. In recent years evidence has been obtained to indicate that prolonged administration of salicylates causes structural and functional alterations in certain glands and tissues of experimental animals[3,4]. The importance of phospholipids in structure and integrity of all membranes, the transport of cholesterol and other lipids in the blood, and possibly in some capacity in terminal oxidation is well recognized.

The present communication deals with the changes in the fatty acid compositions of the phospholipids of liver and brain as a result of prolonged administration of acetylsalicylic acid to young growing rats. In addition, since salicylate has been repeatedly found to cause marked functional changes in the adrenals[5], a preliminary study of the fatty acid composition of their total lipids under these conditions has also been made.

Materials and methods. Male Sprague-Dawley rats were placed on synthetic diet[6] containing 15% corn oil substituted isocalorically for carbohydrate. One half the total number of rats from this group were given daily oral administration of acetylsalicylic acid (0.3 mg/g body weight) mixed with a small amount of corn oil for periods up to 90 days. The daily dosage was determined from the average body weight of the entire group receiving acetylsalicylic acid. Respective controls were given an equivalent amount of corn oil.

Five treated and untreated animals were sacrificed by decapitation at 4, 8 and 12 weeks. Liver, brain and adrenals were taken out immediately. The adrenals were freed from adhering fat and pooled together for extraction of total lipids.

Total lipids were extracted from these tissues by homogenization in a mixture of methylal-methanol (4/1, v/v) containing 0.1 mg/ml of DL-α-tocopherol as an antioxidant. Phospholipids were isolated from the crude extracts of the liver by thin-layer chromatography as described previously[7]. Since the brain tissue contains most of the fatty acids in the form of phospholipids, it was considered adequate to use the whole brain lipid extract

Table I. Fatty acid composition of liver and brain phospholipids of rats administered acetylsalicylic acid (ASA)

Fatty acid	% total fatty acids[a]			
	Liver (4 weeks)		Brain (12 weeks)	
	Control	ASA	Control	ASA
16:al	–	–	1.3 ± 0.0	1.5 ± 0.0
16:0	14.7 ± 0.7	26.4 ± 2.0	32.2 ± 1.1	36.1 ± 0.8
16:1	1.0 ± 0.1	1.0 ± 0.1	0.2 ± 0.0	0.9 ± 0.1
18:al	–	–	1.3 ± 0.3	1.2 ± 0.2
18:0	20.5 ± 1.1	26.5 ± 2.0	19.8 ± 1.4	18.6 ± 0.5
18:1	9.5 ± 0.4	8.3 ± 1.0	32.8 ± 0.8	29.3 ± 0.7
18:2	17.4 ± 0.8	15.4 ± 1.5	0.3 ± 0.0	0.4 ± 0.0
20:4	26.2 ± 1.7	15.4 ± 1.3	4.0 ± 0.2	4.0 ± 0.3
22:6	2.7 ± 0.3	1.2 ± 0.0	3.9 ± 0.4	2.6 ± 0.2
Others	8.0	5.8	4.2	5.4

[a] Results expressed as mean of all animals in the respective groups ± standard error of the mean.

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