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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Table II. Fatty acid composition of adrenal lipids of rats administered acetylsalicylic acid (ASA)

Fatty acid	% total fatty acids*					
	4 weeks		8 weeks		12 weeks	
	Control	ASA	Control	ASA	Control	ASA
16:0	24.5 ± 0.8	28.7 ± 1.2	22.3 ± 1.5	24.5 ± 1.4	18.0 ± 1.0	25.0 ± 1.4
16:1	1.3 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	0.8 ± 0.1	1.2 ± 0.2
18:0	9.9 ± 2.2	6.3 ± 1.9	2.7 ± 0.6	2.4 ± 0.2	2.1 ± 0.3	3.0 ± 0.4
18:1	30.5 ± 1.5	31.0 ± 0.7	32.3 ± 1.0	36.5 ± 0.8	31.3 ± 1.0	35.1 ± 1.3
18:2	27.7 ± 2.0	27.7 ± 2.2	36.5 ± 2.6	32.7 ± 1.4	44.2 ± 1.2	32.4 ± 0.6
20:4	3.7 ± 1.4	2.8 ± 0.6	1.9 ± 0.8	1.1 ± 0.3	1.5 ± 0.3	1.4 ± 0.3
22:6	0.1	0.1	0.1	0.1	0.2	0.2
Others	2.9	2.3	2.9	1.3	1.9	1.7

* Results expressed as mean of all animals in the respective groups ± standard error of the mean.

for the purposes of this study. Also, only total lipid extracts of the adrenals were used for the analysis of fatty acid composition and phospholipids were not separated.

Methyl esters of the fatty acids of the extracts used for the study were prepared by transmethylation and their composition analyzed by means of gas-liquid chromatography as described elsewhere[8].

Results. In the livers of rats, significantly elevated levels of palmitic (16:0) and stearic (18:0) acids in the phospholipids resulted from the administration of acetylsalicylic acid (Table I). Among the unsaturated fatty acids, only arachidonic acid (20:4) showed a significant decrease. The major alterations in the fatty acid composition of liver phospholipids were observed at 4 weeks. Further prolonged administration of acetylsalicylic acid beyond this period failed to bring about any difference in the fatty acids between treated and control animals. On the contrary, the composition of phospholipid fatty acids in the livers of treated animals even approached that of the controls and were similar at 12 weeks.

The fatty acid composition of brain lipids was, as expected, found to be more resistant than liver phospholipids to alterations due to acetylsalicylic acid administration. After prolonged administration up to 12 weeks, however, significantly higher levels of palmitic acid (16:0) and somewhat lower levels of stearic (18:0) and oleic (18:1) acids were found in the brain lipids of rats given acetylsalicylic acid than in the controls.

As distinct from its effects on liver and brain lipids, acetylsalicylic acid caused a more gradual and progressive alteration in the fatty acid composition of adrenal lipids (Table II). After 4 weeks, the only difference noticed was higher level of 16:0 in the adrenals of salicylate-treated animals than in the controls. While this difference remained essentially unchanged, it was accompanied by a larger proportion of 18:1 at 8 weeks. In addition to these changes further alteration, namely, a decrease in the proportion of linoleic acid (18:2) in the adrenals resulted from the continued administration up to 12 weeks.

Discussion. It is well known that liver phospholipids are subject to alterations by various means more readily than brain lipid, and as such it is not surprising that liver phospholipids should respond more readily to acetylsalicylic acid. However, that brain lipids also show certain alterations as a result of prolonged administration of acetylsalicylic acid is notable. Salicylic acid has been shown to penetrate into the brain although less readily so than in the liver[9]. Therefore, one may postulate that the alterations in the fatty acid composition of the brain lipids are due either to a change in the physiological status of the drug-treated animals causing changes in the relative proportions of the various classes of phospholipids; or, this may represent the result of a direct inhibition of the synthesis of unsaturated fatty acids resulting from a suppression of oxidative phosphorylation by acetylsalicylic acid.

Repeated administration of salicylate has been found to cause a marked reduction in adrenal cholesterol[10] and increased blood concentrations of 17-hydroxycorticosteroids in the rat[11]. Since adrenal gland lipids contain a high concentration of cholesterol esters, it would be safe to assume that at least part of the changes noted in this organ is due to preferential utilization of adrenal cholesterol esters for the biosynthesis of corticosteroids[12–15].

Résumé. L'administration prolongée d'acide acétylsalicylique à de jeunes rats (0.3 mg/g) a provoqué des augmentations temporaires d'acide palmitique dans les phospholipides du cerveau et du foie. Des augmentations temporaires d'acide stéarique et des diminutions d'acide arachidonique ont été aussi observées dans les phospholipides du foie. Les changements observés dans les phospholipides du foie permettent de supposer que l'acide acétylsalicylique affecte peut-être les enzymes désaturisant les acides gras.

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